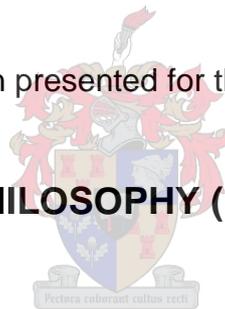


The authentication of regionally unique South African lamb

Sara Wilhelmina Erasmus

Dissertation presented for the degree of

DOCTOR OF PHILOSOPHY (FOOD SCIENCE)



in the Faculty of AgriSciences at Stellenbosch University

Supervisor: Prof L.C. Hoffman (Department of Animal Sciences, Stellenbosch University)

Co-supervisor: Ms M. Muller (Department of Food Science, Stellenbosch University)

March 2017

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained herein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Declaration with signature in possession
of candidate and supervisor

March 2017

Sara Wilhelmina Erasmus

Date

Abstract

Noted for its unique herbaceous flavour which is imbued from a diet of indigenous fragrant plants, Karoo lamb is marketed as one of South Africa's finest meat products. It is also the first fresh meat product to receive Protected Geographical Indication (PGI) status in South Africa. Its distinct quality is imparted through natural grazing of the sheep on the Karoo veld of the Northern parts of the country. Although it is considered common knowledge to South Africans that Karoo lamb is different to that of lamb meat from other regions, there is a lack of scientific evidence to verify these claims. In order for Karoo lamb to receive official recognition and protection as an authentic product, it is vital to confirm such claims. The aim of the study was to validate the authentic nature of regionally unique South African lamb using analytical techniques. Emphasis was placed on Karoo lamb, while the characteristics of other region of origin lamb, such as the Rûens and Free State lamb, were also determined. A key aspect of the study was to link the characteristic diet, related to its origin, to the sensory and chemical profiles of the meat and fat.

The findings show that diet plays an integral part in the sensory characteristics of Karoo lamb meat and hence, have a significant influence on the sensory and chemical profile of South African lamb. Descriptive sensory analysis (DSA), fatty acid analysis, solid-phase microextraction (SPME), isotope ratio mass spectrometry (IRMS), near-infrared reflectance spectroscopy (NIRS) (using a portable MicroNIR spectrometer) and proton transfer reaction-mass spectrometry (PTR-MS) proved to be very successful analytical tools for the authentication of lamb, distinguishing Karoo from Non-Karoo lamb.

A key finding was the detection of volatiles, specifically terpenes, present in both the Karoo bushes and the Karoo lamb meat and fat. Terpenes were prominent in the fat tissue and detected at mass ratios m/z 81 and m/z 137 using PTR-MS. The dominant terpenes were tentatively identified as α -pinene, β -pinene, limonene and trans-caryophyllene using SPME. The highest concentrations of terpenes were detected in Karoo lamb, while the Non-Karoo lamb did not or hardly contained any. Within the Karoo, regional differences were apparent as Hantam Karoo lamb had the highest ratings for herbaceous aroma and flavour and contained the greatest concentration of terpenes. Therefore, it is proposed that Karoo lamb is marketed according to its region of origin. Herbaceous aroma and flavour attributes associate with a diet rich in fragrant Karoo plants which were verified with stable isotope ratio analysis. The stable isotopic ratios were indicative of the extensive grazing diet of the animals where discrimination between diets composed of grass, Karoo bushes, lucerne/alfalfa (*Medicago sativa*) and a combination of grass and Karoo bushes were achieved. The results confirm that Karoo bushes are responsible for the distinct aroma and flavour of Karoo lamb. Hence, the results serve as evidence for its certification and justify the protection of its indicator status.

It is recommended that the meat industry utilise the value linked to origin and invest in the marketing of regionally unique lamb. In order to prevent fraudulence and the misuse of protected names, the meat industry should also implement NIRS and PTR-MS as a rapid and effective origin based testing method. The combination of these two techniques improves the discriminative power and allows reliable origin classification.

Opsomming

Bekend vir sy unieke kruidagtige geur, neergelê deur 'n dieet ryk aan inheemse geurige plante, word Karoolam as een van Suid-Afrika se top vleisprodukte bemark. Karoolam is die eerste vars vleisprodukt in Suid-Afrika om die status van *beskermdede geografiese aanduiding* (*Protected Geographical Indication*; PGI) te ontvang. Die unieke kwaliteit van Karoolam word veroorsaak deur die natuurlike weiding van skape op die Karooveld in die noordelike dele van Suid-Afrika. Alhoewel dit vir die Suid-Afrikaanse verbruiker algemene kennis is dat Karoolam verskil van lamsvleis uit ander produksiestreke, is daar 'n gebrek aan wetenskaplike navorsing om hierdie stellings te verifieer. Dit is egter noodsaaklik om hierdie aansprake te bevestig sodat Karoolam amptelike erkenning en beskerming kan ontvang as 'n unieke produk. Die doel van die studie was om die outentieke aard van streeks-unieke Suid-Afrikaanse lamsvleis te bekragtig deur die gebruik van analitiese tegnieke. Daar is veral klem gelê op Karoolam, maar die eienskappe van ander streeks-unieke lamsvleis, soos dié van Rûens en Vrystaatse lam, is ook bepaal. 'n Belangrike aspek van die studie was om die kenmerkende dieet, verbind met die oorsprong, aan die sensoriese en chemiese profiele van die vleis en vet te koppel.

Daar is gevind dat die dieet 'n integrale rol speel in die sensoriese eienskappe van Karoolam vleis en gevolglik 'n beduidende invloed het op die sensoriese en chemiese profiel van Suid-Afrikaanse lam. Beskrywende sensoriese analise, vetsuur ontledings, soliede fase mikro-ekstraksie (SPME), isotoop verhouding massa spektrometrie (IRMS), naby-infrarooi reflektansie spektroskopie (NIRS) (met behulp van 'n draagbare MicroNIR spektrometer) en proton oordrag reaksie-massa spektrometrie (PTR-MS) is bewys as suksesvolle analitiese tegnieke vir die verifikasie van die unieke aard van lamsvleis met betekenisvolle onderskeid tussen Karoolam en Nie-Karoolam.

'n Betekenisvolle bevinding was die deteksie van vlugtige komponente, bekend as terpene, in die Karoo bossies, asook die Karoolam vleis en vet. Hierdie terpene was veral volop in die vetweefsel en is opgetel by massa verhoudings van m/z 81 en m/z 137 met behulp van PTR-MS. Dominante terpene is tentatief geïdentifiseer as α -pineen, β -pineen, limoneen en trans-caryophyllene met behulp van SPME. Die hoogste konsentrasies van terpene is gevind in Karoolam, terwyl die Nie-Karoolam geen of 'n lae konsentrasie bevat. Streeksverskille het in die Karoo voorgekom met die hoogste graderings vir kruidagtige aroma en geur en konsentrasie van terpene vir Hantam Karoolam. Daarom word daar voorgestel om Karoolam volgens sy streek van herkoms te bemark. Kruidagtige aroma en geureienskappe assosieer met 'n dieet ryk aan geurige Karoo plante wat uiteraard ook deur die stabiele isotoop verhoudings geverifieer is. Die ekstensiewe dieet van die skape is deur die stabiele isotoop verhoudings weerspieël waar onderskeiding getref kon word tussen diëte wat bestaan uit gras, Karoo bossies, lusern (*Medicago sativa*) en 'n kombinasie van gras en Karoo bossies. Die resultate bevestig dat Karoo bossies verantwoordelik is vir die kenmerkende geur en geur van Karoolam. Die resultate dien dus as bewys vir die sertifisering en beskerming van Karoolam as 'n unieke produk.

Die vleisbedryf word aanbeveel om die waarde wat aan oorsprongname gekoppel word, te benut en sodoende te belê in die bemarking van streeksunieke lamsvleis. Die vleisbedryf kan ook NIRS en PTR-MS implementeer as vinnige en doeltreffende oorsprong gebaseerde analitiese metodes om die misbruik van beskermdede name te voorkom. Die kombinasie van hierdie twee tegnieke verbeter die onderskeidingsvermoë en dra by tot betroubare oorsprong klassifikasie.

Acknowledgements

I would like to express my sincere appreciation to the following individuals and organisations for their valuable contributions to the successful completion of this research project.

My supervisor, Prof Louw Hoffman, thank you for introducing me to Meat Science in 2011. You have always been the *Vleiskunde Koning* to me and I treasure all the *Carpe Diem* moments you have created for me. Under your guidance, I have learnt and experienced so much in my four postgraduate years. Thank you for believing in me and trusting in the decisions you (sometimes) allowed me to make. I am truly honoured.

My co-supervisor, Ms Nina Muller, thank you for always being available to help and guide me in the right direction. I have learnt so much from you. Your work ethic, attention to detail and love for students (at the cost of your own work) is truly inspirational. Without your input, this dissertation would not have been possible.

Prof Johann Kirsten, thank you for your contribution and advice. I deeply appreciate your interest in the research and the value you expressed towards the results. I hope that the findings and publications of this study will validate the authentic nature of certified *Karoo Meat of Origin* and help with future research projects.

My highest gratitude goes towards:

Prof Marena Manley for her guidance and support with NIRS analyses. Also, thank you for convincing me to study Food Science in 2009. It has certainly been a great journey.

RIKILT: Prof Saskia van Ruth for her guidance, support and creating the opportunity for me to perform PTR-MS analysis. Dr Martin Alewijn and Mr Alex Koot for their technical guidance at RIKILT. Mr Eric Cuijpers, thank you for all the conversations and making me feel welcome in the Netherlands.

Prof Edi Piasentier for his guidance with the statistical analyses and accommodating me in Udine. I will always have a soft spot for Italy and your rich heritage, culture and, most certainly, the wine and food.

Ms Marieta van der Rijst for the statistical analyses.

Dr Frans Radloff (CPUT), Mr Mike Butler (iThembaLABS) and Mr Osborne (iThembaLABS) for their guidance and help with the stable isotope analyses.

Mr Lucky Mokwena for his help with the solid-phase microextraction. Thank you for your friendly smile and warm heart.

The staff of GWK Groblershoop: Mr Alex Cilliers, Mr Kobus Buys, Mr Flippie Ludick, Mr Martin van Zyl and Mr Willem Papier; and GWK De Aar: Mr Johan Hanekom, Mr Vernon Appolis and Mr Andre van der Walt for assisting me with the sample collection.

Mr Louis Retief of the *Made in the Karoo* brand for supplying meat samples and accommodating me. You have a great brand and your lamb is one of the best I have tasted.

Ms Jolandi Visagie (KLK Calvinia Ramskop Abattoir) and Mr Dian Giliomee (Bredasdorp Abattoir) for assisting me with the sample collection.

Dr P.J. Pieterse for the identification of plants. You were more than willing to help, even though it was not always an easy task.

Mr F.C. Basson (Western Cape Department of Agriculture, South Africa) for creating the maps.

Dr Dirk Troskie for his advice regarding Karoo lamb certification and Mr Derek Carstens of the *Taste of the Karoo* brand for his advice.

The help of staff and post graduate students from the Departments of Animal Sciences and Food Science (Stellenbosch University) and RIKILT (Wageningen University and Research) is highly appreciated. Michael Mlambo, Janine Booyse and Cheryl Muller thank you for all the conversations, jokes and support. I will miss you.

I would like to express my appreciation towards RIKILT (Institute of Food Safety) at Wageningen University and Research in the Netherlands for granting me the valuable opportunity to perform PTR-MS analyses; the Karoo Development Foundation (KDF) and Griekwaland-Wes Korporatief (GWK) for providing meat samples; the Foundation Study Fund (the Netherlands) for South African Students for granting me a bursary to go on a 3-month collaborative research visit to RIKILT in 2016 and the Western Cape Department of Agriculture.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF. NRF funding included the Innovation Scholarship (2013-2014) and Scarce Skills Scholarship (2015-2016).

I am grateful for funding from the Meat Industry Trust (MIT bursary, 2014) and the Brian Koeppen Scholarship awarded to me by the South African Association for Food Science and Technology (SAAFoST). I wish to also acknowledge the financial assistance of Stellenbosch University (Merit bursary, 2013, 2015, 2016; International travel bursary, 2016).

My dear Food Science and Animal Sciences friends: Maxine Jones, Liesel van Emmenes, Altie Burger, Daniel van der Merwe, Henk Smit, Jeannine Marais, Nikki Hogan and Cecil Mitchell. Thank you for all the fun times, conversations and craziness the last couple of years. Without you, my postgraduate years would have been very boring. You will always have a very special place in my heart.

Greta Geldenhuys, thank you for guiding and motivating me all the way. I have learnt so much from you and I am grateful for having you in my life.

Wiesie Burger, Cois Erasmus, Hansie Erasmus, Helena Erasmus, Rozelle Bosch, Eckhardt Bosch, Camen Hunter, Andri Ferreira and all my other friends and family members, thank you for your endless love, prayers and support. I deeply appreciate it.

Oom Francois en tannie Dennise Erasmus, dankie dat julle altyd soveel belangstelling in die studie getoon het en my gehelp het om die Karoo bossies te versamel. Ek het vir nou eers klaar "klei getrap".

My ouers, Koos en Sarie Erasmus, julle het my groot gemaak om onafhanklik, liefdevol, hardwerkend en gemotiveerd te wees sodat ek die beste van die lewe kan maak. Baie dankie vir julle liefde, ondersteuning, gebede, belangstelling en hulp met die studie. Ek sou dit nooit sonder julle kon doen nie. Ek sal altyd die tye onthou wat ons Karoo toe moes gaan om bossies te kry. Pa het dit altyd so geniet om mense te vertel wat ek doen – ek dink pa kan my PhD beter verduidelik as wat ek kan. Ek is baie dankbaar en lief vir julle.

Last, but certainly not the least. All gratitude and honour goes towards our Heavenly Father. This thesis would not have been possible without His help, guidance and graceful time. He has granted me the opportunity to grow intellectually, emotionally, travel the World, experience things I could never imagined and meet the most amazing people on this journey.

Notes and publications

The language and style used in this thesis is in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between the chapters has, therefore, been unavoidable, especially in terms of the materials and methods sections.

Results from this study have been published or presented in the following scientific peer-reviewed journals or symposiums:

Journal articles

Erasmus, S. W., Hoffman, L. C., Muller, M. & Van der Rijst, M. (2016). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems, *Small Ruminant Research*, **144**, 62-74. <http://dx.doi.org/10.1016/j.smallrumres.2016.07.020>.

Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.

Erasmus, S. W., Muller, M. & Hoffman, L. C. (Accepted, In Press). Authentic sheep meat in the European Union: Factors influencing and validating its unique quality. *Journal of the Science of Food and Agriculture*.

Congress oral presentations

Erasmus, S. W., Hoffman, L. C., Muller, M. & Van der Rijst, M. (2015). Exploring the variation in the sensory profile of South African Dorper lamb from extensive grazing systems, presented at the *South African Association for Food Science & Technology (SAAFoST) 21st Biennial International Congress and Exhibition*, 6-9 September 2015, Durban Kwazulu Natal, South Africa.

Congress poster presentations

Erasmus, S. W., Hoffman, L. C., Manley, M. & Muller, M. (2015). MicroNIR spectroscopy: A potential analytical tool for the classification of origin and prediction of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lamb meat, presented at the *17th International Conference on Near Infrared Spectroscopy (ICNIRS)*, 18-23 October 2015, Foz do Iguassu, Brazil.

Erasmus, S. W., Muller, M., Alewijn, M., Koot, A., Van Ruth, S. M. & Hoffman, L. C. (2016). The sensory and chemical profiling of South African lamb: Evidence for its authenticity, presented at the *1st Postgraduate Symposium on Food Fraud* hosted by Wageningen UR (University and Research), 23-24 June 2016, Wageningen, the Netherlands. Awarded 2nd best overall.

Erasmus, S. W., Manley, M., Muller, M. & Hoffman, L. C. (2016). MicroNIR spectroscopy for the authentication of South African lamb, presented at the *62nd International Congress of Meat Science and Technology (ICoMST)*, 14-19 August 2016, Bangkok, Thailand.

Contents

Declaration	ii
Abstract	iii
Opsomming	iv
Acknowledgements	v
Notes and publications	vii
Chapter 1	1
General introduction	
Chapter 2	10
Authentic sheep meat in the European Union: Factors influencing and validating its unique meat quality	
Chapter 3	42
Variation in the sensory profile of South African Dorper lamb from extensive grazing systems	
Chapter 4	70
Fatty acid profile and the volatile aroma compounds of South African Dorper lamb from extensive grazing systems	
Chapter 5	101
Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat	
Chapter 6	123
Near-infrared spectroscopy (NIRS) for the authentication of regionally unique South African lamb	
Chapter 7	141
Stable isotope ratio analysis for the authentication of regionally unique South African lamb	
Chapter 8	160
Proton transfer reaction-mass spectrometry (PTR-MS) for the authentication of regionally unique South African lamb	
Chapter 9	199
General discussion and conclusions	

Chapter 1

General introduction

The origin of food products and quality characteristics linked with it are becoming increasingly important for the consumer with the demand for food shifting from quantity towards quality (i.e. authenticity or quality reassurance) (Verbeke & Vackier, 2004; Grunert, 2005). Labelling plays an important role in origin-based names as it is used to reflect the authentic quality, drawing attention to a product, distinguishing it from similar-type products and exploiting the consumer's positive attitude towards territorial names (Rippon, 2014). In some cases, it can make the consumer believe that the product is better than its counterparts, substantiating a premium selling price for it. Although, authentic products are not necessarily of higher quality, the higher value associated with the name allows opportunities for fraud through false declarations and the misuse of geographical names (Capuano *et al.*, 2013).

The European Union (EU) protect names relating to the origin through a system of Geographical Indications (GI) (Schieber, 2008; Kirsten *et al.*, 2012). Camin *et al.* (2007) explains that certain products from defined regions are highly appreciated by consumers and are therefore more expensive. In EU countries, such products, with a strong regional identity, may have an officially protected denomination of origin (i.e. PDO). Protected Designation of Origin (PDO) is assigned to a foodstuff if all stages of production, processing and preparation is performed in a specific origin (i.e. region, place or country) (Schieber, 2008). Other terms include, Protected Geographical Indication (PGI) where the geographical link must occur in at least one of the stages of production, processing and preparation or Traditional Speciality Guaranteed (TSG) where a product is produced in a traditional manner (Schieber, 2008).

In South Africa, regionally unique products such as *Rooibos tea*, *Honeybush tea* and *Karoo lamb* exist (Joubert & De Beer, 2011; Bramley *et al.*, 2013). Lamb meat originating from the Karoo region is known as Karoo lamb and is recognised as one of the first red meat products to be regarded as a PGI in South Africa. The Karoo covers 30% of the total area of South Africa (Vorster & Roux, 1983; Bramley *et al.*, 2009). The name Karoo is derived from the indigenous Hottentot name, *Karu*, which means dry or arid land (Vorster & Roux, 1983). It is a semi-arid region with a low grazing capacity where the natural vegetation for extensive grazing varies from grassy, dwarf shrublands to dwarf, succulent shrubs (Vorster & Roux, 1983; Cloete & Olivier, 2010). Rainfall is typically low (401-600 mm per annum) and even lower (<200 mm or 201-400 mm per annum) in some parts of the region and greatly influences the plant growth (Palmer & Ainslie, 2005).

Karoo lamb has been part of the South African culture for many years (Du Plessis & Du Rand, 2012). The meat is particularly known for its excellent quality and unique flavour (Kirsten *et al.*, 2008; Weissnar & Du Rand, 2012). It is believed that the typical sensory characteristics of the meat, produced from domesticated sheep (*Ovis aries*), can be ascribed to the free-ranging conditions under which the animals roam, while they graze on the fragrant indigenous plants specific to the Karoo region (Estler *et al.*, 2006; Kirsten *et al.*, 2008; Weissnar & Du Rand, 2012). However, reliable scientific evidence is required to support the statement that

sheep meat of a certain breed produced with a specific feeding system has unique flavour attributes (Fisher *et al.*, 2000). If sufficient evidence could be gathered to support the meat's authenticity; such meat could be eligible for premium quality status (Fisher *et al.*, 2000). Resconi *et al.* (2010) propose that characteristic production systems might have the potential to produce lamb meat with regionally unique flavours. Furthermore, branding of the meat may possibly increase its purchase intent.

The typical Karoo sheep farmer is characterised by having a low-input and extensive production system (Kirsten *et al.*, 2008). Karoo farms characteristically have a very low grazing capacity with the norm in most areas estimated at 35 ha per large stock unit. At the end of the nineteenth century the farming practices of the north-eastern Karoo was changed with the introduction of windmills and wire fencing (Kirsten *et al.*, 2008). By the late 1920's the old shepherding and "kraaling" activities had been replaced with a new grazing system. The new system made use of artificial water sources and camps or paddocks in which the livestock ranged freely. Predictions were that these changes would raise the stocking rates, improve veld cover and reduce soil erosion.

The three main breeds of sheep used for the production of Karoo lamb is the Merino, Dorper and Dohne Merino (Vorster & Roux, 1983; Kirsten *et al.*, 2008; Cloete & Olivier, 2010). The sheep are reared on the field, making the production of Karoo lamb nearly "organic". However, minor doses for the treatment or prevention of sheep diseases are given as required by the Animal Health Act of 2002 (DAFF, 2002). Karoo lamb is generally sold directly from the field as farmers do not endorse the provision of additional feed (Kirsten *et al.*, 2008).

The majority of Karoo lamb farmers use in excess of 1 000 ha with flock sizes comprising more than 200 ewes (Kirsten *et al.*, 2008). Once the ram and ewe lambs reach the desired body weight of 30-40 kg (typically used as guideline by farmers) they are transported and sold to registered abattoirs. The ram lambs are slaughtered pre-puberty and therefore, they are not castrated. Through the abattoirs the meat is finally supplied to wholesalers, retailers and butcheries.

The need to develop an institution to endorse the Karoo brand became a possibility in November 2007, when two research teams united (Atkinson, 2008). One team was led by Prof Johann Kirsten (University of Pretoria) and Dr Dirk Troskie (Western Cape Department of Agriculture) and based at the Department of Agricultural Economics at the University of Pretoria. Their research focussed on the potential to brand products of origin and GI. Their project took place in partnership with CIRAD, a French organisation who focuses on international agricultural research for development (Atkinson, 2008; WIPO, 2009). One of their most important subjects was the branding of Karoo lamb.

By that time the Kirsten and Troskie team had already presented various workshops for Karoo lamb producers and a descriptive sensory analysis was conducted on Karoo lamb and Non-Karoo lamb from other parts of South Africa (Leighton *et al.*, 2007; Atkinson, 2008). They found that 54% of consumers in Cape Town and Johannesburg were aware of Karoo sheep meat, and about 27% of these respondents were willing to pay more for Karoo sheep meat (Kirsten *et al.*, 2008). A strong argument was established for registering Karoo

lamb as a GI, whilst having the potential to increase the profits for Karoo lamb producers (Atkinson, 2008). This could sequentially also be linked to enhanced rural development, such as fair trade registration. Furthermore, studies confirmed the hypothesis that consumers recognise the reputation of Karoo lamb and that they would be willing to pay a premium for it (Kirsten *et al.*, 2012). A strong case was built for a scheme of certified and coordinated marketing as Karoo lamb's identity and image had the potential to extract a premium from the market (Kirsten *et al.*, 2012). These arguments formed part of the motivation for the creation of a Karoo Development Foundation. Ultimately, the Karoo Development Foundation (KDF), part of the Arid Areas Programme (Atkinson, 2008), was established in 2009 as an *inter vivos* trust (nr IT1498/2009) in terms of Section 6 (1) of the Trust Property Control Act (Act 57 of 1988) (KDF, 2013). The KDF launched an origin based certification scheme for Karoo lamb (Kirsten *et al.*, 2012). This scheme enables the farmer to sell his lamb as Karoo lamb, provided that the farm meets the certification requirements set by the KDF (De Villiers, 2012). Under the scheme any producer and/or trader in Karoo sheep meat can apply to be certified to use the mark *Certified Karoo Meat of Origin* (KDF, 2013) (Fig. 1.1).

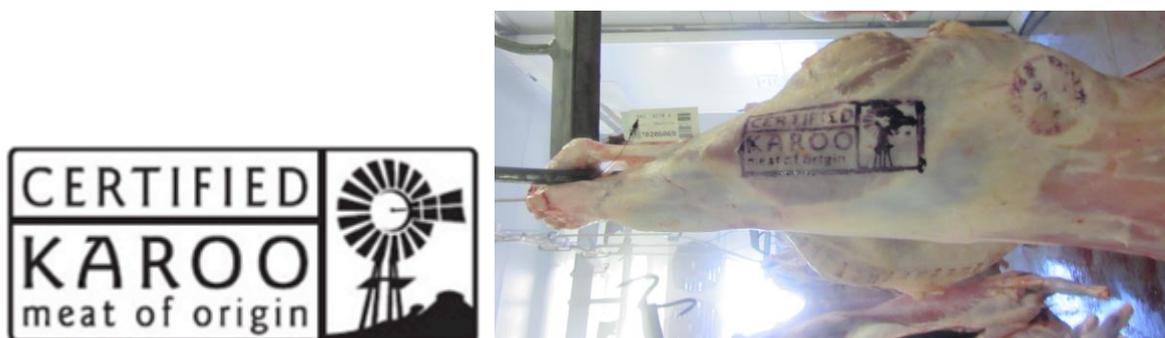


Figure 1.1 *Certified Karoo Meat of Origin* and windmill device stamp (KDF, 2013).

The mark is said to guarantee the origin, quality and unique flavour of Karoo meat (KDF, 2013). A third party auditing company ensures that the required standards of traceability, good manufacturing practices and legal requirements are met. The basis for the minimum standards, as defined by the KDF for the classification of *Karoo Meat of Origin*, was provided by previous studies. These included the work done by Kirsten's research group with the DURAS study (Kirsten *et al.*, 2008) and the subsequent research done for the Western Cape Department of Agriculture (Kirsten, J. 2012, Head of Department of Agricultural Economics, University of Pretoria, South Africa, personal communication, 6 February 2012).

On 1 November 2013 the South African Minister of Trade and Industry, in pursuance of the requirements of Section 13 of the Merchandise Marks Act (Act 17 of 1941), gave notice that the KDF has conveyed a request for the prohibition, in terms of Section 15(1) of the said act, on the use of the words "Karoo Lamb" (DTI, 2013). Certification under the *Karoo Meat of Origin* certification scheme of the KDF ensures that the rules set out in the act are complied with. The act offers valued domestic protection and ensure that the authenticity of Karoo lamb is maintained. A recent press release (10 October 2016), has confirmed that Karoo lamb has also received, the long awaited, international protection through its secured geographic indicator status in the

Economic Partnership Agreement (EPA) between the Southern African Development Community (SADC) EPA Group and the European Union (EU) (DTI, 2014). This development was initiated on 1 October 2014, following the end of the 10-year negotiations on free trade agreements between the EU and groups from the African, Caribbean and Pacific (ACP) countries (Wood, 2014). These developments have made Karoo lamb competitive on an international level and have added further value to its name.

Although the KDF has defined the production area of the Karoo, there is still some controversy regarding the exact boundaries. In fact, tension exist between the need to enclose the region (maintaining exclusivity) and being sufficiently expansive in order to ensure an economically successful production system. Access to international markets requires consistent supply of certified Karoo lamb. This could pose a challenge as times of drought alters the production of lamb, while some Karoo farmers are also not certified. Furthermore, issues such as the boundaries also reduce the availability of Karoo lamb as some regions, such as the Hantam Karoo, are not always accepted as Karoo lamb due to the lack of specific bushes (defined by the KDF) on the farms. As a result, it is important to determine whether different regions within the Karoo should be classified separately, while still conforming to being deemed Karoo lamb.

When official recognition and protection for regionally unique products is required, evidence should be provided to prove that the regional characteristics or “terroir” is responsible for the unique flavour and attributes of the final product. Once this is confirmed, only then can the product be officially protected as the “intellectual property” (brand) of a specific region. Karoo lamb is mainly distinguished from other types of sheep meat based on its unique sensory quality (i.e. flavour and aroma). Therefore, with the aim of providing scientific evidence for its authenticity, exploring the mechanisms and factors involved with the flavour and aroma development in authentic sheep meat is a valid starting point. Such information would provide a better understanding of why region of origin meat may differ on a quality based level, while it also allows for the identification of analytical tools suitable to measure these differences and verify the origin. These aspects will be dealt with in the literature review.

Research objectives for this PhD study

The initial phase of this research took place from 2013 to 2014. The aim of this phase was to compare the sensory and chemical profiles of lamb meat from different geographical origins in South Africa and determine whether the characteristic diet, linked to specific regions, has a significant influence on the sensory profile of South African lamb. In fact, by doing this the study provides scientific evidence to verify the claims (e.g. Karoo lamb has a unique flavour due to the fragrant Karoo bushes they consume) that most South Africans consider as common knowledge. It is crucial to confirm such claims in order for it to receive official recognition and protection as being authentic.

The objectives of the research were to determine the variation in the sensory profiles and proximate composition of lamb meat (Chapter 3, published); to determine the fatty acid profile and volatile aroma compounds of lamb meat (Chapter 4) and to evaluate the stable isotope ratios of lamb meat (Chapter 5,

published). The seven selected farms (each unique in terms of its vegetation and the extensive grazing conditions) and the research layout are shown in Figure 1.2 and 1.3, respectively.

The aims of the second phase of this research (from 2015-2016) were to authenticate regionally unique meat using analytical techniques and to develop a rapid and effective origin based testing method. Abattoirs or meat processors could potentially use such a suitable testing method. Three analytical techniques were selected for this purpose namely, near-infrared reflectance spectroscopy (NIRS) (Chapter 6) using a portable MicroNIR spectrometer, isotope ratio mass spectrometry (IRMS) (Chapter 7) and proton transfer reaction-mass spectrometry (PTR-MS) (Chapter 8). A new set of samples were collected for this phase. The regions selected for evaluation were from the Northern Cape province, the Eastern Cape province and the Western Cape province. Lambs obtained from Namibia (neighbour country), the feedlot and semi-extensive grazing conditions were also included. The selected regions and the research layout are shown in Figure 1.4 and 1.5, respectively.

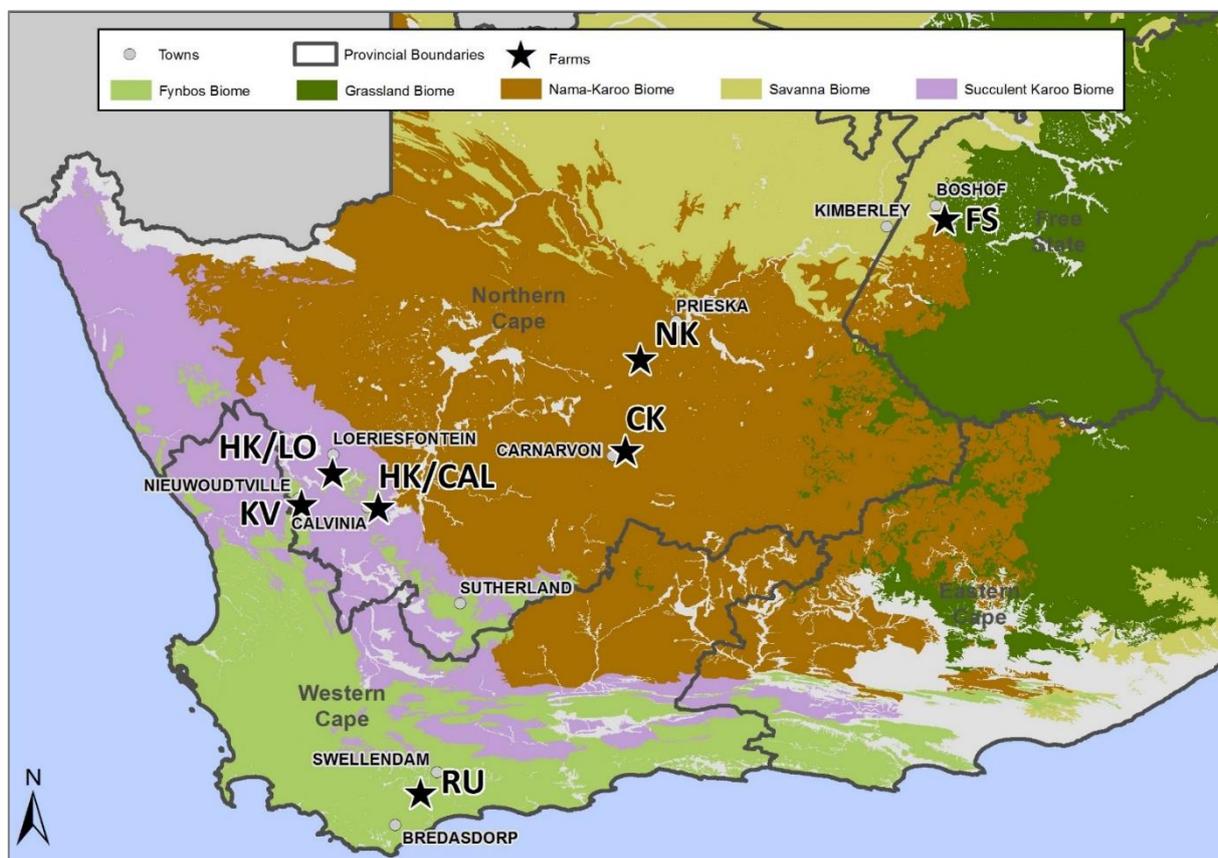


Figure 1.2 Map of the different biomes related to the seven farms selected (phase 1). (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State. Map supplied by Western Cape Department of Agriculture, South Africa (Mucina & Rutherford, 2006).

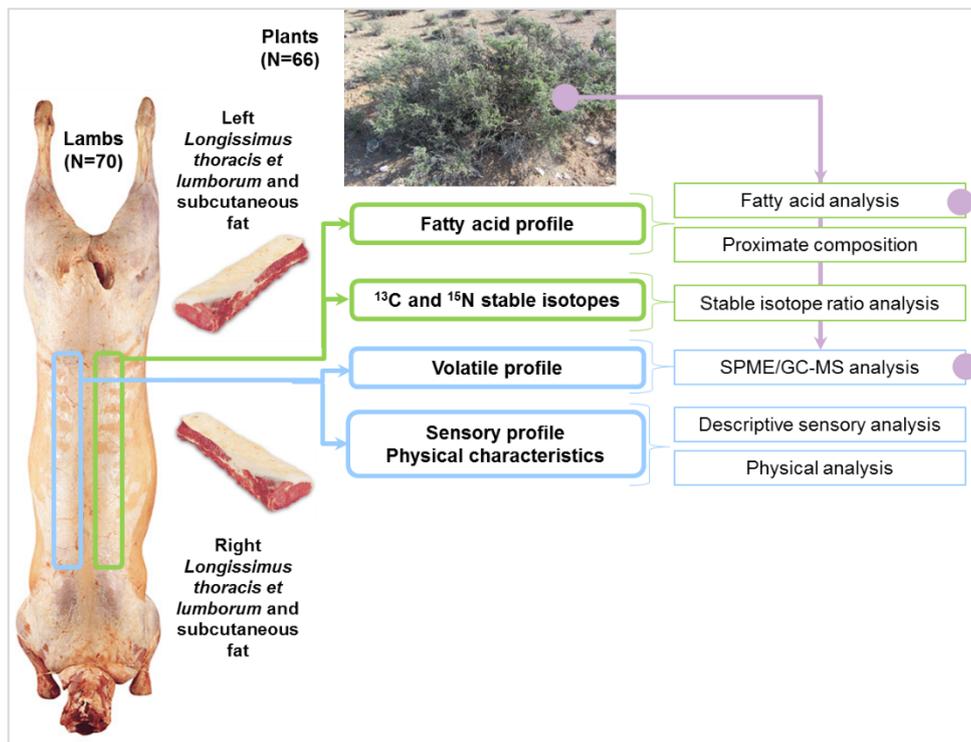


Figure 1.3 Research layout (includes the different aims and analyses performed) for the first phase of the research (Chapter 3, 4 and 5).

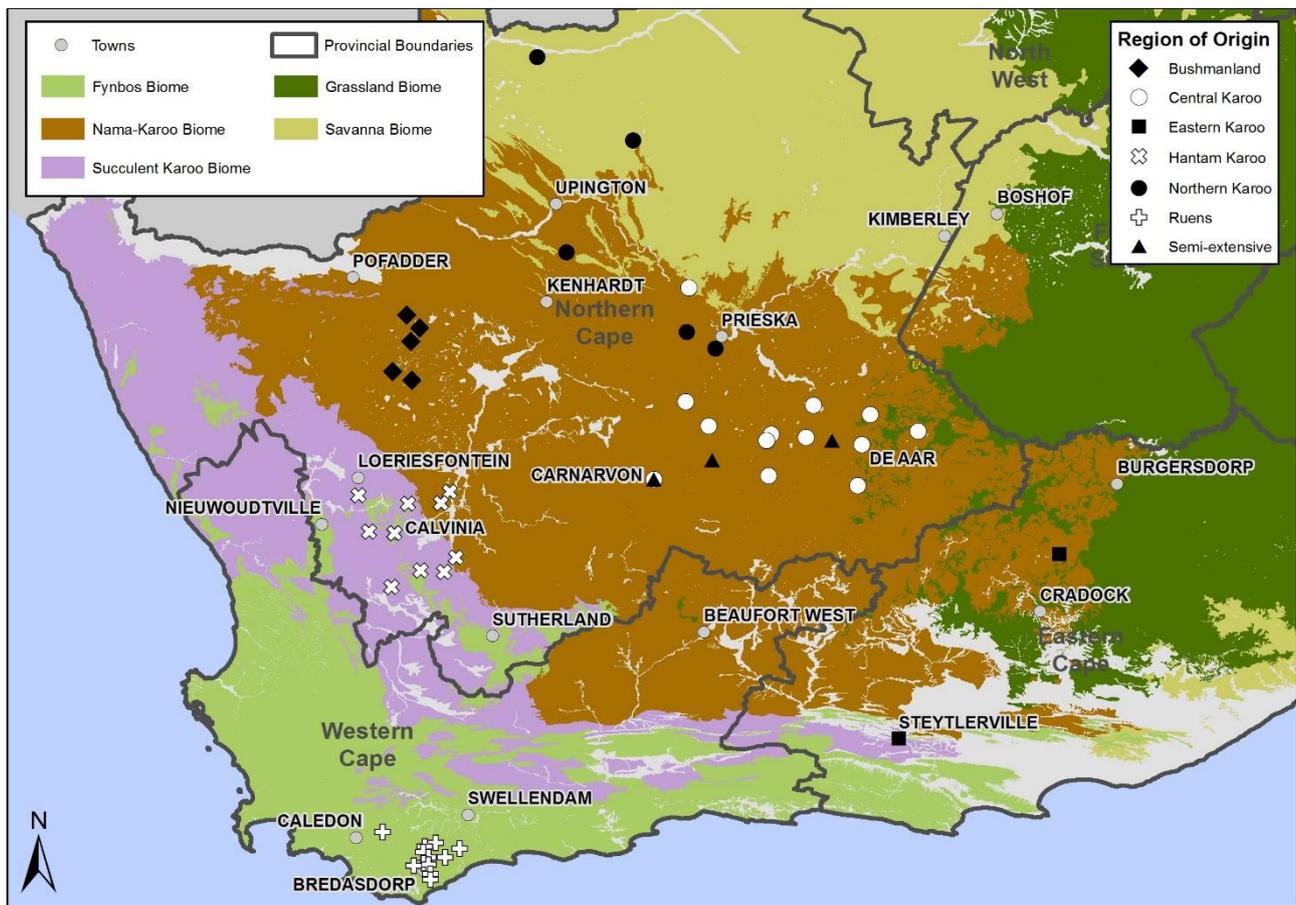


Figure 1.4 Map of the different biomes related to the regions selected (phase 2). Markers indicate the sampling areas. Map supplied by Western Cape Department of Agriculture, South Africa (Mucina & Rutherford, 2006).

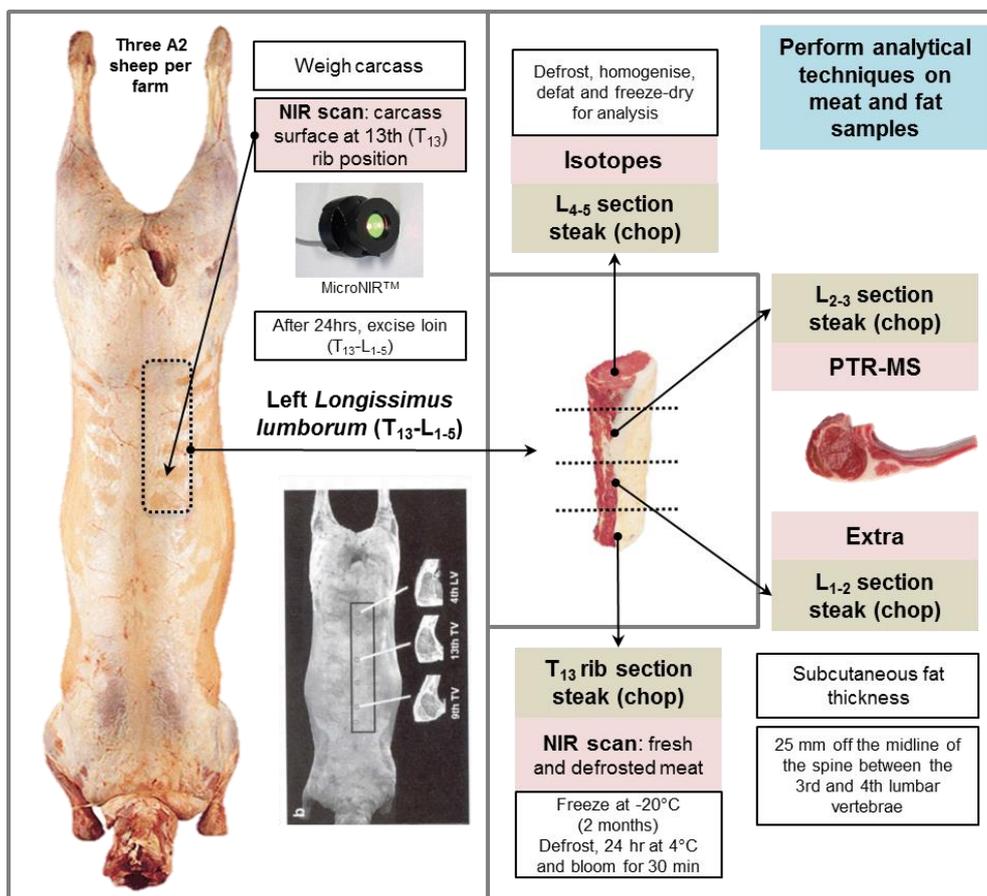


Figure 1.5 Research layout for second phase (new sample set) of the research (Chapter 6, 7 and 8).

References

- Atkinson, D. (2008). The creation of the Karoo Development Foundation Trust. Report. Centre for development support, University of the Free State, South Africa.
- Bramley, C., Bienabe, E. & Kirsten, J. (2009). The economics of geographical indications towards a conceptual framework for geographical indication research in developing countries. In: *The Economics of Intellectual Property*. Pp. 109-141. New York, USA: World Intellectual Property Organization (WIPO).
- Bramley, C., Bienabe, E. & Kirsten, J. (2013). *Developing Geographical Indications in the South: The Southern African Experience*. Dordrecht, the Netherlands: Springer.
- Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S. D., Schlicht, C., Thomas, F., Monahan, F. J., Hoogewerff, J. & Rossmann, A. (2007). Multi-element (H, C, N, S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry*, **389**, 309-320.
- Capuano, E., Boerrigter-Eenling, R., Van der Veer, G. & Van Ruth, S. M. (2013). Analytical authentication of organic products: an overview of markers. *Journal of the Science of Food and Agriculture*, **93**, 12-28.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2002). Animal Health Act, Act No.7 of 2002. Pretoria, South Africa: Government Printer.

- Department of Trade and Industry (DTI). (2013). Merchandise Marks Act (Act No.17 of 1941). *Proposed prohibition on the use of certain words*. Pretoria, South Africa: Government Printer.
- Department of Trade and Industry (DTI). (2014). *Media statements: conclusion of the economic partnership agreement*. Department of trade and industry official website. [Internet document]. URL <http://www.dti.gov.za/editmedia.jsp?id=3079>. Accessed 17/07/2014.
- De Villiers, D. (2012). Made in the Karoo. In: *Farmlink* (edited by L. Louw). Pp. 22-25. Volume 2, No. 4. South Africa: Plaas Publishing cc.
- Du Plessis, H. J. & Du Rand, G. (2012). The significance of traceability in consumer decision making towards Karoo lamb. *Food Research International*, **47**, 210-217.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., Kurt, E., Sinclair, L. A. & Wilkinson, R. G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science*, **55**(2), 141-147.
- Grunert, K. G. (2005). Food quality and safety: consumer perception and demand. *European Review of Agricultural Economics*, **32**(3), 369-391.
- Joubert, E. & De Beer, D. (2011). Rooibus (*Aspalathus linearis*) beyond the farm gate: from herbal tea to potential phytopharmaceutical. *South African Journal of Botany*, **77**, 869-886.
- Karoo Development Foundation (KDF) (2013). Karoo Development Foundation official website. [Internet document]. URL <http://www.karoomeatoforigin.com>. Accessed 24/01/2013.
- Kirsten, J., Troskie, D., Vermeulen, H., Schönfeldt, H. & Bramley, C. (2008). The potential for Karoo lamb as a origin based meat and a geographical indication. Research report, Department of Agricultural Economics and Rural Development, University of Pretoria, South Africa.
- Kirsten, J. F., Vermeulen, H., Van Zyl, K., Du Rand, G., Du Plessis, H. & Weissnar, T. (2012). The economic potential for an origin based marketing and certification system for a meat product in South Africa: perceptions, preferences and experiments. Poster presentation at the *International Association of Agricultural Economists (IAAE) Triennial Conference*, Foz do Iguacu, Brazil, August 18-24, 2012.
- Leighton, C., Schönfeldt, H. C., Van Zyl, J., Van Heerden, S. M., Van Niekerk, J. M. & Morey, L. (2007). Sensory profiles of mutton from different regions in South Africa. Confidential report, Agricultural Research Council, Livestock Business Division – Animal Production, Meat Industry Centre, Irene, South Africa.
- Mucina, L. & Rutherford, M. C. (2006). South African vegetation map. South African National Biodiversity Institute (SANBI). Available from: <http://bgis.sanbi.org/vegmap/map.asp>.
- Palmer, A. R. & Ainslie, A. M. (2005). Grasslands of South Africa. In: *Plant Production and Protection Series: Grasslands of the World* (edited by J. M. Suttie, S. G. Reynolds & C. Batello). No. 34. Pp. 77-120. Rome, Italy: Food and Agriculture Organization of the United Nations.

- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2010). Relationship between odour-active compounds and flavour perception in meat from lamb fed different diets. *Meat Science*, **85**, 700-706.
- Rippon, M. J. (2014). What is the geography of Geographical Indications? Place, production, methods and Protected Food Names. *Area*, **46.2**, 154-162.
- Schieber, A. (2008). Introduction to food authentication. In: *Modern Techniques for Food Authentication* (edited by D.-W. Sun). Pp. 1-26. Oxford, UK: Elsevier Inc.
- Verbeke, W. & Vackier, I. (2004). Profile and effects of consumer involvement in fresh meat. *Meat Science*, **67**, 159-168.
- Vorster, M. & Roux, P. W. (1983). Veld of the Karoo areas. *Proceedings of the Annual Congress of the Grassland Society of South Africa*, **18**, 18-24.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Wood, C. (2014). The way forward for the Southern African Development Community Economic Partnership Agreement. SAIIA Policy Briefing 97, Economic Diplomacy Programme, June 2014.
- World Intellectual Property Organization (WIPO) (2009). *The economics of intellectual property*. WIPO Publication No. 102 (E). ISBN: 978-92-805-1791-0. Geneva, Switzerland.

Chapter 2

Literature Review

Authentic sheep meat in the European Union: Factors influencing and validating its unique meat quality¹

2.1 Introduction

Consumers are becoming increasingly aware of the origin of food products and the quality aspects associated with it (Verbeke & Vackier, 2004). Consequently, the demand for food is shifting from quantity towards quality. Food quality is linked to objective (i.e. must be safe and nutritious) and subjective (i.e. desirable aroma, flavour, texture and colour) parameters (Grunert, 2005). The former can be regulated by law, while the latter is difficult to regulate as it involves consumer perception. Subjective parameters can be measured using objective methods, such as analytical techniques. However, it is not that easy as they first need to be defined by a human who would subjectively describe the aroma, flavour and texture attributes. Subjective parameters are difficult to establish as it may vary considerably depending on the production method of the product and various factors influencing it. Hence, taking the objective and subjective parameters (linked to food quality) into account, food authenticity can be defined as an objective parameter subjectively perceived. This refers to the aspects which make a product authentic such as method of production and processing, specific ingredients and the origin – all of which can be regulated. Yet, the sensory quality, which is influenced by the mentioned aspects, are difficult to regulate as they are bound to vary. Ultimately, the consumer decides whether these aspects are important or not. Consumers who purchase fresh meat are the most concerned about authenticity or quality reassurance (Verbeke & Vackier, 2004). The authentic nature of a product is mostly used as an indicator of food quality through the labelling thereof. This not only draws attention to a product or differentiates it from the others, but can also make the consumer believe that the product is better than its counterparts. Labelling also provides opportunities for fraud relating to false declarations and the misuse of geographical names; especially, since these products are sold at a premium price (Capuano *et al.*, 2013).

The misuse of names of geographical regions has led the European nations to protect names such as *Champagne*, *Port* and *Sherry* through a system of Geographical Indications (GI) by introducing the terms Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG) (Schieber, 2008; Rippon, 2014). Regulations in the European Union such as Regulation (EU) No 1151/2012 in addition to *sue generis* legislation in other countries protect the image of regionally-based agricultural products and foodstuffs. However, this extensive legislative framework is absent in many countries, leaving agro-food products at risk. For instance, in South Africa products such as *Rooibos tea*, *Honeybush tea*, *Karoo lamb*, *Boer goat* and *Klein Karoo ostrich* are at risk. In the case of *Karoo lamb*

¹ Erasmus, S. W., Muller, M. & Hoffman, L. C. (Accepted, In Press). Authentic sheep meat in the European Union: Factors influencing and validating its unique quality. *Journal of the Science of Food and Agriculture*.

(recognised as one of the first red meat products to be regarded as a PGI in South Africa), confidence in quality and excellent aroma and flavour is coupled to this product of origin brand making some consumers more willing to purchase it (Weissnar & Du Rand, 2012).

In the European nations fresh sheep meat types are protected through registration as either a PDO or PGI (DOOR, 2015). These lamb types are born, reared and slaughtered in the specified region of origin, fed a characteristic diet and consequently have a unique sensory quality which is linked to the production system, diet and sheep breed (Erasmus *et al.*, 2016). The existence for some of the lamb types dates back many years. For example, it is believed that the Manx Loaghtan breed of the *Isle of Man Manx Loaghtan lamb* have been on the island for over one thousand years (OJEU, 2006). In general, the sheep breeds are well-adapted to the different environmental conditions of the various geographical areas; especially since most of the breeds have been selected and bred over several years, are hardy and thrive under unfavourable conditions (OJEU, 2006). These sheep breeds tolerate harsh weather conditions, eat what is available and walk long distances to obtain their daily dietary requirements.

In the summer of 1691, Pierre Thomas du Fosse (French scholar and author), explained the distinct nature of *Prés-salés du Mont-Saint-Michel* (Lamb of the salt meadows of Mont-Saint-Michel) as follows: “The grass near the coast is like wild thyme: it gives the sheep meat such an exquisitely delicious taste that one would be tempted to give up partridges and pheasants” (OJEU, 2013). Although the scientific reason for this dietary effect was not well understood at that time, the important role of the vegetation of the salt marshes towards the sensory quality of the meat did not go unnoticed. In fact, other lamb types reared on salt marshes and meadows include: *Orkney lamb*, *Prés-salés de la baie de Somme* (Lamb of the salt marshes of the Somme), *Scotch lamb*, *Shetland lamb* and *Vadehavslam*. *Vadehavslam* has a distinct salty taste due to grazing on the salty meadows in the Wadden Sea region of south-west Denmark (DOOR, 2015).

PDO and PGI sheep meat is protected in the European Union based on its unique meat quality characteristics. Aroma, flavour and texture are sensory quality aspects consumers easily recognise and associate with the product (Grunert, 2005). In general, they are also used to differentiate and market authentic lamb types. The sensory quality is influenced and determined by various factors such as diet and breed, which generally forms the link with the geographical area of origin of the meat. Hence, these factors will be key points in the discussion. The aim of the review is to describe the different authentic lamb types and report on the research performed to verify the authentic nature of the meat. The shortcomings of research studies will also be discussed. In effect, by doing this it would help with the development of PDO and PGI specifications as it points out important aspects to consider. These aspects are also key to the development of Karoo lamb, as well as other regionally unique lamb, as an authentic lamb meat product of South Africa.

2.2 Meat aroma and flavour

Given the important role of sensory quality towards defining authentic lamb meat, it is essential to understand how meat aroma and flavour develops and how these can be influenced. Meat has a complex structure, made

up of macro- (e.g. water, protein, lipids, carbohydrates and inorganic matter) and micro- components (e.g. vitamins, sugars and nucleotides) (Keeton & Eddy, 2004; Pegg & Shahidi, 2004). These and other components are fundamental to the development of flavour and aroma of meat (Mottram 1998; Calkins & Hodgen, 2007). It is known that the application of heat stimulates a series of chemical reactions between the non-volatile constituents (precursors) of lean and adipose tissues in meat (Pegg & Shahidi, 2004; Calkins & Hodgen, 2007). As a result, a diverse system consisting of odour-active and smaller non-volatile chemical compounds is produced and the sensory profile of the meat is formed.

There exists a basic theory that cooked meat from domesticated animal species (i.e. beef, pork and lamb) has a similar flavour profile linked to protein breakdown and the development of heterocyclic compounds (Berg & Walker, 2004). Thus, it is suggested that species-specific flavour is as a result of lipid oxidation products due to intramuscular fat content and not water-soluble precursor compounds (Calkins & Hodgen, 2007; Watkins *et al.*, 2013). Fat also acts as a depot of fat soluble compounds which volatilize upon heating (Pegg & Shahidi, 2004). Furthermore, it is known that new flavours may also be produced as lipid oxidative products interact with the Maillard reaction (i.e. the non-enzymatic browning reaction between a reducing sugar and amino acid group) products.

In terms of the unique flavour of lamb or mutton, a well-defined description of the characteristic sensory attributes of lamb meat is still lacking. This is attributable to the variation within the flavour profiles of lamb meat originating from different regions as well as the fact that consumers differ in their concept of lamb or mutton flavour (Watkins *et al.*, 2013). The details of 48 fresh lamb meat types registered as either PDO or PGI under Regulation (EC) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs are shown in Table 2.1. More detailed descriptions in terms of the PDO or PGI status, geographical area of importance, sheep breeds, the slaughter age or live weight and the carcass weight are provided as Supplementary data (Table S2.1). In each case, the reputation of the lamb type is linked to the name, while the specific product qualities are attributable to the defined geographical area. The geographical area is characterised by its unique climatic conditions combined with knowledge from the local producers. These lamb types have distinct sensory qualities which are largely related to their regionally unique diet (Table 2.1).

2.2.1 Factors affecting the sensory quality of sheep meat

The aroma and flavour of meat is recognised as one of the principal aspects regarding its eating quality (Mottram, 1998). The chemistry behind this phenomenon is not yet completely understood. However, extensive research is continuously being conducted on the factors affecting meat aroma and flavour during the processing and production thereof. A summary of the factors is shown in Figure 2.1. These factors can either have an influence *ante-mortem* or *post-mortem*. *Ante-mortem* factors include the animal's condition at slaughter, method of slaughter, breed, age, gender, plane of nutrition and diet, whereas *post-mortem* factors comprise muscle pH, manner of storage and extent of refrigeration after slaughter and cooking (Madruca &

Mottram, 1995; Spanier *et al.*, 2004). These factors can alter the composition of the meat (i.e. fat content). Compounds derived from the muscle tissue, fat tissue and intramuscular fat (known as precursors) are also affected and in turn they determine the aroma and flavour of the meat (Fig. 2.1). The factors which will be discussed further are diet, breed, age and gender. These factors were selected based on the fact that most ruminant studies focus on these factors and their effect on meat quality. Therefore, it is expected that they might be the main factors distinguishing authentic fresh meat products.

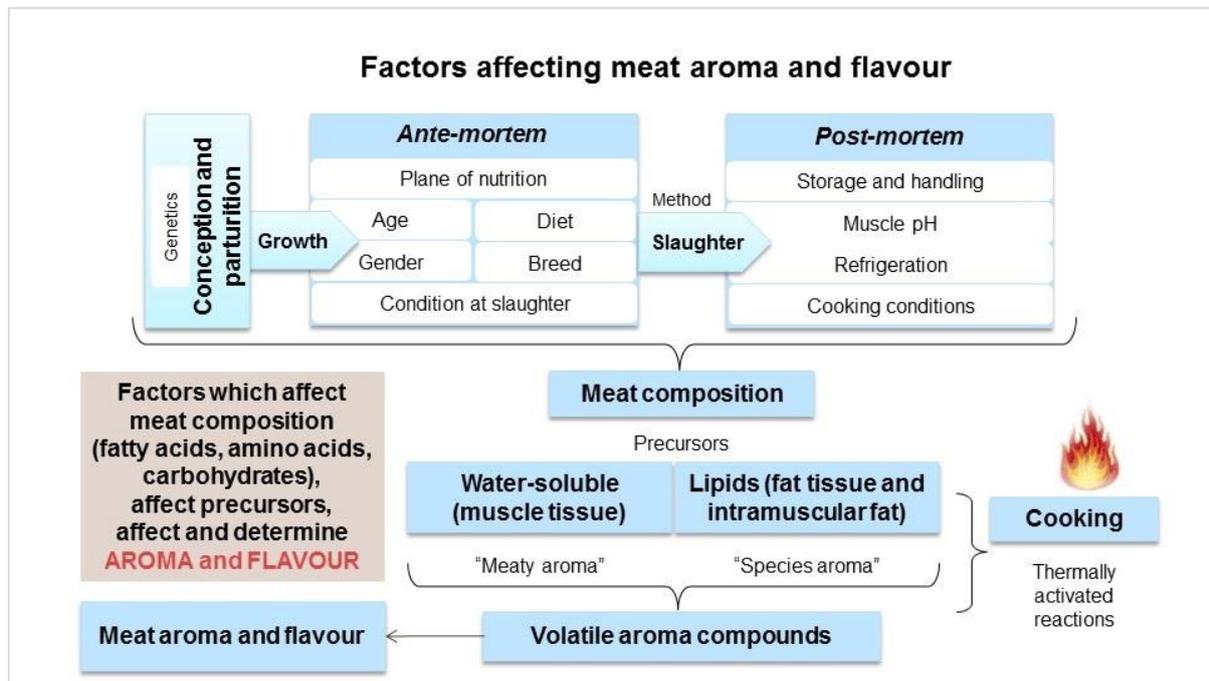


Figure 2.1 Summary of the factors affecting meat aroma and flavour.

2.2.1.1 Diet

The effects of diet on the sensory attributes of meat have been reported in studies as early as the 1930s (Berg & Walker, 2004). Recent studies have illustrated the effect of diet on the sensory attributes of sheep meat (Rousset-Akrim *et al.*, 1997; Fisher *et al.*, 2000; Priolo, *et al.*, 2002; Young *et al.*, 2003; Borton *et al.*, 2005; Resconi *et al.*, 2010). The Polish suckling lambs, *Jagnięcina podhalańska*, owes its exceptional succulence to its marbling, i.e. intramuscular fat. During cooking the intramuscular fat melts but remains within the meat, contributing towards its tenderness and juiciness (Hoffman *et al.*, 2003a). The volatile substances contained in the fat are the main components of taste and aroma (TWV, 2012). Apart from the animal's young age, it is likely that the composition of *Jagnięcina podhalańska*'s diet which essentially determines these characteristics.

The flavour intensity of sheep meat is greatly affected by diet as it can be increased with certain foods (such as legumes and grains) and lessened by keeping the sheep from grazing before they are slaughtered. For instance, legume pastures are known to produce the highest concentrations of indoles in the rumen compared to other fresh forage pastures (Schreurs *et al.*, 2007). An important indole is 3-methylindole, also known as skatole. It has been noted that the concentration of skatole in perirenal fat of male lambs were

higher for lambs fed pasture diets as compared to fat from lambs fed concentrate diets (Young *et al.*, 2003). Researchers have also suggested that skatole is the main cause for pastoral flavour in sheep meat, therefore being a good indicator of pasture diet (Young *et al.*, 1997). Skatole is formed during the degradation of tryptophan in the rumen and excessive amounts are stored in the fat (Schreurs *et al.*, 2008).

Diet mainly influences the fatty acid profile of ruminant meats (Wood *et al.*, 1999). Fisher *et al.* (2000) suggests that the feed and breed have a direct influence on the fatty acid composition of the meat, in that way, affording taste differences. Sañudo *et al.* (2000a) established that the aroma and flavour of meat from grass-fed or grain-fed lamb are influenced by the quantity of *n*-3 or *n*-6 polyunsaturated fatty acids (PUFA) incorporated into the muscle. Concentrate-fed lamb had elevated levels of *n*-6 PUFA, while grass-fed lamb had high levels of *n*-3 PUFA and a more intense aroma and flavour. This effect of diet on the fatty acid profile of meat has also been discussed in a review by Calkins and Hodgen (2007) and similarly reported for *Borrego do Nordeste Alentiano* lamb (Santos-Silva *et al.*, 2006). In a review by Capuano *et al.* (2013) the potential of using the fatty acid profiles for the authentication of organic meat is highlighted. This is based on the marked effect diet has on meat composition, particularly on the fatty acids.

It is known that ruminants have a lower ratio of PUFA to saturated fatty acids (SFA) (P:S) (Keeton & Eddy, 2004). This is as a result of the hydrogenating effect of rumen bacteria on dietary fats (Enser *et al.*, 1998; Keeton & Eddy, 2004). However, one should bear in mind that this effect is different for young suckling lamb given their undeveloped rumen. They behave as monogastrics and the fatty acid profile of the mother's milk is reflected in their fat tissue (Beriain *et al.*, 2000). The meat is initially high in lauric (C12:0) and myristic (C14:0) SFA but as soon as the lambs are weaned and given solid food the concentration of SFA is reduced (Beriain *et al.*, 2000).

Ruminants are known to deposit more unsaturated fatty acids (UFA) in phospholipids than triacylglycerides (TAG) (Enser *et al.*, 1998; Mottram, 1998). Consequently, reasonably high proportions of PUFA are found in the muscle tissue of lean lamb breeds when compared with fatter lambs (Rowe *et al.*, 1999). In the fatter lambs the phospholipid effect is diluted by higher levels of marbling fat. This has a direct influence on the aroma profile of meat as aroma is largely affected by the amount and proportions of different fatty acids in meat (Fisher *et al.*, 2000; Berg & Walker, 2004). The reason for the variation in aroma with increased levels of PUFA (and consequently phospholipids) is linked to the oxidative breakdown (autoxidation) of UFA and their ability to produce volatile aromatic compounds during the meat's cooking process (Mottram, 1998; Wood *et al.*, 1999).

Table 2.1 Details* of fresh lamb meat registered as either Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO)

Fresh sheep meat	Country of origin	PGI or PDO	Description	Diet	Sensory quality
<i>Abbacchio Romano</i>	Italy	PGI	Lambs born, reared and slaughtered within the entire territory of the Region of Lazio	Mother's milk and supplementary grazing on natural foods and wild plants. Ewes graze on natural and sown pasture and meadow land and may receive supplementary dried fodder and concentrates.	Meat is light pink and texture fine with small deposits of intramuscular fat
<i>Agneau de lait des Pyrénées</i> (Pyrenees suckling lamb)	France	PGI	Suckling lambs (Manech red head, Manech black head or Basque-Béarnaise breeds) born and reared with mothers raised by traditional methods in the Western edge of the Pyrenean chain and its foothills. Dairy breeds are adapted to the mountain areas and transhumance	Solely mother's milk, where the ewes graze freely on high- and medium-altitude natural grassland in spring, summer and autumn, grassland supplemented with preserved fodder, grain cereals, straw and compound feedingstuffs in winter	Meat is white or barely pink, succulent and tender with a low-grain texture. Characteristics are due to milk feeding and young slaughter age of the animals
<i>Agneau de l'Aveyron</i> (Lamb of Aveyron)	France	PGI	Lacaune breed lambs born, reared and slaughtered within the Department of Aveyron and adjacent cantons	Mother's milk (70 days minimum) and reared in sheep-fold	Meat is rose-pink, tender and tasty linked to traditional production system and diet
<i>Agneau de Lozère</i> (Lamb of Lozère)	France	PGI	Lambs of the Blanche du Massif Central breed (on the threshold between suckling and grass-fed lamb) born, reared and slaughtered within the specified regions	Mother's milk, grassland or range, supplemented with other feed resources produced from range, grassland, fodder crops or dry fodder	Meat is white to light pink with a fine and firm texture and 'perfumed with the scent of grass' attributed to the purity of the breed and diversity of the diet
<i>Agneau de Pauillac</i> (Pauillac lamb)	France	PGI	Suckling lambs born and reared with mothers raised by traditional methods within the Department of Gironde	Mother's milk, supplemented with cereal concentrates. Ewes graze on natural pasture	Meat is light, with a distinct taste and flavour compared to traditional heavy lamb and milk-fed lamb from dairy farms. Fat is firm and white. Qualities due to production system and diet
<i>Agneau de Sisteron</i> (Sisteron lamb)	France	PGI	Lambs born, reared and slaughtered within the specified regions	Mother's milk (60 days minimum), supplemented with grass and/or fodder, together with a cereal feed supplement	Meat is light pink, non-greasy, fine and smooth textured with a mild flavour attributed to diet
<i>Agneau du Bourbonnais</i> (Bourbonnais lamb)	France	PGI	Lambs born, reared (in natural grassland meadows) and slaughtered within the specified regions	Mother's milk and natural fodder, mainly meadow grass	Meat is light red and fat is white and firm. Unique characteristics of meat linked to production system and diet
<i>Agneau du Limousin</i> (Limousin lamb)	France	PGI	Lambs born, reared and slaughtered in the Limousin region	Mother's milk (60 days minimum), grass feeding (grazing or preserved hay) with varying quantities of feed supplements for the lambs according to the season and weather conditions	Unique characteristics of meat linked to production system and diet
<i>Agneau du Périgord</i> (Lamb of Périgord)	France	PGI	Lambs born and reared in the Périgord area in a traditional way: a suckling period with their mothers followed by a finishing period in a sheepfold.	Mother's milk (for at least 60 days), where the ewes graze on natural or temporary meadows, after which lambs receive specific supplemental feeding with whole or flattened grain and fodder.	Meat is white to light pink, tender and juicy with a delicate aroma and flavour of lamb and 'melt-in-the-mouth' texture. Fat is firm and white. Qualities attributed to age and diet
<i>Agneau du Poitou-Charentes</i> (Lamb of Poitou-Charentes)	France	PGI	Lambs (Charmoise breed produces lambs with stocky, well-formed legs) born, reared and slaughtered within the Poitou-Charentes area which extends to neighbouring municipalities	Mother's milk (60 days minimum) and grass	Meat is light pink and texture fine attributed to the tradition of semi-open-air livestock farming
<i>Agneau du Quercy</i> (Quercy lamb)	France	PGI	Causse du Lot breed lambs born, reared (on the dry and arid area of the Causse du Quercy) and slaughtered within the specified regions	Mother's milk (70 days minimum) and feed available on the dry and arid area of the Causse du Quercy complemented with hay and cereals	Unique characteristics of meat linked to production system and diet

Table 2.1 (Continued)

Fresh sheep meat	Country of origin	PGI or PDO	Description	Diet	Sensory quality
<i>Agnello del Centro Italia</i> (Lamb of Central Italy)	Italy	PGI	Lambs born, reared and slaughtered in the regions of Central Italy	Mother's milk, fodder made up of wild plants from the meadows, sown pasture, pulses and/or grasses	Unique characteristics of meat linked to diet
<i>Agnello di Sardegna</i>	Italy	PGI	Lambs born, reared and slaughtered in the Island region of Sardinia region	Suckling lambs: solely mother's milk (30-35 days); Light lambs and lambs for cutting: mother's milk (30-35 days), fresh or dried natural feed (fodder and cereals), grasses and native wild and aromatic plants	Meat is white, tender, succulent, fine-textured and firm with a delicate aroma, distinct wild flavour, pleasant taste and small deposits of intramuscular fat linked to the production system and diet
<i>Αρνάκι Ελασσόνας</i> (Elassonas lamb)	Greece	PDO	Suckling lambs born and reared within the Province of Elassona with ewes bearing the phenotypic characters of indigenous Greek breeds	Solely mother's milk, where the ewes graze freely on mountain pastures with grasses, herbs and aromatic plants (above 250 m) and on artificial grassland. Complimentary feedingstuffs, as well as vitamins and minerals, are given for 3-5 months	Meat is white to light pink, tender and juicy. Herbaceous plants give the ewe's milk and, particularly, the lamb's meat a particular and characteristic aroma and taste
<i>Barèges-Gavarnie</i>	France	PDO	Mutton of the Barègeoise breed born, reared (spending at least two summers in the mountain pasture) and slaughtered within the specified regions	Mother's milk until weaned. Winter: hay meadows supplemented with dried fodder, whole or kibbled grain cereals, Spring and autumn: highland areas with pasture, Summer: mountain pastures	Meat is bright red and marbled, but without excessive fat. No strong smell of sheepmeat or wool grease. Qualities due to production system and diet
<i>Borrego da Beira</i>	Portugal	PGI	Merino and Churra (do Campo and Mondegueira) lambs born, reared and slaughtered within the specified regions	Spontaneous pastures due to the edaphological-climatic conditions of the region	Unique characteristics of meat linked to production system and diet
<i>Borrego de Montemor-o-Novo</i>	Portugal	PGI	Menino Branco Regional breed lambs born, reared and slaughtered in the Parishes in the subdistrict of Montemor-o-Novo, Évora, Arraiolos and Moura	Spontaneous Mediterranean pastures	
<i>Borrego do Baixo Alentejo</i> (Lamb of Baixo Alentejo)	Portugal	PGI	Merina and Campaniça breed lambs born, reared and slaughtered within the specified regions	Mother's milk, natural pasture, feed and woodland in the area	Unique characteristics of meat linked to production system, breed and diet
<i>Borrego do Nordeste Alentejano</i>	Portugal	PGI	Lambs of the Merino Branco breed born, reared and slaughtered in the northern region of Alentejo	Mother's milk, natural pastures and local forages among oak groves (plantations of cork-oak, holm oak and oak), supplemented with concentrates	Meat is tender, juicy and has a smooth texture with a unique flavour and small deposits of intramuscular fat linked to the production system and diet
<i>Borrego Serra da Estrela</i>	Portugal	PDO	Lambs of the Bordaleira breed born, reared and slaughtered in the Beira plateau region	Mother's milk, natural pastures composed of spontaneous perennial grasses and cultivated pastures (based on white clover and subterranean clovers)	Unique characteristics of meat linked to production system and diet
<i>Borrego Terrincho</i>	Portugal	PDO	Lambs of the Churra da Terra Quente breed born, reared and slaughtered within the specified regions	Traditional methods of feeding and rearing on natural pastures, fallow ground, uncultivated land and in woods, arising from the edaphological-climatic conditions peculiar to the region	
<i>Cordeiro Bragançano</i> (Bragançano lamb)	Portugal	PDO	Churra Galego Bragançano breed lambs born, reared and slaughtered in the Terra Fria tramontane region	Reared in the region and fed with whole mother's milk	
<i>Cordeiro de Barroso, Anho de Barroso or Cordeiro de leite</i>	Portugal	PGI	Lambs born, reared and slaughtered within the specified regions	Mother's milk and the utilisation of fallow land, natural and improved pastures and the spontaneous vegetation of the Barroso region	Meat is tender, juicy and very tasty, with a typical flavour linked to the production system and diet

Table 2.1 (Continued)

Fresh sheep meat	Country of origin	PGI or PDO	Description	Diet	Sensory quality
<i>Cordeiro Mirandês</i> or <i>Canhono Mirandês</i> (Mirandês lamb)	Portugal	PDO	Lambs of the Churra Galega Mirandesa breed, born and reared in a traditional extensive farming system on the Miranda plateau	Class A: mothers' milk (suckling lambs), Class B and C: mother's milk, regional flora and supplemented with cereal grains grown on the farm	Meat is pink, extremely tender, succulent and very tasty, with uniform deposits of intramuscular fat and fat which is consistent and does not 'sweat'. Qualities linked to breed and diet
<i>Cordero de Extremadura</i> (Lamb of Extremadura)	Spain	PGI	Lambs are born, reared in an extensive grazing system (while suckling, 40-50 days) and finished intensively using concentrates and cereal straw in the Extremadura region	Mother's milk, herbaceous pasture and where necessary, feed supplements, composed of straw, grain, fodder, by-products and concentrates whose main constituents are cereals, oilseeds and protein crops	Meat is pink to light pink, succulent and tender with a moderate level of intramuscular fat deposits, pleasant taste and aroma attributed to the extensive and semi-extensive production system
<i>Cordero de Navarra</i> or <i>Nafarroako Arkumea</i>	Spain	PGI	Suckling lamb (Lechal, Navarra and Lacha breeds) and the light lamb (Ternasco, Navarra breed) born, reared and slaughtered within the entire province of Navarre	Mother's milk, mountain pastures, upland pasture, meadows, stubble fields, scrub, polyphytic cultivated meadows and supplemented with dried fodder (alfalfa hay, grass or vetch-oats) and natural feed (cereals and pulses)	Lechal: Meat is pearly white to light pink, tender, very juicy, smooth texture and distinctive flavour. Ternasco: Meat is light pink, tender, very juicy, smooth texture and distinctive flavour with small deposits of intramuscular fat.
<i>Cordero Manchego</i> (Manchego lamb)	Spain	PGI	Suckling lambs of the Manchego breed, born and reared for at least 30 days on their mother's milk supplemented by white straw and authorised concentrates	Mother's milk (30 days minimum), supplemented with white straw and concentrates, and the utilisation of natural resources, flora and meadows, forage crops, fallow land, stubble and coppices	Meat is pale pink, very tender and juicy, with incipient intramuscular fattiness imparting a characteristic, highly pleasant flavour. Qualities linked to the varied diet and special features of the breed
<i>Cordero Segureño</i> (Segureño lamb)	Spain	PGI	Segureño breed lambs born, reared under extensive or semi-extensive conditions and slaughtered in the area hedged in by the range of hills known as Cordilleras Béticas Orientales	Mother's milk supplemented with products rich in fibre and spontaneous vegetation native to the area, pasture of stubble fields of cereal and legume crops and, occasionally, irrigated pastureland	Meat is pink, succulent, tender with a good level of fat coverage. Unique characteristics of meat linked to breed and diet
<i>Diepholzer Moorschnucke</i>	Germany	PDO	Heidschnucke and traditional breeds such as weiße hornlose Moorschnucke sheep born, reared in the Moors and wetlands, and slaughtered in the area	Mother's milk, heather, bent, cotton, grass, sedge, various herbs and grasses, pine, birch, frángula and other woody plants. Supplemented with feed produced on the farm in the winter	Meat is tender, with a characteristic gamey flavour attributable to the production system and typical diet
<i>Hånnlamb</i>	Sweden	PDO	Lambs of the Gutefår breed born, reared and slaughtered in the Gotland region	Natural vegetation with herbs (i.e. thyme) and grasses of the Gotland region. Hay/silage and concentrates as supplementary feed	Meat is fine grained, juicy, darker in colour and flavours of butter, metal (liver and blood), herbs, chestnut, gamey and woody (earth, moss and mushrooms) and naturally salty and acidic taste. Qualities are linked to breed and diet
<i>Isle of Man Manx Loaghtan lamb</i>	United Kingdom	PDO	Lambs of the Manx Loaghtan breed born, reared and slaughtered on the Isle of Man	Mother's milk, characteristic island vegetation of unimproved pasture, gorse and bracken scrub, and heather Moorland	Meat is fine grained, less fatty and darker in colour with a distinct gamey flavour
<i>Jagnięcina podhalańska</i> (Podhalańska lamb)	Poland	PGI	Suckling lambs born and reared with mothers raised by traditional methods in a part of the Western Carpathians, where the Podhale area is the microregion which forms the centre of the whole production area	Solely mother's milk, where the ewes graze freely on mountain pastures (green fodder) in summer and autumn, hay, hay silage and concentrated feed in winter and early spring	Meat is light pink, succulent with a delicate flavour and characteristic taste and aroma similar to game. Qualities are linked to breed and diet

Table 2.1 (Continued)

Fresh sheep meat	Country of origin	PGI or PDO	Description	Diet	Sensory quality
<i>Karoo lamb</i>	South Africa	PGI	Lambs (Dorper, South African Mutton Merino, Merino, Dormer or Dohne Merino breeds) reared extensively and slaughtered in the Karoo region of South Africa	Mother's milk, natural Karoo vegetation with fragrant bushes. Supplemented with cereals, silage or any other natural plant matter (less than 30% of the total daily requirements) to assist during dry spells and to improve the condition of animals during the reproductive cycle	Meat is tender and has a characteristic herbaceous aroma and flavour due the natural grazing on the indigenous fragrant Karoo bushes
<i>Lakeland Herdwick</i>	United Kingdom	PDO	Sheep bred (Herdwick breed), born, reared and slaughtered in the country of Cumbria	Mother's milk, mountain flora, herbage of the fells including grasses, heather and plants such as bilberry. Finished on locally sourced grass, hay or silage	Meat is pink to dark pink, fine grained, succulent and tender with a gamey flavour resulting from slow maturation and a long grazing period
<i>Lechazo de Castilla y León</i>	Spain	PGI	Churra, Castellana and Ojalada breed lambs born, reared and slaughtered in various agricultural districts in the Autonomous Community of Castilla y León	Solely mother's milk, where the ewes are fed cereals supplemented by natural grazing and stubble	Meat is pearly white or light pink, very tender, succulent, smooth in texture with small deposits of intramuscular fat. Qualities due to breed and diet
<i>Lička janjetina</i> (Lika lamb)	Croatia	PGI	Lambs (Lika pramenka breed) born, reared and slaughtered in the country of Lika-Senj	Mother's milk, indigenous pastures in the summer and hay in the winter	Characteristic aroma and flavour linked to the production system and diet
<i>Lüneburger Heidschnucke</i>	Germany	PDO	Heidschnucke lambs born, reared on the dry heathland of the Lüneburg region and slaughtered in the area	Mother's milk and dry heathland of the Lüneburg region, consisting of woody-stemmed heather and grassland	Characteristic gamey flavour linked to the production system and diet
<i>Orkney lamb</i>	United Kingdom	PDO	Lambs born, reared and slaughtered in Orkney (group of islands off the North Coast of Scotland)	Mother's milk, seaweed, grass and herbage	Distinct texture and flavour linked to diet with specific characteristics due to the topography, geology and climate of the Orkney Islands
<i>Paška janjetina</i> (Pag lamb)	Croatia	PDO	Lambs born, reared and slaughtered on the island of Pag in the northern Adriatic Sea	Mother's milk (reduced) and vegetation growing on the karst, bare rocky ground such sage, nightingale and stingy short grass sprinkled with salt from the sea	Meat is light pink, tender, tasty and herbaceous due to the nature of its diet (aromatic and medicinal plants)
<i>Prés-salés de la baie de Somme</i> (Lamb of the salt marshes of the Somme)	France	PDO	Lambs reared on the salt marshes of the Baie de Somme and the Baie d'Authie for a minimum of 75 days in a manner that respects the natural balance of this environment	Mother's milk, halophytic pasture of the salt marshes (especially alkali grass) and finished on forage and concentrates	Meat is pink, juicy and produce intense, lasting flavours in the mouth. Fat is firm and white
<i>Prés-salés du Mont-Saint-Michel</i> (Lamb of the salt meadows of Mont-Saint-Michel)	France	PDO	Lambs reared on the salt marshes of the Mont-Saint-Michel bay for a minimum of 70 days in a manner that respects the natural balance of this environment	Mother's milk, halophytic pasture of the salt marshes (plants include puccinellie, troscart and obione) and finished on forage and concentrates	Meat is pink to dark pink with a marbled appearance, juicy and produce intense, lasting flavours in the mouth, with no taste of wool grease
<i>Scotch lamb</i>	United Kingdom	PGI	Lambs are finished (not less than 2 months), slaughtered in the Mainland of Scotland including the islands off the West Coast (Orkney and the Shetland)	Mother's milk and grassland and finished in Scotland for a period not less than 2 months	Flavour and tenderness attributed to lambs raised extensively on grassland
<i>Shetland lamb</i>	United Kingdom	PDO	Shetland or Shetland/Cheviot breed lambs born, reared and slaughtered in Shetland (group of islands off the North Coast of Scotland)	Mother's milk, seaweed, grass and herbage	Distinct texture and flavour linked to diet with specific characteristics due to the topography, geology and climate of the Shetland Islands
<i>Ternasco de Aragón</i> (Lamb from Aragón)	Spain	PGI	Lambs of the Rasa Aragonesa, Ojinegra de Teruel and Roya Bilbilitana breeds born, reared and slaughtered in the Autonomous Community of Aragon	Mother's milk (50 days minimum), supplemented with white straw and concentrates	Meat is light pink, tender, juicy, soft in texture with small deposits of intramuscular fat. Qualities attributed to production system and diet
<i>Uain Sléibhe Chonamara</i> (Connemara hill lamb)	Ireland	PGI	Light lambs of a black faced breed are bred, born and reared in Connemara (West of Ireland)	Mother's milk, mountain grasses, sedges, heathers and herbs	Meat is rose red in colour and has a solid deep texture. Taste, flavour and colour linked to local flora grazed

Table 2.1 (Continued)

Fresh sheep meat	Country of origin	PGI or PDO	Description	Diet	Sensory quality
<i>Vadehavslam</i>	Denmark	PGI	Lambs born and reared in the Wadden Sea region of south-west Denmark	Mother's milk, plants and grasses in salt meadows, maize, silage and hay supplemented with barley in the finishing period	Distinct salty taste due to grazing on the salty meadows
<i>Welsh lamb</i>	United Kingdom	PGI	Lambs bred (traditional hardy Welsh breeds), born and reared in Wales	Mother's milk and grassland which flourishes as a result of the wet and mild Welsh climate	Unique character attributed to Welsh breeds that dominate flock and lambs raised extensively on grassland
<i>West Country lamb</i>	United Kingdom	PGI	Lambs born, reared and finished (not less than 2 months) in the West Country region of England	Mother's milk and grassland	Specific grass-based diet gives the meat a more richly flavour and creamy coloured fat

(PGI) Protected Geographical Indication; (PDO) Protected Designation of Origin. * Details obtained online (http://ec.europa.eu/agriculture/quality/schemes/index_en.htm) from DOOR database (Database of Origin and Registration) under Regulation (EC) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs (excluding details for Karoo lamb).

In addition to the important contribution of phospholipids towards aroma, species aroma of sheep meat has been linked to certain volatile branched-chain fatty acids (BCFA) (Young *et al.*, 1997; Young *et al.*, 2003). Wong *et al.* (1975) were the first to report the importance of methyl-branched saturated fatty acids, such as 4-methyloctanoic (MOA) and 4-methylnonanoic (MNA) acid towards lamb and mutton aroma. These compounds contribute to the typical sweet or sour flavour note of the meat which they described as “soo”. In the same way other branched and unsaturated medium chain (C8-C10) fatty acids, such as 4-ethyloctanoic acid (EOA), contributes to lamb or mutton aroma (Young *et al.*, 1997; Watkins *et al.*, 2010). Watkins *et al.* (2014) found that, with an increasing concentration of MOA and EOA in cooked lamb, its consumer acceptability decreased. BCFA are produced by means of lipid hydrolysis from the triacylglycerol component of the fat (Wong *et al.*, 1975). Brennan and Lindsay (1992) reported lower quantities of MOA and MNA in shoulder and leg of lamb compared to subcutaneous fat. Therefore, the contribution of BCFA towards overall aroma would be greater in fat than muscle tissues. In addition, Ha and Lindsay (1991) demonstrated that a mixture of BCFA (i.e. EOA and MOA) and alkylphenols produce a distinct mutton-like aroma. Alkylphenols are said to be responsible for sheepyard-like aromas in sheep fat (Ha & Lindsay, 1991). Alkylphenols may also intensify the characteristic sheep-meat flavour caused by BCFA (Young *et al.*, 1997).

Changes in the diet of lambs could produce variation in the composition of their body fat. This is supported by the established fact that diet is known to alter the volatile fatty acid ratio in the rumen, resulting in the biosynthesis of these volatile acids into different long chain fatty acids (Cramer & Marchello, 1964). Consequently, the change in rumen fermentation linked with dietary changes results in the modification of the fat composition. Findings by Webb *et al.* (1994) suggest that an increase in the metabolisable energy (maize meal being the source of energy) of the diet causes a decrease in ruminal pH. This enhances the activity of lactate utilizing bacteria with a change in the ruminal fermentation end-products, as the acetate to propionate ratio decrease. High concentrations of propionate and methylmalonate are not completely metabolised in the liver but synthesised to unsaturated, odd numbered and/or BCFA. This modification has direct implications for aroma as differences in muscle fatty acid composition results in differences in the final perceived aroma. It is difficult to say which type of diet has the greatest effect on the BCFA as the concentration of BCFA in meat produced from animals raised on different diets vary. Young *et al.* (2003) found the concentrations of BCFA in subcutaneous fat of concentrate-fed lambs to be higher than that detected in pasture-fed lambs. Yet, in an earlier study BCFA were shown to be highest in slow-grown pasture-fed lamb compared to that of grain-fed lamb (Young *et al.*, 1997). The reason for the variation in BCFA could be due to the difference in sample preparation. Fat samples were cooked differently in the two studies, with more glycerol being generated in the earlier study (Young *et al.*, 1997). Two important BCFA, 4-methyloctanoic and 4-methylnonanoic acid, were also not detected in the one study (Young *et al.*, 2003).

The distinct sensory profile of *Karoo lamb* is produced through the consumption of aromatic Karoo bushes (indigenous plants) (Estler *et al.*, 2006; Erasmus *et al.*, 2016). An early report suggests that the palatability of the plants may vary according to season (Henrici, 1952). In summer, sheep prefer to eat grass

or the shoots of bushes rather than the older twigs. Depending on the condition of the veld and the prevalence of plants, some species may only be consumed if there is nothing else to eat, for example *Eriocephalus spp.* Although plants species, such as the latter may be considered as quite good fodder plants, they have a taste which the sheep do not like and is consumed less in mixed veld conditions (Henrici, 1952). In the case of *Borrego do Nordeste Alentejano* lamb in Portugal, season also has an effect on diet and ultimately meat quality. During spring lambs are raised on pasture and their dams supplemented with concentrate, while in autumn the lambs are weaned and fed with concentrate (Santos-Silva *et al.*, 2006). The spring lambs had more tender meat compared to autumn lambs. Furthermore, when carcass weight (12 and 15 kg) was taken into account consumers preferred the meat from lighter lambs when raised in pasture, and from heavier lambs when raised in confinement.

Similar to *Karoo lamb*, Sardinian lamb (*Agnello di Sardegna*) and Elassonas lamb (*Αρνάκι Ελασσόνας*) has a distinct wild flavour and aroma due to the consumption of native wild and aromatic plants (Table 2.1). The aromatic compounds are likely transferred from the diet to the meat (Ha & Lindsay, 1991). In other cases, the diet could produce characteristic gamey attributes. Examples include *Lüneburger Heidschnucke*, *Isle of Man Manx Loaghtan lamb* and *Diepholzer Moorschnucke*, where the latter is interestingly produced from a varied diet which includes heather, bent, cotton, grass, sedge, various herbs and grasses, pine, birch, frángula and other woody plants (Table 2.1).

Given the important role of diet and its link to origin, some important questions should be asked regarding the specifications set out for the different PDO/PGI lamb types. As mentioned above with *Borrego do Nordeste Alentejano* lamb, season is an important effect to consider together with the diversity in farming practises (Santos-Silva *et al.*, 2006). For example, late weaning and pasture-feeding is practised in spring as opposed to early-weaning and concentrate-feeding performed in autumn. Therefore, can lamb meat from autumn-born lambs really be linked to the geographical area, as they are weaned early and fattened indoors with concentrates? Similarly, the *Barèges-Gavarnie* sheep types in France follow different diets depending on season. In winter the diet is hay meadows supplemented with dried fodder and grain cereals (Table 2.1). In spring and autumn the sheep graze on highland areas with pasture and in summer they graze on mountain pastures (Table 2.1). In winter, the *Diepholzer Moorschnucke* also receives supplementary feed (Table 2.1). For *Prés-salés de la baie de Somme* and *Prés-salés du Mont-Saint-Michel* lamb, farmers are allowed to finish the sheep on forage and concentrates (Table 2.1). A variation in the sensory profile of the meat is certainly expected and research is needed to determine the effect and a threshold for supplementation so that the characteristics of the product is not lost. Although some PDO lamb meat is unquestionably linked to its geographical production area, such as *Prés-salés* produced on the salt marshes, for other lamb types (as pointed out) their diet may not necessarily form the link with the origin. The inclusion of regional breeds forms part of the specifications and may provide the required link. For instance, *Agneau de l'Aveyron* lambs are reared in a sheep-fold but only the Lacaune breed may be used. The effect of breed is discussed in the next section 2.2.1.2. The questions raised above highlight how different the lamb types are and also reflect how

broad the specifications can be. This is a problem as the link with origin could in effect be lost and it is questionable whether these lamb types should be classed as PDO/PGI or not.

2.2.1.2 Breed

Breed was initially reported to have some effect on flavour with results indicating that mutton flavour is greatest in fine-wool sheep breeds (Sink & Caporaso, 1977). The most important differences between breeds can be seen in the amount of fat and the place where it is deposited (Sañudo *et al.*, 1998). Depending on the breed, more or less fat can be placed subcutaneously, intermuscularly, intramuscularly or internally. Considering the important role of fat in terms of meat quality, variation in the sensory quality of sheep meat from different breeds may be expected.

Young *et al.* (1993) found that breed (Coopworth vs Merino) has an effect on the ultimate pH and flavour of the meat. The Coopworth breed (a combined meat and wool breed) was stronger in mutton-meat flavour and lower in muscle pH (5.77), whereas the Merino (very fine wool breed) was stronger in foreign flavour and higher in muscle pH (6.16). Breed did not have an influence on muscle toughness. In fact, texture could be outshone by flavour and aroma as seen with *Lakeland Herdwick lamb* (Wood *et al.*, 1997). Although 20 months old Herdwicks were tougher than 8 months old Herdwicks, they had a similar liking to 6 months old Suffolks, suggesting that the flavour and aroma (due to higher fatty acid content) were superior to texture attributes.

Fisher *et al.* (2000) established that only one flavour profile (Soay breed) differed from three extensively produced breeds. The flavour profile of the different breeds were generated using a combination of sensory attributes (i.e. toughness, juiciness, overall liking and flavour descriptive terms) (Fisher *et al.*, 2000). When the fatty acid composition of the meat is combined with the flavour profile, it is possible to determine how the fatty acid profile affects the sensory quality of the meat. The Soay breed had a particular high score for the livery flavour attribute. They suggest that flavour may be related to the overall composition of muscle tissue since the Soay meat were high in PUFA and much darker in colour than the other breeds. Similarly the Swedish *Hånnlamb*, *Isle of Man Manx Loaghtan lamb* and *Lakeland Herdwick lamb* have also been described as having darker coloured meat with distinct gamey and meat (liver and blood) flavours (Table 2.1). The darker meat may be due to a high myoglobin content or high ultimate pH of the meat (Young *et al.*, 1993; Iqbal, 2005). The high ultimate pH may be as a result of pre-slaughter stress or the inherent meat quality as studies have shown that some breeds (Young *et al.*, 1993; Hoffman *et al.*, 2003a) and reproduction lines (Hoffman *et al.*, 2003b) are more affected than others. The livery flavour is likely consequential of *n*-6 PUFA and *n*-3 PUFA peroxidation and the myoglobin content as explained below. Game meat, another meat dark in colour and high in myoglobin (Hoffman *et al.*, 2005), has livery as a common descriptor for its flavour. The extensive production system results in the muscles of the animal working harder, in that way developing more red oxidative fibres, which are higher in myoglobin (Warriss, 2010). Myoglobin is an iron-containing haem protein. Therefore, with an increase in myoglobin content, an increase in iron concentration also takes place. Iron contributes to the perceived livery flavour attributes and catalyses lipid oxidation in the muscles (Warriss, 2010). Young *et al.*

(1993) described cooked Merino meat, which was dark in colour, as “bland”, “fishy/stale/rancid” and “bloody”. *Prés-salés du Mont-Saint-Michel* is also strongly coloured with intense, lasting flavours due to physical exercise during extensive grazing on the salt marshes (Table 2.1).

Teixeira *et al.* (2005) compared the meat quality of two Portuguese PDO lamb breed types, *Cordeiro Bragançano* (Bragançano lambs) and *Cordeiro Mirandês* (Mirandesa lambs). In terms of sensory quality, Mirandesa meat was more acceptable than that of Bragançano. Bragançano meat was tougher, more stringy and had a higher odour intensity. A possible source of the variation could be related to diet as different breeds may have different dietary traits. Du Toit (1998) reported the selective dietary traits of the Merino and Dorper. They found that Merino sheep are more selective grazers and prefer to graze on soft-leaved grasses, Karoo bush leaves and thin bush twigs. The Dorper sheep are more general or non-selective grazers, focussing on the woody plant types of the veld, such as Karoo bushes, trees and shrubs. Similarly, Brand (2000) found distinct differences in the selective grazing behaviour of the Merino and Dorper. Under sufficient grazing conditions the Dorsers walk less than the Merinos in search of food. However, they cover greater distances when food is scarce reflecting their adaptability to unfavourable climatic conditions. These findings confirm that the diets of the two breeds of sheep differ. Moreover, the type of plants the sheep consume could contribute to the final flavour and aroma of the meat.

Another important aspect of breed to highlight is that a large number of the suckling and light lamb types listed in Table 2.1 are derived from dairy breeds, especially in the Mediterranean area. Some of the most productive dairy breeds include the Lacaune and Manech from France (producing *Agneau de l'Aveyron*, *Agneau de Pauillac* and *Agneau de lait des Pyrénées*, respectively), Sarda from Italy (producing *Abbacchio Romano* and *Agnello di Sardegna*), Awassi from Israel, East Friesian from Northern Europe, and Chios or Sfakiano from Greece (Tzanidakis *et al.*, 2014). Milk yields can range from 250-600 litres per ewe and is largely used for making dairy products apart from raising the lambs. In Spain the local Churra and Castellana dairy breeds (used for the production of *Lechazo de Castilla y León*) are continuously being replaced by crossbreeds and foreign dairy breeds such as the Assaf, Awassi, Lacaune and East Friesian (Ugarte *et al.*, 2001). Although these foreign breeds may produce more milk, some of them have difficulties adapting to the environmental conditions while others may exploit the natural vegetation. This may affect growth traits and meat quality. Therefore, inclusion of such breeds as part of the registered denomination of origin lamb types should be carefully considered, as regional characteristics may be lost. More details of dairy breeds and different lamb types are provided in the next section given that the lambs are slaughtered at a very young age. Hence, when compared to other meat and/or wool sheep breeds, age is an important factor to consider.

2.2.1.3 Age

A study by Schönfeldt *et al.* (1993) suggests that the meat of younger animals contains less fibrous tissue residue, is more tender and has less typical species flavour than that of older animals. The subcutaneous fat is also less saturated, where that of older animals is typically more saturated, with increased levels of

triacylglycerides which contain BCFA responsible for species-specific aroma (Young *et al.*, 2003). Sutherland and Ames (1996) found that the BCFA concentrations did not differ in the adipose tissue of wethers and rams slaughtered at 12 weeks. Nevertheless, once the animals reached sexual maturity at 30 weeks, the rams had much greater concentrations of MOA and MNA. Similar results were reported by Young *et al.* (2006) where BCFA concentrations differed significantly between wethers and rams aged 13 months or older.

Wood *et al.* (1997) found differences in the sensory qualities of 8 and 20 months old *Lakeland Herdwick* lamb. The 20 months old Herdwicks had a lower overall liking and tougher meat than the 8 months old lambs. This was due to the variation in fatty acid content, where the older animals contained increased levels of PUFA. PUFA are prone to lipid oxidation and likely the source of the “rancid” and “stale” flavours detected. However, in another study where younger Altamura lambs (40 and 75 days old) from the Apulia region in Southern Italy were compared, the youngest lambs had a higher content of unsaturated fatty acids (MUFA and PUFA) than the oldest lambs (Della Malva *et al.*, 2016). However, no significant difference were detected for the aroma and flavour of the different aged Altamura lambs. These results suggest that although the younger Altamura lambs contained high levels of PUFA, the sensory effect is not detected at this young age (less than 2 months) compared to the study investigating the older Lakeland Herdwick lambs (older than 8 months).

Another factor to take into account is the increase in fat with increase in age as natural forages contain several odour-active compounds that are deposited in fat tissue (Berg & Walker, 2004). As these compounds accumulate, they have a more profound contribution towards flavour hence, resulting in distinct (positive and negative) flavour differences between young and older animals. With regards to texture, Devine *et al.* (1993) concluded that age-related tenderness effects of young and older lambs (up to 14 months) were minor. The aforementioned attributes are expected to make the meat of younger animals more desirable than that of their older counterparts. In certain places, such as Sardinia, suckling lambs are slaughtered at an early age (30-40 days) in order to maximize the amount of milk for the production of traditional cheese (Manca *et al.*, 2013). These young lambs, known as *Agnello di Sardegna*, are well sought after for their tenderness, juiciness and sensory qualities. In Spain and the Canary Islands, the meat of suckling lamb (*Lechal*) and light lamb (*Ternasco*) is known for its light pink colour, soft texture and smooth flavour. Spanish consumers associate young lamb with adjectives such as “natural, tasty and healthy”, while they are also willing to pay more for it (Sañudo *et al.*, 1998).

Diet affects the growth rate, and different growth rates results in differences in weight at a specific age. Therefore, when lambs from different diet groups are slaughtered at constant age they would most likely have different weights. This is also related to the suckling length as *Kıvırcık* lambs of the western Anatolian region of Turkey weaned later had higher weights than those weaned earlier (Ekiz *et al.*, 2012). Lambs weaned earlier have to adapt to the consumption of solid foods, while the non-weaned lambs still receive a higher energy intake from the milk. In Croatia and other Mediterranean countries, traditional sheep production systems depend largely on the diet, breed (i.e. milk and/or meat producing) and slaughter weight of the animal (Vnučec *et al.*, 2014). On the island of Pag, the milk-producing *Paška janjetina* (Pag lamb) are solely fed

mother's milk and slaughtered at 7-15 kg (28-40 days). Whereas, the milk- and meat-producing Istrian sheep breed is raised intensively and slaughtered at 20-25 kg (60-80 days). The meat-producing Dalmatian Pramenka sheep breed is also slaughtered at 20-25 kg although, being raised extensively, they are 90-120 days old. Conversely, when lambs are raised on the same diet and slaughtered at different weights, they will vary in age. This will also lead to other variation, such as the proportion of fat in the various fat depots; *Cordeiro Mirandês* lamb showed an increase in subcutaneous fat with the increase in carcass weight (≤ 7 kg and 7-10 kg) (Santos *et al.*, 2015). However, the influence on the sensory quality appears to be minor as the increase in carcass weight (8.0-13.4 kg) of *Ternasco de Aragón* (the first Spanish fresh meat given a denomination of origin) has little effect on the sensory profile of the meat, although the colour of the meat appears darker with increase in weight (related to the increase in age) (Sañudo *et al.*, 1996; Beriain *et al.*, 2000) and the flavour intensity and tenderness increased with fat class for only the leanest lambs (Sañudo *et al.*, 2000b). Similarly, *Cordero de Extremadura*'s sensory quality was unaffected by slaughter weight (24 and 29 kg) or gender, but meat from the lighter lambs were more acceptable and juicier (Tejeda *et al.*, 2008).

2.2.1.4 Gender

Initial studies propose that the flavour intensity of meat decrease from rams to ewes to wethers, especially where meat samples have a high fat content (Sink & Caporaso, 1977). Nonetheless, Claasen (2008) observed no aroma differences for young (approximately 160 days old) Dorper sheep of different gender (ewes, rams and wethers). This could be because of age, since gender does not greatly affect the levels of fatty acids in the subcutaneous fat of lambs up to an age of 10 to 12 months (Cramer & Marchello, 1964). At and after this age, the levels of fatty acids began to vary, which could result in aroma differences. Also, the flavour intensity is likely related to the concentration of volatile compounds as Ha and Lindsay (1991) found that ram fat and lamb fat contained similar phenolic compounds, but with higher concentrations in that of ram fat. Nevertheless, the age affording differences is not definite as Cloete *et al.* (2012) found no differences ($P > 0.05$) in the sensory attributes of the meat from 20-months old rams and ewes. On the contrary, male *Kivircik* lambs had higher PUFA levels than the female lambs after receiving intensive feeding for 10 weeks after weaning (Yarali *et al.*, 2014). A general conclusion is that gender starts to have an effect on aroma after a certain physiological age (i.e. sexual maturity) is reached. This is linked with an increase in fat with increase in age causing an increase in lipid-derived aroma precursors (Ba *et al.*, 2012).

However, in the case of suckling lamb (up to 3 months old), the *Cordero Manchego* female lambs tend to be fatter than the males (Díaz *et al.*, 2003). This is because the female lambs have a slower growth rate (i.e. were older at slaughter), but mature and accumulate fat earlier than the male lambs. In this regard, gender differences would be greater for suckling lambs than for heavier lambs. The females also have more α -linolenic acid (C18:3 *n*-3) and PUFA in their subcutaneous fat and consequently are regarded to be healthier than the male lambs (Díaz *et al.*, 2003). It is important to note that the rumen of a suckling lamb is not yet completely functional (Lane *et al.*, 2000). Therefore, the fat composition of the meat from these suckling lambs is similar

to the composition of their mother's milk as dietary unsaturated fatty acids are not greatly hydrogenated by the ruminal micro-organisms (Osorio *et al.*, 2007). The milk fat from pasture-grazing ewes are also more likely to be higher in *n*-3 fatty acids (compared to ewes fed concentrates) given the higher amounts of α -linolenic acid in grass (Enser *et al.*, 1998). Compared to milk-replacers, ewes' milk is also higher in BCFA, SFA and *n*-3 fatty acids, all of which have a direct influence on the suckling lamb meat quality (Osorio *et al.*, 2007). Studies have been performed, where the effect of milk source (e.g. mother's milk vs. artificial) on the meat quality is assessed (Napolitano *et al.*, 2002; Lanza *et al.*, 2006). A main finding is that the milk source has a significant effect on the fatty acid composition of the meat. This is an important effect to take into account as farmers tend to use artificial milk to raise lambs so that more of the mother's milk can be used for the production of regional dairy products. However, the lamb meat quality and sensory characteristics (Napolitano *et al.*, 2002) is altered and questions are raised whether the meat could still be regarded as a true region of origin product.

2.3 Authentication

A major shortcoming of the information provided in the applications published in the Official Journal of the European Union (Table 2.1) is the lack of sound scientific research. In fact, the unique sensory qualities of the authentic lamb types were mainly described based on anecdotal data, press release articles (general and specialist) and testimonies from leading restaurateurs and chefs. Although it is considered common knowledge to locals that a specific authentic lamb type of a particular origin has unique characteristics (i.e. aroma, flavour etc.) due to the traditional production system and plants they consume, reliable scientific evidence is still required to verify these claims in order for it to receive official recognition in the scientific community. According to Franke *et al.* (2005), it is possible to determine the origin of meat using analytical tools as several qualities are influence by geographically specific factors. Research performed on the authentic fresh lamb meat types registered as either PGI or PDO is shown in Table 2.2. Attention was given to methods used to verify the sensory and chemical characteristics of the meat.

Five volatiles were identified in Lika lamb which have not been found in other volatile studies of lamb (Krvavica *et al.*, 2015). Furthermore, the meat of Lika lamb had a different volatile profile compared to that of the Dalmatian lamb leading to the conclusion that origin has an effect on volatile profile and therefore, the aroma and flavour of the meat (Krvavica *et al.*, 2015). However, a limitation of the study was that a small number of meat samples were used and Lika lamb was only compared to one other breed.

A descriptive sensory analysis study was performed comparing the following three authentic Spanish breeds: Rasa Aragonesa (local meat breed of *Ternasco de Aragón*), Churra (local dairy breed of *Lechazo de Castilla y León*) and Spanish Merino (specialised meat breed of *Cordero de Extremadura*) (Martínez-Cerezo *et al.*, 2005). The effect of breed as well as slaughter weight were compared. The meat of Churra suckling lambs (10-12 kg) were the most tender, likely as a result of it being an early maturing dairy breed. However, at higher weights the Spanish Merino's meat was the most tender and juicy. For the Rasa Aragonesa breed, meat tenderness and juiciness improved with the increase in slaughter weight. Yet for Churra the opposite

was seen as higher weights produced less tender meat. However, the decrease in tenderness can be improved with ageing as in all cases, ageing improved the tenderness scores (Martínez-Cerezo *et al.*, 2005). An ageing period of 4 days proved to be sufficient, whilst suckling lambs should not be aged too long (16 days) as an off-flavour (although not analysed, this could be linked to oxidation – an aspect that warrants further research) starts to develop.

Although sensory analysis proved to be a valid method for determining the sensory quality and comparing different breeds, the subjectivity of the judges could have influenced the results as they were not accustomed to the characteristic milky taste of meat from suckling lambs (Martínez-Cerezo *et al.*, 2005). The same subjectivity were shown in a study where lamb meat of different lamb types were assessed by consumers in six European countries (Greece, Italy, Spain, France, Iceland and United Kingdom) (Sañudo *et al.*, 2007). It was found that two categories of consumers of lamb exist: those who prefer “milk or concentrate taste” and those who prefer “grass taste” (Sañudo *et al.*, 2007). Therefore, it is important to bear in mind the consumer’s cultural aspects and habits in any analysis of flavour and overall acceptability.

The use of isotopes to discriminate lamb from different European regions proved to be very successful (Piasentier *et al.*, 2003). By examining the carbon stable isotopes suckling lambs (*Lechazo de Castilla y León* and *Elassonas lamb*) and lambs supplemented with concentrates (*Ternasco de Aragón* and *Agneau de l’Aveyron*) could be distinguished from pasture-fed lamb (*Welsh lamb*) as the former contained elevated $\delta^{13}\text{C}$ values. However, one limitation of the analysis is that a great deal of variation may exist within a region given the different production methods. For example, depending on the season lambs may be raised on pasture in the summer or supplemented with grains in the winter (Table 2.1). A study on *Agnello di Sardegna* PGI suckling lamb showed a wide range of variation of both the physicochemical parameters and the fatty acid composition of intramuscular fat which were related to the fact that the lambs came from different areas of Sardinia and were reared in two different seasons (winter and spring) (Addis *et al.*, 2013). Hence, when a reference database is built for authentic lamb meat, it is crucial that sampling is done to include most of the variation within a specific region.

An untrained panel (in triangle tests) were unable to detect significant difference between Churra suckling lambs raised on maternal milk compared to milk replacer (*Lechazo de Castilla y León*) (Osorio *et al.*, 2008). Yet, it is expected the meat of lambs raised on maternal milk may have a different flavour to lambs raised on milk replacer based on the variation in their volatile profiles (Osorio *et al.*, 2008). Given that suckling lamb should be raised solely on maternal milk, it is important that analytical techniques be implemented to test the authenticity of the meat.

Another shortcoming of the scientific work is that some information is not published in English. For example, in the regulatory documents of *Cordero de Extremadura* studies which have highlighted the uniqueness of the meat is referenced (OJEU, 2010). Table 2.2 only contains results published in English. Whilst in other cases (*Elassonas lamb*) analyses performed are described in the regulations but not found in literature.

Table 2.2 Research performed on authentic fresh lamb meat types registered as either Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO) in the Database of Origin and Registration (DOOR)

Fresh sheep meat	Sensory and/or chemical analyses performed	Findings
<i>Abbacchio Romano</i>	*	
<i>Agneau de lait des Pyrénées</i>	*	
<i>Agneau de l'Aveyron</i> (Lamb of Aveyron)	Stable isotope ratio analysis (C, N) ⁷ ; Consumer sensory analysis ²³	Concentrate and straw-fed Lacaune breed have elevated $\delta^{13}\text{C}$ values. ⁷ Isotope ratios classified meat according to origin and feeding regime. ⁷ Lamb meat of concentrate-fed Lacaune breed rated higher for juiciness compared to grass-fed sheep. ²³
<i>Agneau de Lozère</i>	*	
<i>Agneau de Pauillac</i>	*	
<i>Agneau de Sisteron</i>	*	
<i>Agneau du Bourbonnais</i>	*	
<i>Agneau du Limousin</i> (Limousin lamb)	Stable isotope ratio analysis (C, N, H, S) ⁶	Isotope ratios discriminate lamb from lamb of other European regions. ⁶ Hydrogen isotope ratios are related to the colder and more humid climate of the region. ⁶ Supplementation with maize were detected in some samples. ⁶
<i>Agneau du Périgord</i>	*	
<i>Agneau du Poitou-Charentes</i>	*	
<i>Agneau du Quercy</i>	*	
<i>Agnello del Centro Italia</i> (Lamb of Central Italy)	Stable isotope ratio analysis (C, N, H, S) ⁶ ; Stable isotope ratio analysis (C, N) ⁷ ; Consumer sensory analysis ²³	Isotope ratios discriminate lamb from lamb of other European regions. ^{6,7} Hydrogen isotope ratios are related to the colder and more humid climate of the Tuscany region. ^{6,7} Milk fed lambs from Tuscany and older Bergamasca breed fed pasture and crop (maize, soybean) residues had elevated $\delta^{13}\text{C}$ values. ^{6,7} Bergamasca breed (30.5 kg carcass, 12 months old) received lowest aroma liking and juiciness scores (age affect quality) ²³ Meat of concentrate-fed Appenninica more juicy than grass-fed Bergamasca. ²³
<i>Agnello di Sardegna</i> (Lamb of Sardinia)	Fatty acid analysis, CIE L*a*b*, Vitamin E and chemical composition ¹² ; Fatty acid analysis and chemical composition ²²	The meat tends to have lower L* (43.86) (lightness), higher a*(15.15) (red index) and lower b* (4.80) (yellow index) values than those reported for other lamb breeds with similar weight and rearing system. ¹² The meat has double the amount of vitamin E compared to Churra suckling lambs (<i>Lechazo de Castilla y León</i>). ¹² The high nutritional quality of intramuscular fat in the lamb meat is related to the Sardinian ewe feeding system, based mainly on grazing. ¹² Meat from Mouflon x Sarda is leaner and has a lower cholesterol content than Sarda x Sarda lambs. ²² The polyunsaturated to saturated and n-6 to n-3 fatty acids ratios of the intramuscular lipids were optimal in both groups. ²²
<i>Αρνάκι Ελασσόνας</i> (Elassonas lamb)	Stable isotope ratio analysis (C, N) ⁷ ; Fatty acid analysis ⁸ (described in regulations but not found in literature); Consumer sensory analysis ²³	Milk-fed Karangouniki breed have elevated $\delta^{13}\text{C}$ values. ⁷ Isotope ratios classified meat according to origin and feeding regime. ⁷ The meat has a characteristic aroma and a pleasant smell and taste; it is tender, juicy, has a pH of 7.1-7.3, a very thin layer of fat and high levels of α -linolenic (C18:3) acid (compared to lambs from lowland areas). ⁸ Aromatic plants give the ewe's milk (particularly, the lamb's meat) a characteristic aroma and taste. ⁸ Suckling lamb (8.1 kg carcass) received lower aroma liking than lighter (5.4 kg carcass) Spanish Churra lamb. ²³ Lamb meat rated higher for juiciness compared to grass-fed sheep. ²³
<i>Barèges-Gavarnie</i>	*	
<i>Borrego da Beira</i>	*	
<i>Borrego de Montemor-o-Novo</i>	Water-holding capacity (WHC), Warner-Bratzler shear force (WBSF) and CIE L*a*b* ¹⁸	Meat of pure Merino Branco breed has a lower shear force. ¹⁸ WHC is lower for pasture-fed Merino Branco lambs ¹⁸ Meat becomes darker with the increase in slaughter weight (L* value decrease) but it is still within the range of light pink for heavier lamb (30 kg) meat. ¹⁸
<i>Borrego do Baixo Alentejo</i> (Lamb of Baixo Alentejo)	Water-holding capacity (WHC), Warner-Bratzler shear force (WBSF) and CIE L*a*b* ¹⁸	Shear force lower for pure Merino Branco breed compared to Merino Branco crossbred. ¹⁸ Lambs supplemented with concentrates grows faster. ¹⁸ Meat becomes darker with the increase in slaughter weight (L* value decrease) but it is still within the range of light pink for heavier lamb (30 kg) meat. ¹⁸
<i>Borrego do Nordeste Alentejano</i>	Water-holding capacity (WHC), Warner-Bratzler shear force (WBSF) and CIE L*a*b* ¹⁸	Shear force lower for pure Merino Branco breed compared to Merino Branco crossbred. ¹⁸ Lambs supplemented with concentrates grows faster. ¹⁸ Meat becomes darker with the increase in slaughter weight (L* value decrease) but it is still within the range of light pink for heavier lamb (30 kg) meat. ¹⁸
<i>Borrego Serra da Estrela</i>	*	
<i>Borrego Terrincho</i>	CIE L*a*b* and chemical composition ²¹	The pH of Churra da Terra Quente lamb meat is not affected by gender. ²¹ The meat is pale (light) in colour. ²¹ Female lambs and weight classes 8-11 kg and >11 kg have higher proportions of intramuscular fat, which were the minimum amount required to ensure satisfactory organoleptic qualities of the meat. ²¹ A slaughter weight below 8 kg should not be used. ²¹

Table 2.2 (Continued)

Fresh sheep meat	Sensory and/or chemical analyses performed	Findings
<i>Cordeiro Bragançano</i> (Bragançano lamb)	Descriptive sensory analysis, Warner-Bratzler shear force (WBSF), CIE L*a*b* and chemical composition ²⁷	L* values (lightness) decrease with the increase in live weight and light lambs have higher b* values (yellow index). ²⁷ Bragançano lambs had higher b* values than <i>Mirandesa lambs</i> (likely linked to the milk diet of low-iron content received by the suckling lambs). ²⁷ Shear force increase with live weight. ²⁷ Bragançano lambs had higher shear force values than <i>Mirandesa lambs</i> . ²⁷ Heavy carcasses had more flavour intensity than light ones. ²⁷
<i>Cordeiro de Barroso</i>	*	
<i>Cordeiro Mirandês or Canhão Mirandês</i> (Mirandês lamb)	CIE L*a*b* and chemical composition ^{24,27} ; Descriptive sensory analysis and Warner-Bratzler shear force (WBSF) ²⁷	The subcutaneous and intermuscular fat Churra Galega Mirandesa meat increased with increase in carcass weight. ²⁴ Lambs (7 kg carcass) have higher L* values (lightness) and lambs (7-10 kg carcass) have higher a* values (red index). ²⁴ Tenderness is not affected by weight or gender. ²⁴ Lamb carcasses from both class weights have comparable quality attributes. ²⁴ L* values decrease with the increase in live weight and light lambs have higher b* values (yellow index). ²⁷ Shear force increase with live weight. ²⁷ Mirandesa lambs had lower shear force values than <i>Bragançano lambs</i> . ²⁷ Heavy carcasses had more flavour intensity than light ones. ²⁷ Mirandesa lambs have lower toughness, stringiness and aroma intensity than <i>Bragançano lambs</i> . ²⁷
<i>Cordero de Extremadura</i> (Lamb of Extremadura)	Descriptive sensory analysis ^{5,25} ; CIE L*a*b*, haem pigment and chemical composition ¹⁹ ; Fatty acid analysis ²⁶	Aroma and flavour intensities increase with the increase in slaughter weight. ⁵ Suckling lambs should not be aged too long. ⁵ Meat colour is lighter for younger lambs. ¹⁹ The meat of the Spanish Merino is redder than that of Churra and Rasa Aragonesa lambs. ¹⁹ Intramuscular fat increase and moisture decrease for heavier lambs. ¹⁹ Spanish Merino meat is discriminated from Churra Lebrijana and similar to Segureño meat. ²⁵ Spanish Merino had a higher content of α -linolenic (C18:3) acid (intramuscular fat) compared to other Spanish breeds. ²⁶
<i>Cordero de Navarra or Nafarroako Arkumea</i>	Descriptive sensory analysis ^{28,29}	The Ternasco lamb meat is harder, mealier, more cohesive and more difficult to swallow than milkfed lamb meat (Lechal). ^{28,29} The increase in live weight produced wool aroma and flavour and more intense aftertaste in cooked lamb meat. ^{28,29} Lechal lambs produce meat with a more characteristic aroma and flavour than Ternasco lambs. ^{28,29}
<i>Cordero Manchego</i> (Manchego lamb)	Water-holding capacity (WHC), Warner-Bratzler shear force (WBSF) and CIE L*a*b* ²⁷	Unweaned lambs were heavier and younger at slaughter than lambs reared under a milking-suckling system. ²⁷ WHC and WBSF did not vary between lambs weaned lambs at 35 days old and unweaned lambs. ²⁷ The meat of unweaned lambs were lighter (higher L* values) than that of weaned lambs. ²⁷ As consumers of light carcass meat prefer paler meat, unweaned rearing system yields better quality. ²⁷
<i>Cordero Segureño</i> (Segureño lamb)	Solid-phase microextraction (SPME) ¹⁵ ; Subjective meat colour evaluation ²⁰ ; Descriptive sensory analysis ²⁵ ; Fatty acid analysis ²⁶	The cooked meat of the Segureño breed raised semi-extensively on pasture of the Segura Mountains had a different volatile profile to that of the Segureño breed raised intensively on a grain-based concentrate (supplemented with barley, maize, orange peel and straw). ¹⁵ The total volatile concentration was higher in pasture-fed lambs. ¹⁵ The major volatiles in pasture-fed lambs were phenol, 4-methylphenol and hexanoic acid, and in concentrate-fed lambs were 3-hydroxy-2-butanone. ¹⁵ Segureño lamb meat is pink in colour and colour is not significantly influenced by carcass weight and gender. ²⁰ Segureño meat is discriminated from Churra Lebrijana and similar to the Spanish Merino meat. ²⁵ Segureño had the highest linoleic (C18:2 n-6) acid content for intermuscular, subcutaneous, omental and kidney knob fat depots compared to other Spanish breeds. ²⁶
<i>Diepholzer Moorschnucke Hännlamb</i>	*	
	Sensory analyses and/or fatty acid analyses ⁹ (described in regulations but not found in literature)	The taste of the meat is affected by the water content of the feed. ⁹ The abundance of herbs (i.e. wild thyme) in the natural pastures gives the meat a distinct gamey taste (a quality mentioned in historical texts). ⁹ Grass and herbs contain high levels of polyunsaturated fatty acids and antioxidants (i.e. vitamin E) which are deposited in the meat, giving it a different fatty acid profile and characteristic taste. ⁹
<i>Isle of Man Manx Loaghtan lamb</i>	*	
<i>Jagnięcina podhalańska</i>	*	
<i>Karoo lamb</i>	Descriptive sensory analysis ¹ ; Stable isotope ratio analysis (C, N) ²	Karoo lamb differs from lamb not raised in the Karoo region. ¹ Karoo lamb has a distinct herbaceous aroma and flavour linked to diet (which is linked to origin). ¹ Isotope ratios could discriminate the meat based on origin. ²
<i>Lakeland Herdwick</i>	Descriptive sensory analysis ³ ; Fatty acid analysis ³	Superior flavour and high levels of eating quality compared to crossbred Suffolks. ³ Meat contains high contents of stearic (C18:0) and α -linolenic (C18:3) fatty acids compared to beef and pork. ³ Higher proportion of total fat (marbling fat) and polyunsaturated fatty acids are positive attributes. ³

Table 2.2 (Continued)

Fresh sheep meat	Sensory and/or chemical analyses performed	Findings
<i>Lechazo de Castilla y León</i>	Descriptive sensory analysis ⁵ ; Stable isotope ratio analysis (C, N) ⁷ ; Sensory analysis (triangle tests), Volatile compound analysis, CIE L*a*b*, Vitamin E and chemical composition ¹⁶ ; CIE L*a*b*, haem pigment and chemical composition ¹⁹ ; Consumer sensory analysis ²³	Aroma and flavour intensities increase with the increase in slaughter weight, while tenderness decrease. ⁵ Churra suckling lambs (10-12 kg) have the most tender and juicy meat (early maturing dairy breed). ⁵ Suckling lambs should not be aged too long. ⁵ Churra have elevated $\delta^{13}\text{C}$ values. ⁷ Isotope ratios classified meat according to origin and feeding regime. ⁷ The meat of Churra suckling lambs showed higher L* (lightness), lower a* (red index) higher b* (yellow index) values, higher vitamin E, less lipid-oxidative stability and more volatile compounds (derived from lipid oxidation) than lamb reared on milk replacer. ¹⁶ An untrained panel were unable to detect significant difference between lambs raised on maternal milk compared to milk replacer. ¹⁶ Lambs raised on maternal milk may have a different flavour to lambs raised on milk replacer. ¹⁶ L* values were higher in the Churra breed at all slaughter weights compared to Spanish Merino and Rasa Aragonesa. ¹⁹ Intramuscular fat increase and moisture decrease for heavier lambs. ¹⁹ Churra have higher total and insoluble collagen contents compared to Spanish Merino and Rasa Aragonesa. ¹⁹ ; Churra lamb meat rated higher for juiciness compared to grass-fed sheep. ²³
<i>Lička janjetina</i> (Lika lamb)	Solid-phase microextraction (SPME) ⁴	Lika lamb has a unique volatile profile compared to the Dalmatian lamb. ⁴ Variation is as a result of diet. ⁴
<i>Lüneburger Heidschnucke</i>	*	
<i>Orkney lamb</i>	Stable isotope ratio analysis (C, N, H, S) ⁶	Isotope ratios discriminate lamb from lamb of other European regions. ⁶ Hydrogen isotope ratios are related to the distance from the sea. ⁶ High $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values may be due to fertilization of the grasslands with seaweed and sea-spray sulphate, respectively. ⁶
<i>Paška janjetina</i> (Pag lamb)	Sensory analyses and/or chemical composition ¹⁰ (described in regulations but not found in literature); CIE L*a*b* ¹⁷	Pag lamb meat is classified as pale pink according to established average colour values L* (lightness), a* (red index) and b* (yellow index). ^{10,17} The meat has a high fat content. ¹⁰ The climatic conditions and vegetation (which includes aromatic and medicinal plants) affects the quality of the meat, giving it a distinct "Mediterranean" taste. ¹⁰
<i>Prés-salés de la baie de Somme</i>	*	
<i>Prés-salés du Mont-Saint-Michel</i>	*	
<i>Scotch lamb</i>	*	
<i>Shetland lamb</i>	*	
<i>Ternasco de Aragón</i> (Lamb from Aragón)	Descriptive sensory analysis ^{5,13,28,29} ; Stable isotope ratio analysis (C, N) ⁷ ; Fatty acid analysis ¹³ ; Gas Chromatographic-olfactometric (GC-O) ¹⁴ ; CIE L*a*b*, haem pigment and chemical composition ¹⁹ ; Consumer sensory analysis ²³	Aroma, flavour, tenderness and juiciness increase with the increase in slaughter weight of the Rasa Aragonesa breed. ⁵ Rasa Aragonesa have the strongest fat flavour intensity compared to Churra and the Spanish Merino. Suckling lambs should not be aged too long. ⁵ Concentrate-fed Rasa Aragonesa have elevated $\delta^{13}\text{C}$ values. ⁷ Isotope ratios classified meat according to origin and feeding regime. ⁷ Concentrate-fed Rasa Aragonesa breed meat have a high content of long chain polyunsaturated n-6 fatty acids compared to grass-fed <i>Welsh lamb</i> . ¹³ Spanish sensory panel prefer the aromas and flavours associated with linoleic (C18:2) acid and grain feeding. ¹³ Key volatile aroma compounds identified in fresh and frozen Rasa Aragonesa lamb meat. ¹⁴ Meat colour is lighter for younger lambs. ¹⁹ Intramuscular fat increase and moisture decrease for heavier lambs. ¹⁹ Lamb meat rated higher for juiciness compared to grass-fed sheep. ²³ Rasa Aragonesa breed perceived mealier than lamb from the Lacha breed (<i>Cordero de Navarra</i> or <i>Nafarroako Arkumea</i>). ^{28,29} The increase in live weight produced wool aroma and flavour and more intense aftertaste in cooked lamb meat. ²⁸ Livery flavour develops in heavier (24 kg live weight) lambs. ²⁹ The increase in live weight produced wool aroma and more intense aftertaste in cooked lamb meat. ²⁹
<i>Uain Sléibhe Chonamara</i> (Connemara hill lamb)	Stable isotope ratio analysis (C, N, H, S) ⁶	Isotope ratios discriminate lamb from lamb of other European regions. ⁶ Low $\delta^{13}\text{C}$ values due to higher humidity in region. ⁶
<i>Vadehavslam</i> (Wadden Sea lamb)	*	
<i>Welsh lamb</i>	Stable isotope ratio analysis (C, N) ⁷ ; Descriptive sensory analysis ¹³ ; Fatty acid analysis ¹³ ; Consumer sensory analysis ²³	Pasture-fed Welsh Mountain breed have lower $\delta^{13}\text{C}$ values. ⁷ Isotope ratios classified meat according to origin and feeding regime. ⁷ Grass-fed Welsh Mountain breed have a high content of long chain polyunsaturated n-3 fatty acids compared to concentrate-fed <i>Ternasco de Aragón</i> lamb. ¹³ British sensory panel prefer the intense aromas and flavours associated with α -linolenic (C18:3) acid and grass feeding. ¹³ Meat of grass-fed Welsh Mountain rated high for tenderness and less juicy compared to milk- and concentrate fed sheep. ²³

Table 2.2 (Continued)

Fresh sheep meat	Sensory and/or chemical analyses performed	Findings
<i>West Country lamb</i>	Stable isotope ratio analysis (C, N, H, S) ⁶ ; Sensory analyses and/or fatty acid analysis ¹¹ (described in regulations but not found in literature)	Isotope ratios discriminate lamb from lamb of other European regions. ⁶ Low $\delta^{13}\text{C}$ values in Cornwall lamb due to higher humidity in region. ⁶ Grass-fed lambs have a distinct fatty acid profile with a higher content of α -linolenic (C18:3) acid compared to concentrate-fed lambs. ¹¹ Lamb meat and fat have a higher concentration of vitamin E. ¹¹ The taste of lamb is better in grass-finished sheep than grain-fed ones. ¹¹

(PGI) Protected Geographical Indication; (PDO) Protected Designation of Origin; * Sensory and/or chemical research were not found in English scientific published articles. Details of lambs obtained online (http://ec.europa.eu/agriculture/quality/index_en.htm) from DOOR database (Database of Origin and Registration) under Regulation (EC) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs.

- Erasmus, S. W., Hoffman, L. C., Muller, M. & Van der Rijst, M. (2016). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems. *Small Ruminant Research*, **144**, 62-74 DOI: 10.1016/j.smallrumres.2016.07.020.
- Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat. *Food Chemistry*, **192**, 997-1005 DOI: 10.1016/j.foodchem.2015.07.121.
- Wood, J. D., Enser, M. B. & Nute, G. R. (1997). An investigation of flavours in meat from sheep grown slowly or more quickly on grass diets. *Research project conducted for Ministry of Agriculture Fisheries and Food. Division of Food Animal Science*, University of Bristol.
- Krvavica, M., Bradaš, M., Rogošić, J., Jug, T., Vnučec, I. & Radovčić, N. M. (2015). Volatile aroma compounds of Lika lamb. *MESO: The first Croatia meat journal*, **17**(3), 264-271 <http://hrcak.srce.hr/141126>.
- Martínez-Cerezo, S., Sañudo, C., Medel, I. & Oletta, J. L. (2005). Breed, slaughter and ageing time effects on sensory characteristics of lamb. *Meat Science*, **69**, 571-578.
- Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S. D., Schlicht, C., Thomas, F., Monahan, F. J., Hoogewerff, J. & Rossmann, A. (2007). Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry*, **389**, 309-320.
- Piasentier, E., Valusso, R., Camin, F. & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science*, **64**, 239-247.
- Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Αρνάκι Ελασσόνας* (Arnaki Elassonas), EC No: EL-PDO-0005-0735-14.01.2009.
- Official Journal of the European Union (OJEU). (2016). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. *Hännlamb*, EU No: SE-PDO-0005-01327-21.4.2015.
- Official Journal of the European Union (OJEU). (2016). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. *Paška janjetina*, EU No: HR-PDO-0005-01347-19.6.2015.
- Official Journal of the European Union (OJEU). (2013). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. *West Country lamb*, EU No: UK-PGI-0005-0667-21.12.2007.
- Addis, M., Fiorr, M., Manca, C., Riu, G. & Scintu, M. F. (2013). Muscle colour and chemical and fatty acid composition of "Agnello di Sardegna" PGI suckling lamb. *Small Ruminant Research*, **115**, 51-55.
- Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I. & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, **54**, 339-346.
- Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V. & Escudero, A. (2011). Gas chromatographic-olfactometric characterisation of headspace and mouthspace key aroma compounds in fresh and frozen lamb meat. *Food Chemistry*, **129**, 1909-1918.
- Almela, E., Jordán, M. J., Martínez, C., Sotomayor, J. A., Bedia, M. & Bañón, S. (2010). Ewe's diet (pasture vs grain-based feed) affects volatile profile of cooked meat from light lambs. *Journal of Agricultural and Food Chemistry*, **58**, 9641-9646.
- Osorio, M. T., Zumalacárregui, J. M., Cabeza, A., Figueira, A. & Mateo, J. (2008). Effect of rearing system on some meat quality traits and volatile compounds of suckling lamb meat. *Small Ruminant Research*, **78**, 1-12.
- Vnučec, I., Držaić, V., Mioč, B., Prpić, Z., Pavić, V. & Antunović, Z. (2014). Carcass traits and meat colour of lambs from diverse production systems. *Vet arhiv*, **84**(3), 251-263.
- Santos-Silva, J., Mendes, I. A. & Bessa, R. J. B. (2002). The effect of genotype, feeding system and slaughter weight on the quality of light lambs. *Livestock Production Science*, **76**, 17-25.
- Martínez-Cerezo, S., Sañudo, C., Panea, B., Medel, I., Delfa, R., Sierra, I., Beltrán, J. A., Cepero, R. & Olleta, J. L. (2005). Breed, slaughter and ageing time effects on physico-chemical characteristics of lamb. *Meat Science*, **69**, 325-333.
- Peña, F., Cano, T., Domenech, V., Alcalde, M. J., Martos, J., García-Martínez, A., Herrera, M. & Rodero, E. (2005). Influence of sex, slaughter weight and carcass weight on "non-carcass" and carcass quality in segureña lambs. *Small Ruminant Research*, **60**, 247-254.
- Santos, V. A. C., Silva, S. R., Mena, E. G. & Azevedo, J. M. T. (2007). Live weight and sex effects on carcass and meat quality of "Borrego terrincho-PDO" suckling lambs. *Meat Science*, **77**, 654-661.
- Vacca, G. M., Carcangiu, V., Dettori, M. L., Pazzola, M., Mura, M. C., Luridiana, S. & Tilloca, G. (2008). Productive performance and meat quality of Mouflon x Sarda and Sarda x Sarda suckling lambs. *Meat Science*, **80**, 326-334.
- Sañudo, C., Alfonso, M., Julián, R. S., Thorkelsson, G., Valdimarsdottir, T., Zygoiannis, D., Stamataris, C., Piasentier, E., Mills, C., Berge, P., Dransfield, E., Nute, G. R., Enser, M. & Fisher, A. V. (2007). Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. *Meat Science*, **75**, 610-621.
- Santos, V. A. C., Cabo, A., Raposo, P., Silva, J. A., Azevedo, J. M. T. & Silva, S. R. (2015). The effect of carcass weight and sex on carcass composition and meat quality of "Cordeiro Mirandês"-Protected designation of origin lambs. *Small Ruminant Research*, **130**, 136-140.
- Alcalde, M. J., Morena-Indias, I., Horcada, A., Molina, A. & Juárez, M. (2014). Generalized procrustes analysis (GPA) as a tool to discriminate among sheep breeds. *Archiv Tierzucht*, **28**, 1-10.
- Juarez, M., Horcada, A., Alcalde, M. J., Aldai, N., Polvillo, O., Valera, M. & Molina, A. (2010). Short communication. Fatty acid composition of lamb fat depots as an origin discriminator. *Spanish Journal of Agricultural Research*, **8**(4), 976-980.
- Teixeira, A., Batista, S., Delfa, R. & Cadavez, V. (2005). Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. *Meat Science*, **71**, 530-536.
- Beriain, M. J., Gorraiz, C., Horcada, A. & Purroy, A. (2000). Sensory quality of fresh lamb meat. In: *Sheep and Goat Nutrition: Intake Digestion, Quality of Products and Rangelands* (edited by I. Ledin & P. Morand-Fehr). Pp. 125-128. Zaragoza: CIHEAM.
- Gorraiz, C., Beriain, M. J., Chasco, J. & Iraizoz, M. (2000). Descriptive analysis of meat from young ruminants in Mediterranean systems. *Journal of Sensory Studies*, **15**, 137-150.

Considering all the different lamb types in Table 2.2, the most research were performed on the Spanish lamb types. The sample sizes were sufficient and different breeds, live weights and meat ageing times were taken into account when chemical as well as sensory characteristics were determined. For future research on authentic lamb types such an approach should be considered.

Overall, it is evident that a combination of analytical tools is required to link lamb meat with its geographic origin as all methods have some sort of limitation. Whilst some methods (i.e. isotope analysis) are directly linked to the origin, other provide more descriptive information (i.e. sensory analysis and volatile profiling) about the chemical drivers causing the variation in the sensory quality.

2.4 Conclusions

The effect of origin on the meat quality characteristics of PDO and PGI fresh sheep meat have been discussed. It is evident from the discussion that diet is a key factor in terms of its effect on the sensory quality of meat. Concerning suckling lambs, lighter lambs and heavier lambs, variation in the sensory characteristics may exist due to differences in age, time of weaning and the type of feeding system adopted. The fatty acid profile (including volatile branched-chain fatty acids) of ruminant meat play an important role in the development of aroma and flavour. The texture (i.e. tenderness and juiciness) and colour of meat should also not be overlooked as they contribute to the overall quality of the product. In all, the diverse and authentic nature of the lamb types were reflected as well as their link with the geographical area of origin. In some cases, the descriptive data was experimentally substantiated, but in quite a number of other cases the information is based on anecdotal data. This is a major concern given that an adequate experimental design (i.e. representative sample size, enough replications per treatment, appropriate control treatments etc.) is required in order to verify the authenticity of the meat. Consequently, the numerous provenance claims cannot be confirmed without reliable scientific evidence. Other concerns are the effect of supplementary feed and concentrates on the sensory quality, how the replacement of traditional breeds with foreign breeds affects the growth traits and meat quality, the effect of using artificial milk to raise lambs instead of mother's milk on the meat quality and whether the PDO/PGI specifications should be adjusted to account for these. Therefore, more research is needed to prove how the different factors can influence the meat quality and also validate the link with the origin.

In the case of regionally unique South African lamb, these factors should be taken into account when the authentic nature of the meat and fat is determined. Diet plays a very important role, as it varies depending on the region of origin. For instance, Karoo lamb is characterised by its herbaceous aroma and flavour due to the consumption of fragrant indigenous Karoo plants.

2.5 References

Addis, M., Fiorr, M., Manca, C., Riu, G. & Scintu, M. F. (2013). Muscle colour and chemical and fatty acid composition of "Agnello di Sardegna" PGI suckling lamb. *Small Ruminant Research*, **115**, 51-55.

- Ba, H. V., Hwang, I., Jeong, D. & Touseef, A. (2012). Principle of meat aroma flavors and future prospect. In: *Latest Research into Quality Control* (edited by M. S. F. Nezhad). ISBN: 978-953-51-0868-9. InTech, DOI: 10.5772/51110.
- Berg, E. P. & Walker, E. L. M. (2004). Sheep. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 1291-1295. Oxford, UK: Elsevier.
- Beriain, M. J., Horcada, A., Purroy, A., Lizaso, G., Chasco, J. & Mendizabal, J. A. (2000). Characteristics of Lacha and Rasa Aragonesa lambs slaughtered at three live weights. *Journal of Animal Science*, **78**, 3070-3077.
- Borton, R. J., Loerch, S. C., McClure, K. E. & Wulf, D. M. (2005). Comparison of characteristics of lambs fed concentrate or grazed on ryegrass to traditional or heavy slaughter weights. I. Production, carcass, and organoleptic characteristics. *Journal of Animal Science*, **83**(3), 679-685.
- Brand, T. S. (2000). Grazing behaviour and diet selection by Dorper sheep. *Small Ruminant Research*, **36**, 147-158.
- Brennand, C. P. & Lindsay, R. C. (1992). Distribution of volatile branched-chain fatty acids in various lamb tissues. *Meat Science*, **31**, 411-421.
- Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, **77**, 63-80.
- Capuano, E., Boerrigter-Eenling, R., Van der Veer, G. & Van Ruth, S. M. (2013). Analytical authentication of organic products: an overview of markers. *Journal of the Science of Food and Agriculture*, **93**, 12-28.
- Claasen, B. (2008). *The effect of agricultural production system on the meat quality of Dorper lambs*. MSc thesis, Stellenbosch University, South Africa.
- Cloete, J. J. E., Hoffman, L. C. & Cloete, S. W. P. (2012). A comparison between slaughter traits and meat quality of various sheep breeds: wool, dual-purpose and mutton. *Meat Science*, **91**, 318-324.
- Cramer, D. A. & Marchello, J. A. (1964). Seasonal and sex patterns in fat composition of growing lambs. *Journal of Animal Science*, **23**, 1002-1010.
- Database of Origin and Registration (DOOR). (2015). Database under Regulation (EC) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs. European Commission Agriculture and Rural Development official website. [Internet document]. URL http://ec.europa.eu/agriculture/quality/schemes/index_en.htm. Accessed 03/02/2016.
- Della Malva, A., Albenzio, M., Caroprese, M., Muscio, A., Santillo, A. & Marino, R. (2016). Relationship between slaughtering age, nutritional and organoleptic properties of Altamura lamb meat. *Small Ruminant Research*, **135**, 39-45.
- Devine, C. E., Graafhuis, A. E., Muir, P. D. & Chrystall, B. B. (1993). The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Science*, **35**, 63-77.
- Díaz, M. T., Velasco, S., Pérez, C., Lauzurica, S., Huidobro, F. & Cañeque, V. (2003). Physio-chemical characteristics of carcass and meat Manchego-breed suckling lambs slaughtered at different weights. *Meat Science*, **65**, 1085-1093.

- Du Toit, P. C. V. (1998). Diets selected by Merino and Dorper sheep in Karoo veld. *Archivos de Zootecnia*, **47**, 21-32.
- Ekiz, B., Ekiz, E. E., Yalcintan, H., Kocak, O. & Yilmaz, A. (2012). Effects of suckling length (45, 75 and 120 d) and rearing type on cortisol level, carcass and meat quality characteristics in Kivircik lambs. *Meat Science*, **92**, 53-61.
- Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J.D. & Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relating to production system and implications for human nutrition. *Meat Science*, **49**, 329-341.
- Erasmus, S. W., Hoffman, L. C., Muller, M. & Van der Rijst, M. (2016). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems. *Small Ruminant Research*, **144**, 62-74. <http://dx.doi.org/10.1016/j.smallrumres.2016.07.020>.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., Kurt, E., Sinclair, L. A. & Wilkinson, R. G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science*, **55**(2), 141-147.
- Franke, B. M., Gremaud, G., Hadorn, R. & Kreuzer, M. (2005). Geographic origin of meat – elements of an analytical approach to its authentication. *European Food Research Technology*, **221**, 493-503.
- Grunert, K. G. (2005). Food quality and safety: consumer perception and demand. *European Review of Agricultural Economics*, **32**(3), 369-391.
- Ha, J. K. & Lindsay, R. C. (1991). Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *Journal of Food Science*, **56**(5), 1197-1202.
- Henrici, M. (1952). Karroid and Karoo veld as nutrition to farm animals. *Materiae vegetabiles*, **1**(1), 5-16.
- Hoffman, L. C., Kritzing, B. & Ferreira, A. V. (2005). The effects of region and gender on the fatty acid, amino acid, mineral, myoglobin and collagen contents of impala (*Aepyceros melampus*) meat. *Meat Science*, **69**(3), 551-558.
- Hoffman, L. C., Muller, M., Cloete, S. W. P. & Schmidt, D. (2003a). Comparison of six crossbred lamb types: sensory, physical and nutritional meat quality characteristics. *Meat Science*, **65**, 1265-1274.
- Hoffman, L. C., Schmidt, D., Muller, M. M., Cloete, J. J. E. & Cloete, S. W. P. (2003b). Sensory and objective mutton quality characteristics of SA Merino sheep selected for and against reproductive fitness. *South African Journal of Animal Science*, **33**(1), 52-64.
- Iqbal, S. A. (2005). Chapter 7. In: *Food Chemistry*. Pp. 160-161. New Delhi, India: Discovery Publishing House.
- Keeton, J. T. & Eddy, S. (2004). Flavour development. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 210-218. Oxford, UK: Elsevier.
- Krvavica, M., Bradaš, M., Rogošić, J., Jug, T., Vnučec, I. & Radovčić, N. M. (2015). Volatile aroma compounds of Lika lamb. MESO: *The first Croatia meat journal*, **17**(3), 264-271 <http://hrcak.srce.hr/141126>.

- Lane, M. A., Baldwin, R. L. & Jesse, B. W. (2000). Sheep rumen metabolic development in response to age and dietary treatments. *Journal of Animal Science*, **78**, 1990-1996.
- Lanza, M., Bella, M., Priolo, A., Barbagallo, D., Galofaro, V., Landi, C. & Pennisi, P. (2006). Lamb meat quality as affected by a natural or artificial milk feeding regime. *Meat Science*, **73**, 313-318.
- Madruca, M. S. & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture*, **68**, 305-310.
- Manca, C., Addis, M., Riu, G., Fiori, M. & Scintu, M. F. (2013). Physiochemical properties of different muscles from Sarda suckling lambs covered by the protected geographical indication "Agnello di Sardegna". *Journal of Food Quality*, **36**, 369-374.
- Martínez-Cerezo, S., Sañudo, C., Medel, I. & Oletta, J. L. (2005). Breed, slaughter and ageing time effects on sensory characteristics of lamb. *Meat Science*, **69**, 571-578.
- Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, **62**(4), 415-424.
- Napolitano, F., Cifuni, G. F., Pacelli, C., Riviezzi, A. M. & Girolami, A. (2002). Effect of artificial rearing on lamb welfare and meat quality. *Meat Science*, **60**, 307-315.
- Official Journal of the European Union (OJEU). (2006). Publication of an application for registration pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. *Isle of Man Manx Loaghtan Lamb*, EC No: UK/00340/05.04.2004.
- Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Cordero de Extremadura*, EC No: ES-PGI-0005-0725-09.10.2008.
- Official Journal of the European Union (OJEU). (2013). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs. *Prés-salés du Mont-Saint-Michel*, EC No: FR-PDO-0005-0813-25.06.2010.
- Osorio, M. T., Zumalacárregui, J. M., Cabeza, A., Figueira, A. & Mateo, J. (2008). Effect of rearing system on some meat quality traits and volatile compounds of suckling lamb meat. *Small Ruminant Research*, **78**, 1-12.
- Osorio, M. T., Zumalacárregui, J. M., Figueira, A. & Mateo, J. (2007). Fatty acid composition in subcutaneous, intermuscular and intramuscular fat deposits of suckling lamb meat: Effect of milk source. *Small Ruminant Research*, **74**, 127-134.
- Pegg, R. B. & Shahidi, F. (2004). Flavour development. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 570-578. Oxford, UK: Elsevier.
- Piasentier, E., Valusso, R., Camin, F. & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science*, **64**, 239-247.

- Priolo, A., Micol, D., Agabriel, J., Prache, S. & Dransfield, E. (2002). Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science*, **62**(2), 179-185.
- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2010). Relationship between odour-active compounds and flavour perception in meat from lamb fed different diets. *Meat Science*, **85**, 700-706.
- Rippon, M. J. (2014). What is the geography of Geographical Indications? Place, production, methods and Protected Food Names. *Area*, **46.2**, 154-162.
- Rousset-Akrim, S., Young, O. A. & Berdagué, J.-L. (1997). Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science*, **45**(2), 169-181.
- Rowe, A., Macedo, F. A. F., Visetainer, J. V., Souza, N. E. & Matsushita, M. (1999). Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. *Meat Science*, **51**, 283-288.
- Santos, V. A. C., Cabo, A., Raposo, P., Silva, J. A., Azevedo, J. M. T. & Silva, S.R. (2015). The effect of carcass weight and sex on carcass composition and meat quality of “Cordeiro Mirandês” – Protected designation of origin lambs. *Small Ruminant Research*, **130**, 136-140.
- Santos-Silva, J., Esteves, A., Alexandre, N., Alves, S., Portugal, A. P., Mendes, I. A., Silva Pereira, M., Vacas de Carvalho, M. & Bessa, R.J.B. (2006). *Contribution to a better definition of the production standards of the lamb “Borrego do Nordeste Alentejano – PGI”*. *Animal products from the Mediterranean area*. Pp. 377-383. EAAP publication No. 119. Wageningen, the Netherlands: Wageningen Academic Publishers.
- Sañudo, C., Alfonso, M., Julián, R. S., Thorkelsson, G., Valdimarsdottir, T., Zygoiannis, D., Stamataris, C., Piasentier, E., Mills, C., Berge, P., Dransfield, E., Nute, G. R., Enser, M. & Fisher, A. V. (2007). Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. *Meat Science*, **75**, 610-621.
- Sañudo, C., Alfonso, M., Sánchez, A., Delfa, R. & Teixeira, A. (2000b). Carcass and meat quality in light lambs from different fat classes in the EU carcass classification system. *Meat Science*, **56**, 89-94.
- Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., Maria, G., Sierra, I. & Wood, J. D. (2000a). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, **54**, 339-346.
- Sañudo, C., Sanchez, A. & Alfonso, M. (1998). Small ruminant production systems and factors affecting lamb meat quality. *Meat Science*, **49**(Suppl.1), S29-S64.
- Sañudo, C., Santolaria, M. P., María, G., Osorio, M. & Sierra, I. (1996). Influence of carcass weight on instrumental and sensory lamb meat quality in intensive production systems. *Meat Science*, **42**(2), 195-202.
- Schieber, A. (2008). Introduction to food authentication. In: *Modern Techniques for Food Authentication* (edited by D.-W. Sun). Pp. 1-26. Oxford, UK: Elsevier Inc.
- Schönfeldt, H. C., Naudé, R. T., Bok, W., Van Heerden, S. M. & Smit, R. (1993). Flavour- and tenderness-related quality characteristics of goat and sheep meat. *Meat Science*, **34**, 363-379.

- Schreurs, N. M., Lane, G. A., Tavendale, M. H., Barry, T. N. & McNabb, W. C. (2008). Pastoral flavour in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology*, **146**, 193-221.
- Schreurs, N. M., Marotti, D. M., Tavendale, M. H., Lane, G. A., Barry, T. N., Lopez-Villalobos, N. & McNabb, W. C. (2007). Concentration of indoles and other rumen metabolites in sheep after a meal of fresh white clover, perennial ryegrass, or *Lotus corniculatus* and the appearance of indoles in the blood. *Journal of the Science of Food and Agriculture*, **87**, 1042-1051.
- Sink, J. D. & Caporaso, F. (1977). Lamb and mutton flavour: contributing factors and chemical aspects. *Meat Science*, **1**(2), 119-127.
- Spanier, A. M., Flores, M., Toldrá, F., Aristoy, M.-C., Bett, K. L., Bystricky, P. & Bland, J. M. (2004). Meat flavor: contribution of proteins and peptides to the flavor of beef. In: *Quality of Fresh and Processed Foods* (edited by F. Shahidi, A. M. Spanier, C.-T. Ho & T. Braggins). Pp. 33-49. Vol. 542. New York, USA: Kluwer Academic/Plenum Publishers.
- Sutherland, M. M. & Ames, J. M. (1996). Free fatty acid composition of the adipose tissue of intact and castrated lambs slaughtered at 12 and 30 weeks of age. *Journal of Agricultural and Food Chemistry*, **44**, 3113-3116.
- Teixeira, A., Batista, S., Delfa, R. & Cadavez, V. (2005). Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. *Meat Science*, **71**, 530-536.
- Tejeda, J. F., Peña, R. E. & Andrés, A. I. (2008). Effect of live weight and sex on physio-chemical and sensorial characteristics of Merino lamb meat. *Meat Science*, **80**, 1061-1067.
- The Warsaw Voice (TWV). (2012). Regional and Traditional Products: Jagnięcina Podhalańska – Podhale Lamb. The Warsaw Voice official website. [Internet document].
URL <http://www.warsawvoice.pl/WVpage/pages/articlePrint.php/25578/article>. Accessed 29/06/2016.
- Tzanidakis, N., Stefanakis, A. & Sotiraki, S. (2014). Dairy sheep breeding. LowInputBreeds technical note. [Internet document].
URL <http://www.lowinputbreeds.org/lib-technical-notes.html>. Accessed 27/07/2016.
- Ugarte, E., Ruiz, R., Gabiña, D. & Beltrán de Heredia, I. (2001). Impact of high-yielding foreign breeds on the Spanish dairy sheep industry. *Livestock Production Science*, **71**, 3-10.
- Vnučec, I., Držaić, V., Mioč, B., Prpić, Z., Pavić, V. & Antunović, Z. (2014). Carcass traits and meat colour of lambs from diverse production systems. *Veterinarski Arhiv*, **84**(3), 251-263.
- Verbeke, W. & Vackier, I. (2004). Profile and effects of consumer involvement in fresh meat. *Meat Science*, **67**, 159-168.
- Warriss, P. D. (2010). *Meat Science*. 2nd ed. Cambridge, UK: Cambridge University Press.
- Watkins, P. J., Frank, D., Singh, T. K., Young, O. A. & Warner, R. (2013). Sheep meat flavor and the effect of different feeding systems: a review. *Journal of Agricultural and Food Chemistry*, **61**, 3561-3579.

- Watkins, P. J., Kearney, G., Rose, G., Allen, D., Ball, A. J., Pethick, D. W. & Warner, R. D. (2014). Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat. *Meat Science*, **96**, 1088-1094.
- Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., Dunshea, F. R. & Pethick, D. W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat Science*, **86**, 594-599.
- Webb, E. C., Casey, N. H. & Van Niekerk, W. A. (1994). Fatty acids in the subcutaneous adipose tissue of intensively fed SA mutton merino and dorper wethers. *Meat Science*, **38**, 123-131.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Wong, E., Nixon, L. N. & Johnson, C. B. (1975). Volatile medium chain fatty acids and mutton flavor. *Journal of Agricultural Food Chemistry*, **23**(3), 495-498.
- Wood, J. D., Enser, M. B. & Nute, G. R. (1997). An investigation of flavours in meat from sheep grown slowly or more quickly on grass diets. Research project conducted for Ministry of Agriculture Fisheries and Food. Division of Food Animal Science, University of Bristol.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I. & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society*, **58**, 363-370.
- Yarali, E., Yilmaz, O., Cemal, I., Karaca, O. & Taskin, T. (2014). Meat quality characteristics in Kivircik lambs. *Turkish Journal of Veterinary and Animal Sciences*, **38**, 452-458.
- Young, O. A., Berdague, J.-L., Viallon, C., Rousset-Akrim, S. & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, **45**(2), 183-200.
- Young, O. A., Lane, G. A., Podmore, C., Fraser, K., Agnew, M. J., Cummings, T. L. & Cox, N. R. (2006). Changes in composition and quality characteristics of ovine meat and fat from castrates and rams aged to 2 years. *New Zealand of Agricultural Research*, **49**, 419-430.
- Young, O. A., Lane, G. A., Priolo, A. & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food Agriculture*, **83**, 93-104.
- Young, O. A., Reid, D. H. & Scales, G. H. (1993). Effect of breed and ultimate pH on odour and flavour of sheep meat. *New Zealand Journal of Agricultural Research*, **36**, 363-370.

Supplementary data

Table S2.1 Details* of fresh lamb meat registered as either Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO)

Fresh sheep meat	Country of origin	EU scheme	Status	Description	Geographical area	Sheep breeds	Diet	Sensory quality	Slaughter age or live weight	Carcass weight	Official Journal of the European Union source
<i>Abbacchio Romano</i>	Italy	PGI	Registered	Lambs born, reared and slaughtered in the area	The entire territory of the Region of Lazio	Sarda, Comisana, Sopravissana, Massese and Merinizzata Italiana breeds and crosses	Mother's milk and supplementary grazing on natural foods and wild plants. Ewes graze on natural and sown pasture and meadow land and may receive supplementary dried fodder and concentrates.	Meat is light pink and texture fine with small deposits of intramuscular fat	28-40 days	8 kg maximum (with head and red offal)	Official Journal of the European Union (OJEU). (2008). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Abbacchio Romano</i> , EC No: IT-PGI-005-0293-21.05.2003.
<i>Agneau de lait des Pyrénées</i> (Pyrenees suckling lamb)	France	PGI	Registered	Suckling lambs born and reared with mothers raised by traditional methods	Western edge of the Pyrenean chain and its foothills	Manech red head, Manech black head or Basque-Béarnaise (hardy and local dairy sheep breeds)	Solely mother's milk, where the ewes graze freely on high- and medium-altitude natural grassland in spring, summer and autumn, grassland supplemented with preserved fodder, grain cereals, straw and compound feedingstuffs in winter	Meat is white or barely pink, succulent and tender with a low-grain texture. Characteristics are due to milk feeding and young slaughter age of the animals	Up to 45 days	4.5-11 kg	Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Agneau de lait des Pyrénées</i> , EC No: FR-PGI-005-0665-10.12.2007.
<i>Agneau de l'Aveyron</i> (Lamb of Aveyron)	France	PGI	Registered	Lambs born, reared (in a sheep-fold) and slaughtered in the area	Department of Aveyron and adjacent cantons	Lacaune	Mother's milk (70 days minimum) and reared in sheep-fold	Meat is rose-pink, tender and tasty linked to traditional production system and diet		±17 kg	Application for registration, Art. 17, PGI, National file No: IG/32/94
<i>Agneau de Lozère</i> (Lamb of Lozère)	France	PGI	Registered	Lambs (on the threshold between suckling and grass-fed lamb) born, reared and slaughtered in the area	Département de la Lozère (185 municipalities), Département du Cantal (33 municipalities), Département de l'Ardèche (11 municipalities), Département de la Haute Loire (30 municipalities)	Blanche du Massif Central	Mother's milk, grassland or range, supplemented with other feed resources produced from range, grassland, fodder crops or dry fodder	Meat is white to light pink with a fine and firm texture and perfumed with the scent of grass' attributed to the purity of the breed and diversity of the diet	Up to 130 days	7-19 kg	Official Journal of the European Union (OJEU). (2007). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Agneau de Lozère</i> , EC No: FR/PGI/005/0501/06.10.2005.
<i>Agneau de Pauillac</i> (Pauillac lamb)	France	PGI	Registered	Suckling lambs born and reared with mothers raised by traditional methods	Department of Gironde	Ewes: Lacaune meat, Tarasconnaise, Blanche du Massif Central crossed with butcher-quality rams: Bérichon du Cher, Charolais, Suffolk, Rouge de l'Ouest	Mother's milk (formula milk is not allowed), supplemented with cereal concentrates. Ewes graze on natural pasture	Meat is light, with a distinct taste and flavour compared to traditional heavy lamb and milk-fed lamb from dairy farms. Fat is firm and white/slightly pinkish. Qualities due to production system and diet	Up to 80 days	11-15 kg	Official Journal of the European Union (OJEU). (2016). Publication of an amendment application pursuant to Article 50(2)(a) of Regulation (EC) No 1151/2012 on the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs. <i>Agneau de Pauillac</i> , EC No: FR-PGI-0105-01316-24.2.2015.
<i>Agneau de Sisteron</i> (Sisteron lamb)	France	PGI	Registered	Lambs born, reared and slaughtered in the area	Whole of the department of Hautes-Alpes and Alpes de Haute-Provence. Certain cantons of the department of Alpes Maritimes, Var, Bouches du Rhône, Vaucluse and Drôme	Breeding flocks: Mérinos d'Arles, Préalpes du Sud, Mouréous, or crosses of these breeds. Rams for the production of meat: Ile de France, Charollais, Suffolk or Berrichon	Mother's milk (60 days minimum), supplemented with grass and/or fodder, together with a cereal feed supplement	Meat is light pink, non-greasy, fine and smooth textured with a mild flavour attributed to diet	70-150 days	13-19 kg	Official Journal of the European Union (OJEU). (2005). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Agneau de Sisteron</i> , EC No: FR/00316/15.10.2003.
<i>Agneau du Bourbonnais</i> (Bourbonnais lamb)	France	PGI	Registered	Lambs born, reared (in natural grassland meadows) and slaughtered in the area	Department of Allier and adjacent cantons in the Departments of Creuse, Saône et Loire, Cher and Nièvre		Mother's milk and natural fodder, mainly meadow grass	Meat is light red and fat is white and firm. Unique characteristics of meat linked to production system and diet	90-120 days		Application for registration, Art. 17, PGI, National file No: IG/33/94
<i>Agneau du Limousin</i> (Limousin lamb)	France	PGI	Registered	Lambs born, reared and slaughtered in the Limousin region	Limousin region which is made up of the departments of Corrèze, Creuse and Haute-Vienne and cantons of other surrounding departments		Mother's milk (60 days minimum), grass feeding (grazing or preserved hay) with varying quantities of feed supplements for the lambs according to the season and weather conditions	Unique characteristics of meat linked to production system and diet			Official Journal of the European Union (OJEU). (1999). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Agneau du Limousin</i> , National file No: IG/11/95.
<i>Agneau du Périgord</i> (Lamb of Périgord)	France	PGI	Registered	Lambs born and reared within the area in a traditional way: a suckling period with their mothers followed by a finishing period in a sheepfold	Périgord area	Rams: Berrichon, Charollais, Ile de France, Rouge de l'Ouest, Suffolk, and Texel (meat breeds). Ewes: Lacaune viande, Blanche du Massif central (pure-bred hardy breeds) or semi-hardy breeds, i.e. cross between hardy and meat breeds	Mother's milk (for at least 60 days), where the ewes graze on natural or temporary meadows, after which lambs receive specific supplemental feeding with whole or flattened grain and fodder	Meat is white to light pink, tender and juicy with a delicate aroma and flavour of lamb and 'meat-in-the-mouth' texture. Fat is firm and white. Qualities attributed to age and diet	80-180 days	15-21 kg	Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Agneau du Périgord</i> , EC No: FR-PGI-0005-0711-10.07.2008.
<i>Agneau du Poitou-Charentes</i> (Lamb of Poitou-Charentes)	France	PGI	Registered	Lambs born, reared and slaughtered in the area	Poitou-Charentes (Charente, Charente-Maritime, Deux-Sèvres and Vienne) area and extends to neighbouring municipalities	Charmoise (produces lambs with stocky, well-formed legs), Ile de France, Mouton Vendéen Rouge de l'Ouest, Suffolk, Texel	Mother's milk (60 days minimum) and grass	Meat is light pink and texture fine attributed to the tradition of semi-open-air livestock farming	10 months maximum	14-22 kg	Official Journal of the European Union (OJEU). (2003). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Agneau du Poitou-Charentes</i> , EC No: FR/00177/00.12.21.
<i>Agneau du Quercy</i> (Quercy lamb)	France	PGI	Registered	Lambs born, reared (on the dry and arid area of the Causses du Quercy) and slaughtered in the area	Department of Lot and adjoining cantons (excluding Cantal) plus Sarlat in the Dordogne comprising the natural region of Quercy	Causse du Lot	Mother's milk (70 days minimum) and feed available on the dry and arid area of the Causses du Quercy complemented with hay and cereals	Unique characteristics of meat linked to production system and diet	90-180 days		Council Regulation (EEC) No 2081/92, Application for registration, Art. 17, PGI, National file No: IG/34/94
<i>Agnello del Centro Italia</i> (Lamb of Central Italy)	Italy	PGI	Registered	Lambs born, reared and slaughtered in Central Italy	Regions: Abruzzo, Lazio, Marche, Tuscany, Umbria, Emilia-Romagna, including the entire territories of the provinces of Bologna, Rimini, Forlì-Cesena, Ravenna and, to a certain extent, the territories of the provinces of Modena, Reggio Emilia and Parma	Appenninica, Bergamasca, Biellese, Fabrianese, Merinizzata Italiana, Pomarancina, Sopravissana, Zerasca, Comisana, Cornella Bianca, Cornigliese (Corniglio), Garfagnina Bianca, Gentile di Puglia, Massese, Pagliarota, Pecora delle Langhe.	Mother's milk, fodder made up of wild plants from the meadows, sown pasture, pulses and/or grasses	Unique characteristics of meat linked to diet	Up to 12 months		Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Agnello del Centro Italia</i> , EC No: IT-PGI-0005-0806-18.05.2010.
<i>Agnello di Sardegna</i>	Italy	PGI	Registered	Lambs born, reared and slaughtered in Sardinia	Island region of Sardinia	Sarda or first generation crosses with Ile De France and Berrichon Du Cher meat breeds or other highly specialised and well-tested meat breeds	Suckling lambs: solely mother's milk (30-35 days); Light lambs and lambs for cutting: mother's milk (30-35 days), fresh or dried natural feed (fodder and cereals), grasses and native wild and aromatic plants	Meat is white, tender, succulent, fine-textured and firm with a delicate aroma, distinct wild flavour, pleasant taste and small deposits of intramuscular fat linked to the production system and diet		Suckling lambs: 4.5-8.5 kg, Light lambs: 8.5-10 kg, Lambs for cutting: 10-13 kg	Official Journal of the European Union (OJEU). (2014). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Agnello di Sardegna</i> , EC No: IT-PGI-0205-01227-6.5.2014.
<i>Αρνάκι Ελασσόνας</i> (Arnaki Elassonas) (Elassonas lamb)	Greece	PDO	Registered	Suckling lambs born and reared with ewes bearing the phenotypic characters of indigenous Greek breeds	Province of Elassona	Indigenous mainland Greek breeds: Karagouniki, Vahiki, Sarakatsaniki, Boutsiko or crossbreeding between these breeds or with the Greek breeds: Hiotiko, Seron, Mithinis or Ftrizaris	Solely mother's milk, where the ewes graze freely on mountain pastures with grasses, herbs and aromatic plants (above 250 m) and on artificial grassland. Complimentary feedingstuffs, as well as vitamins and minerals, are given for 3-5 months	Meat is white to light pink, tender and juicy. Herbaceous plants give the ewe's milk and, particularly, the lamb's meat a particular and characteristic aroma and taste	30-45 days	6.5-10.5 kg	Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Αρνάκι Ελασσόνας</i> (Arnaki Elassonas), EC No: EL-PDO-0005-0735-14.01.2009.
<i>Barèges-Gavarnie</i>	France	PDO	Registered	Mutton born, reared (spending at least two summers in the mountain pasture) and slaughtered in the area	Municipalities of the department of Hautes-Pyrénées: Barèges, Bepouey, Chèze, Esquièze-Sère, Esterre, Gavarnie, Gèdre, Grust, Luz-St-Sauveur, Saligès, Sassis, Szozs, Sers, Viella, Viey, Viscos, Vicos, and part of the municipality of Cauterets	Barègeoise	Mother's milk until weaned. Winter: hay meadows supplemented with dried fodder, whole or kibbled grain cereals, Spring and autumn: highland areas with pasture, Summer: mountain pastures	Meat is bright red and marbled, but without excessive fat. No strong smell of sheepmeat or wool grease. Qualities due to production system and diet	Ewes: 2-6 years, Castrated males: 18 months minimum	Ewes: 22 kg minimum, Castrated males: 23 kg minimum	Official Journal of the European Union (OJEU). (2006). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Barèges-Gavarnie</i> , EC No: FR/PDO/005/0366/30.7.2003.
<i>Borrego da Beira</i>	Portugal	PGI	Registered			Merino, Churra do Campo and Churra Mondgueira	Spontaneous pastures due to the edaphological-climatic conditions of the region	Unique characteristics of meat linked to production system and diet	40-45 days		Application for registration, Art. 17, PGI, National file No: 51/94
<i>Borrego de Montemor-o-Novo</i>	Portugal	PGI	Registered		Parishes in the subdistrict of Montemor-o-Novo, Évora, Arraiolos and Moura	Merino Branco Regional	Spontaneous Mediterranean pastures			9-12 kg	Application for registration, Art. 17, PGI, National file No: 7/93

Supplementary data

Table S2.1 (Continued)

Fresh sheep meat	Country of origin	EU scheme	Status	Description	Geographical area	Sheep breeds	Diet	Sensory quality	Slaughter age or live weight	Carcass weight	Official Journal of the European Union source
<i>Borrego do Baixo Alentejo</i> (Lamb of Baixo Alentejo)	Portugal	PGI	Registered	Lambs born, reared and slaughtered in the area	Municipalities of Aljustrel, Almodôvar, Alvíto, Barrancos, Beja, Castro Verde, Cuba, Ferreira do Alentejo, Mértola, Moura, Ourique, Serpa, Viana do Alentejo and Vidigueira and certain parishes of the municipalities of Alcácer do Sal, Grândola, Mourão, Odemira, Portel and Santiago do Cacém	Merina and Campaniça and their crosses with other breeds derived from Merino	Mother's milk, natural pasture, feed and woodland in the area	Unique characteristics of meat linked to production system, breed and diet	3-4 months	8-10 kg or 10.1-13 kg	Official Journal of the European Union (OJEU). (1997). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Lamb of Baixo Alentejo</i> , National application No: 75/96.
<i>Borrego do Nordeste Alentejano</i>	Portugal	PGI	Registered	Lambs born, reared and slaughtered in the Northern Region of Alentejo	The communes of Alter do Chão, Arronches, Avis, Campo Maior, Castelo de Vide, Crato, Elvas, Fronteira, Gavião, Marvão, Montorpe, Nisa, Ponte de Sor, Portalegre and Sousel in the district of Portalegre	Merino Branco (pure bred or cross-bred with other breeds)	Mother's milk, natural pastures and local forages among oak groves (plantations of cork-oak, holm oak and oak), supplemented with concentrates	Meat is tender, juicy and has a smooth texture with a unique flavour and small deposits of intramuscular fat linked to the production system and diet	90-120 days	9-13 kg or 13.1-15 kg	Official Journal of the European Union (OJEU). (2002). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Borrego do Nordeste Alentejano</i> , National application No: 80/89.
<i>Borrego Serra da Estrela</i>	Portugal	PDO	Registered	Lambs born, reared and slaughtered in the area	Beira plateau	Bordaleira	Mother's milk, natural pastures composed of spontaneous perennial grasses and cultivated pastures (based on white clover and subterranean clovers)	Unique characteristics of meat linked to production system and diet	Up to 30 days, 12 kg minimum		Application for registration, Art. 17, PDO, National application No: 16/94
<i>Borrego Terrincho</i>	Portugal	PDO	Registered	Lambs born, reared and slaughtered in the area		Churra da Terra Quente	Traditional methods of feeding and rearing on natural pastures, fallow ground, uncultivated land and in woods, arising from the edaphological-climatic conditions peculiar to the region		6-8 weeks, 12 kg	4-8 kg	Application for registration, Art. 17, PDO, National application No: 12/93
<i>Cordeiro Bragançano</i> (Bragança lamb)	Portugal	PDO	Registered	Lambs born, reared and slaughtered in the area	Terra Fria tramontane region	Churra Galega Bragançano	Reared in the region and fed with whole mother's milk		3-4 months		Application for registration, Art. 17, PDO, National application No: 22/94
<i>Cordeiro de Barroso, Anho de Barroso or Cordeiro de leite</i>	Portugal	PGI	Registered	Lambs born, reared and slaughtered in the area	Vila Real district municipalities of Boicás, Chaves, Mondim de Basto, Montalegre, Murça, Ribeira de Pena, Valpaços and Vila Pouca de Aguiar	Crosses of Churra Gelga and Bordaleira de Entre Douro e Minho	Mother's milk and the utilisation of fallow land, natural and improved pastures and the spontaneous vegetation of the Barroso region	Meat is tender, juicy and very tasty, with a typical flavour linked to the production system and diet	30-120 days	4-12 kg	Official Journal of the European Union (OJEU). (2006). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Cordeiro de Barroso, Anho de Barroso or Cordeiro de leite</i> , EC No: PT/00231/6.5.2002.
<i>Cordeiro Mirandês or Canhão Mirandês</i> (Mirandês lamb)	Portugal	PDO	Registered	Lambs of the Churra Galega Mirandesa breed, born and reared in a traditional extensive farming system on the Miranda plateau	Municipalities of Miranda do Douro, Mogadouro and Vimioso and the district of Bragança	Churra Galega Mirandesa	Class A: mothers' milk (suckling lambs), Class B and C: mother's milk, regional flora and supplemented with cereal grains grown on the farm	Meat is pink, extremely tender, succulent and very tasty, with uniform deposits of intramuscular fat and fat which is consistent and does not 'sweat'. Qualities linked to breed and diet	Up to 4 months	Class A: 4-7 kg, Class B: 7.1-10 kg, Class C: 10.1-12 kg	Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Cordeiro Mirandês/Canhão Mirandês</i> , EC No: PT-PDO-0005-0787-12.08.2009.
<i>Cordero de Extremadura</i> (Lamb of Extremadura)	Spain	PGI	Registered	Lambs are born, reared in an extensive grazing system (while suckling, 40-50 days) and finished intensively using concentrates and cereal straw	Extremadura region	For dams: Merino or Merino crossed with Merino Precord, Merino Fleischschaf, and Ile de France, provided that at least half of the lamb's progenitors are Merinos. For sires: pure-bred or simple hybrids of any strains of the Merino breed (Merino, Merino Precord, Merino Fleischschaf, Ile de France and Berrichon du Cher)	Mother's milk, herbaceous pasture and where necessary, feed supplements, composed of straw, grain, fodder, by-products and concentrates whose main constituents are cereals, oilseeds and protein crops	Meat is pink to light pink, succulent and tender with a moderate level of intramuscular fat deposits, pleasant taste and aroma attributed to the extensive and semi-extensive production system	Up to 100 days	Male: 16 kg maximum, Female: 14 kg maximum	Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Cordero de Extremadura</i> , EC No: ES-PGI-0005-0725-09.10.2008.
<i>Cordero de Navarra or Nafarroako Arkumea</i>	Spain	PGI	Registered	Suckling lamb (Lechal) and the light lamb (Ternasco) born, reared and slaughtered in the area	Entire province of Navarra	Lechal: Navarra and Lacha, Ternasco Navarra	Mother's milk, mountain pastures, upland pasture meadows, stubble fields, scrub, polyphytic cultivated meadows and supplemented with dried fodder (alfalfa hay, grass or vetch-oats) and natural feed (cereals and pulses)	Lechal: Meat is pearly white to light pink, tender, very juicy, smooth texture and distinctive flavour. Ternasco: Meat is light pink, tender, very juicy, smooth texture and distinctive flavour with small deposits of intramuscular fat.	Lechal: 25-30 days (Lacha) and 40-45 days (Navarra), Ternasco: 45 days and fattened to 9-12 kg carcass weight	Lechal: 5-8 kg (Lacha) and 6-8 kg (Navarra), (including head and offal), Ternasco: 9-12 kg (excluding head or offal)	Official Journal of the European Union (OJEU). (2006). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Cordero de Navarra/Nafarroako Arkumea</i> , EC No: ES/0212/17.10.2001.
<i>Cordero Manchego</i> (Manchego lamb)	Spain	PGI	Registered	Suckling lambs of the Manchego breed, born and reared for at least 30 days on their mother's milk supplemented by white straw and authorised concentrates	Provinces of Albacete, Ciudad Real, Cuenca and Toledo, in an area comprising the La Mancha, Manchuela, Centro and Almansa districts of Albacete, the Mancha, Campo de Calatrava and Campo de Montiel districts of Ciudad Real, the Manchuela, Mancha Baja and Mancha Alta districts of Cuenca and the La Mancha district of Toledo	Manchego	Mother's milk (30 days minimum), supplemented with white straw and concentrates, and the utilisation of natural resources, flora and meadows, forage crops, fallow land, stubble and coppices	Meat is pale pink, very tender and juicy, with incipient intramuscular fattiness imparting a characteristic, highly pleasant flavour. Qualities linked to the varied diet and special features of the breed	Up to 3 months, 22-28 kg	10-14 kg	Official Journal of the European Union (OJEU). (1998). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Cordero Manchego</i> .
<i>Cordero Segureño</i> (Segureño lamb)	Spain	PGI	Registered	Lambs born, reared under extensive or semi-extensive conditions and slaughtered in the area	Meeting-point of the provinces of Albacete, Almería, Granada, Jaén and Murcia, hedged in by the range of hills known as the Cordilleras Béticas Orientales, characterised by a minimum altitude of 500 metres	Segureño	Mother's milk supplemented with products rich in fibre and spontaneous vegetation native to the area, pasture of stubble fields of cereal and legume crops and, occasionally, irrigated pastureland	Meat is pink, succulent, tender with a good level of fat coverage. Unique characteristics of meat linked to breed and diet		9-13 kg	Official Journal of the European Union (OJEU). (2013). Publication of an application pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Cordero Segureño</i> , EC No: ES-PGI-0005-0871-01.04.2011.
<i>Diepholzer Moorschnucke</i>	Germany	PDO	Registered	Sheep born, reared in the moors and wetlands, and slaughtered in the area	Diepholzer Moorniederung nature reserve which covers the wetlands in the Landkreise of Diepholz and Nienburg (Weser)	Heidschnucke and traditional breeds such as weiße hornlose Moorschnucke	Mother's milk, heather, bent, cotton, grass, sedge, various herbs and grasses, pine, birch, frangula and other woody plants. Supplemented with feed produced on the farm in the winter	Meat is tender, with a characteristic gamey flavour attributable to the production system and typical diet			Council Regulation (EEC) No 2081/92, Application for registration, Art. 17, PDO, EC No: G/DE/1096/26.01.94
<i>Hännilamb</i>	Sweden	PDO	Applied	Lambs born, reared and slaughtered in the Gotland	The island of Gotland with adjacent islands in the Baltic sea	Gutefår	Natural vegetation with herbs (i.e. thyme) and grasses of the Gotland region. Hay/silage and concentrates as supplementary feed	Meat is fine grained, juicy, darker in colour and flavours of butter, metal (liver and blood), herbs, chestnut, gamey and woody (earth, moss and mushrooms), an acidic and naturally salty taste	30-50 kg	±15-30 kg	Official Journal of the European Union (OJEU). (2016). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. <i>Hännilamb</i> , EU No: SE-PDO-0005-01327-21.4.2015.
<i>Isle of Man Manx Loaghtan lamb</i>	United Kingdom	PDO	Registered	Lambs born, reared and slaughtered on the Isle of Man	The Isle of Man	Manx Loaghtan (believed to have been on the island for over one thousand years and ideally adapted to the unique environment of the island)	Mother's milk, characteristic island vegetation of unimproved pasture, gorse and bracken scrub, and heather moorland	Meat is fine grained, less fatty and darker in colour with a distinct gamey flavour	6-15 months	13-18 kg	Official Journal of the European Union (OJEU). (2006). Publication of an application for registration pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Isle of Man Manx Loaghtan Lamb</i> , EC No: UK/00340/05.04.2004.
<i>Jagnięcina podhalańska</i> (Podhalańska lamb)	Poland	PGI	Registered	Suckling lambs born and reared with mothers raised by traditional methods	Part of the Western Carpathians, where the Podhale area is the microregion which forms the centre of the whole production area	Polish Mountain Sheep, Coloured Mountain Sheep and/or Podhale Zackel	Solely mother's milk, where the ewes graze freely on mountain pastures (green fodder) in summer and autumn, hay, hay silage and concentrated feed in winter and early spring	Meat is light pink, succulent with a delicate flavour and characteristic taste and aroma similar to game. Qualities are linked to breed and diet	Up to 2 months	4-8 kg	Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Jagnięcina podhalańska</i> , EC No: PL-PGI-0005-0837-12.11.2010.

Supplementary data

Table S2.1 (Continued)

Fresh sheep meat	Country of origin	EU scheme	Status	Description	Geographical area	Sheep breeds	Diet	Sensory quality	Slaughter age or live weight	Carcass weight	Official Journal of the European Union source
<i>Karoo lamb</i>	South Africa	PGI	Applied (Not shown in DOOR)	Lambs reared extensively and slaughtered in the Karoo region of South Africa	Municipalities (listed by the Karoo Development Foundation) forming the main Karoo region and farms with typical Karoo vegetation located in bordering municipalities	Dorper, South African Mutton Merino, Merino, Dormer, Dohne Merino or preferably meat breed types with good bone to muscle ratio and an even fat distribution	Natural vegetation with fragrant bushes of the Karoo region and cereals, silage or any other natural plant matter as supplementary feed to assist during dry spells and to improve the condition of animals during the reproductive cycle	Meat is tender and has a characteristic herbaceous aroma and flavour due to the natural vegetation of the indigenous Karoo bushes	Up to 12 months	14-31 kg	Department of Trade and Industry (DTI). (2013). Merchandise Marks Act (Act No.17 of 1941). Proposed prohibition on the use of certain words, Pretoria, South Africa: Government Printer.
<i>Lakeland Herdwick</i>	United Kingdom	PDO	Registered	Sheep bred, born, reared and slaughtered in country of Cumbria	Country of Cumbria	Herdwick	Mother's milk, mountain flora, herbage of the fells including grasses, heather and plants such as bilberry. Finished on locally sourced grass, hay or silage	Meat is pink to dark pink, fine grained, succulent and tender with a gamey flavour resulting from slow maturation and a long grazing period	Lambs: 8-12 months, Shearlings: 15-24 months, Mutton: more than 24 months	Lambs: 14-22 kg, Shearlings/mutton: 8 kg minimum	Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Lakeland Herdwick</i> , EC No. UK-PDO-0005-0891-06.09.2011.
<i>Lechazo de Castilla y León</i>	Spain	PGI	Registered	Lambs born, reared and slaughtered in the area	Various agricultural districts in the Autonomous Community of Castilla y León	Churra, Castellana and Ojalada	Solely mother's milk, where the ewes are fed cereals supplemented by natural grazing and stubble	Meat is purely white or light pink, very tender, succulent, smooth in texture with small deposits of intramuscular fat. Qualities due to breed and diet			Official Journal of the European Union (OJEU). (1998). Publication of an application for registration pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. <i>Lechazo de Castilla y León</i> .
<i>Lika janjetina</i> (Lika lamb)	Croatia	PGI	Applied	Lambs born, reared and slaughtered in the area	Lika-Senj County	Lika pramenka	Mother's milk, indigenous pastures in the summer and hay in the winter	Characteristic aroma and flavour linked to the production system and diet	3-4 months, 24-28 kg		Kravnica, M., Bradaš, M., Rogošić, J., Jug, T., Vnučec, I. & Radović, N. M. (2015). Volatile aroma compounds of Lika lamb. <i>MESO: The first Croatian meat journal</i> , 17(3), 264-271 http://hrcak.srce.hr/141126 .
<i>Lüneburger Heidschnucke</i>	Germany	PDO	Registered	Lambs born, reared on the dry heathland of the Lüneburg region and slaughtered in the area	Former principality of Lüneburg (now known as the Lüneburger Heide), covering the rural districts of Celle, Gifhorn, Harburg, Lüchow-Danzenberg, Uelzen, Lüneburg and Sottau-Fallingb. bostel	Heidschnucke	Mother's milk and dry heathland of the Lüneburg region, consisting of woody-stemmed heather and grassland	Characteristic gamey flavour linked to the production system and diet			Council Regulation (EEC) No 2081/92. Application for registration. Art. 17. PDO, EC No: G/D/658/260194
<i>Orkney lamb</i>	United Kingdom	PDO	Registered	Lambs born, reared and slaughtered in Orkney	Group of islands in the North Atlantic off the North Coast of Scotland	North Ronaldsay or cross breed of the Shetland/North Country Cheviot/North Ronaldsay breeds	Mother's milk, seaweed, grass and herbage	Distinct texture and flavour linked to diet (seaweed) with specific characteristics due to the topography, geology and climate of the Orkney Islands			Council Regulation (EEC) No 2081/92. Application for registration. Art. 17. PDO, National file No: 00811
<i>Paška janjetina</i> (Pag lamb)	Croatia	PDO	Applied	Lambs born, reared and slaughtered on the island of Pag	Croatian island of Pag in the northern Adriatic Sea	Island's original Pramenka and Merino ram cross	Mother's milk (reduced) and vegetation growing on the karst, bare rocky ground such as sage, nightingale and stinky short grass sprinkled with salt from the sea	Meat is light pink, tender, tasty and herbaceous due to the nature of its diet (aromatic and medicinal plants)	25-45 days, 7-16 kg	4-10 kg	Official Journal of the European Union (OJEU). (2016). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. <i>Paška janjetina</i> , EU No: HR-PDO-0005-01347-19.6.2015
<i>Prés-salés de la baie de Somme</i> (Lamb of the salt marshes of the Somme)	France	PDO	Registered	Lambs reared on salt marshes for a minimum of 75 days in a manner that respects the natural balance of this environment	Cantons and municipalities surrounding the salt marshes of the Baie de Somme and the Baie d'Authie	Suffolk, Hampshire, Roussin, Ile de France, Route de l'Ouest, Boulonnais and Vendéen	Mother's milk, halophytic pasture of the salt marshes (especially alkali grass) and finished on forage and concentrates	Meat is pink, juicy and produce intense, lasting flavours in the mouth. Fat is firm and white	Up to 12 months (75 days minimum)	16 kg minimum	Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Prés-salés de la baie de Somme</i> , EC No: FR-PDO-0005-0604-11.05.2007.
<i>Prés-salés du Mont-Saint-Michel</i> (Lamb of the salt meadows of Mont-Saint-Michel)	France	PDO	Registered	Lambs reared on salt marshes for a minimum of 70 days in a manner that respects the natural balance of this environment	Cantons and municipalities surrounding the salt marshes of the Mont-Saint-Michel bay	Suffolk, Roussin, Rouge de l'Ouest, Vendéen, Cotentin, Avranchin, Charollais	Mother's milk, halophytic pasture of the salt marshes (plants include puccinelle, troscart and aboune) and finished on forage and concentrates	Meat is pink to dark pink with a marbled appearance, juicy and produce intense, lasting flavours in the mouth, with no taste of wool grease	Up to 12 months	14 kg minimum	Official Journal of the European Union (OJEU). (2013). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. <i>Prés-salés du Mont-Saint-Michel</i> , EU No: FR-PDO-0005-0813-25.06.2010.
<i>Scotch lamb</i>	United Kingdom	PGI	Registered	Lambs are finished (not less than 2 months), slaughtered and dressed in Scotland	Mainland of Scotland including the islands off the West Coast, Orkney and the Shetlands Isles		Mother's milk and grassland and finished in Scotland for a period not less than 2 months	Flavour and tenderness attributed to lambs raised extensively on grassland			Council Regulation (EEC) No 2081/92. Application for registration. Art. 17. PGI, National file No: PGI/00411
<i>Shetland lamb</i>	United Kingdom	PDO	Registered	Lambs born, reared and slaughtered in Shetland	Group of islands in the North Atlantic off the North Coast of Scotland	Shetland or Shetland/Cheviot	Mother's milk, seaweed, grass and herbage	Distinct texture and flavour linked to diet with specific characteristics due to the topography, geology and climate of the Shetland Islands	Up to 12 months	Pure Shetland: 7-14 kg, Crossbred: 20 kg maximum	Council Regulation (EEC) No 2081/92. Application for registration. Art. 17. PDO, National file No: 01211
<i>Ternasco de Aragón</i> (Lamb from Aragón)	Spain	PGI	Registered	Lambs born, reared and slaughtered in the area	Autonomous Community of Aragón	Rasa Aragonesa, Ojinegra de Teruel and Roya Bilbiliana	Mother's milk (50 days minimum), supplemented with white straw and concentrates	Meat is light pink, tender, juicy, soft in texture with small deposits of intramuscular fat. Qualities attributed to production system and diet	70-90 days	8-12.5 kg	Official Journal of the European Union (OJEU). (2012). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Ternasco de Aragón</i> , EC No: ES-PGI-0217-0096-17.03.2010.
<i>Uain Sílibhe Chonamara</i> (Connemara hill lamb)	Ireland	PGI	Registered	Lambs are bred, born and reared in Connemara and light in weight	Area in the West of Ireland known as Connemara	Black faced breed, born and raised in the area	Mother's milk, mountain grasses, sedges, heathers and herbs	Meat is rose red in colour and has a solid deep texture. Taste, flavour and colour linked to local flora grazed	10-14 weeks	±10 kg	Official Journal of the European Union (OJEU). (2006). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Connemara hill lamb/Uain Sílibhe Chonamara</i> , EC No: IE/00366/03.09.2004.
<i>Vadehavslam</i> (Wadden Sea lamb)	Denmark	PGI	Registered	Lambs born and reared in the area	Wadden Sea region of south-west Denmark	Texel breed or Texel crossed with other breeds (Suffolk or Gotland pett)	Mother's milk, plants and grasses in salt meadows, maize, silage and hay supplemented with barley in the finishing period	Distinct salty taste due to grazing on the salty meadows	19-25 kg		Official Journal of the European Union (OJEU). (2011). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Vadehavslam</i> , EC No: DK-PGI-0005-0771-25.03.2008.
<i>Welsh lamb</i>	United Kingdom	PGI	Registered	Lambs bred, born and reared in Wales	Wales	Traditional hardy Welsh breeds (Welsh Mountain, Welsh Mules, Welsh Halfbreeds, Beulah, Welsh Hill Speckled Face, Lleyn Sheep, Llanwenog and Radnor), may be crossed with Texel or Suffolk rams	Mother's milk and grassland (interspersed with heathers and indigenous fragrant wild herbs) which flourishes as a result of the wet and mild Welsh climate	Unique character attributed to Welsh breeds that dominate flock and lambs raised extensively on grassland	Up to 12 months		Official Journal of the European Union (OJEU). (2010). Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. <i>Welsh lamb</i> , EC No: UK-PGI-0105-0081-17.04.2007.
<i>West Country lamb</i>	United Kingdom	PGI	Registered	Lambs born, reared and finished (not less than 2 months) in the West Country region of England	Six counties of Cornwall, Devon, Dorset, Gloucestershire, Somerset and Wiltshire	Polled Dorset and Dorset Horn	Mother's milk and grassland	Specific grass-based diet gives the meat a more richly flavour and creamy coloured fat	Up to 12 months	9-26 kg	Official Journal of the European Union (OJEU). (2013). Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council quality schemes for agricultural products and foodstuffs. <i>West Country lamb</i> , EU No: UK-PGI-0005-0667-21.12.2007.

(PGI) Protected Geographical Indication; (PDO) Protected Designation of Origin; * Details obtained online (http://ec.europa.eu/agriculture/quality/schemes/index_en.htm) from DOOR database (Database of Origin and Registration) under Regulation (EC) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs.

Chapter 3

Variation in the sensory profile of South African Dorper lamb from extensive grazing systems²

Abstract

Descriptive sensory analysis was used to determine the variation in the sensory profiles of extensively produced South African Dorper lamb. The *Longissimus thoracis et lumborum* and subcutaneous fat of lambs from seven different farms in South Africa were assessed by rating its sensory attributes, determining proximate composition and measuring pH, thaw loss, cooking loss and Warner-Bratzler shear force. Discriminant analysis grouped treatments in terms of sensory and physical characteristics. The Northern Cape farms [except for Hantam Karoo/Calvinia (HK/CAL)] clustered closely with less discrimination and were rated highest ($P \leq 0.05$) in lamb-like characteristics, tenderness and juiciness. Mutton-like, sheep wool and herbaceous attributes were prominent for HK/CAL, while Rûens (RU) and Free State (FS) received high ratings for mutton-like, oily and barnyard/kraal attributes. These groupings are likely owing to dietary differences between the regions. The results suggest the possibility of region of origin classification for South African lamb such as Karoo lamb with herbaceous attributes related to a fragrant Karoo plant diet.

Keywords: Aroma; Descriptive sensory analysis; Extensive grazing; Flavour; Lamb; Meat quality

3.1 Introduction

In South Africa, several extensive sheep grazing systems, varying on account of diet, exist. Sheep farming is a dominant enterprise in 82.0% of the Northern Cape province, mainly due to the limitation of alternative farming ventures (Cloete & Olivier, 2010). It also comprises the largest area of the “Karoo”. The Karoo constitutes a variety of different vegetation types, which form part of about 30% of the total area of South Africa (Vorster & Roux, 1983; Bramley *et al.*, 2009). The name Karoo is derived from the indigenous Hottentot name, Karu, which means dry or arid land (Vorster & Roux, 1983). This semi-arid region has a low carrying capacity of less than one large stock unit per 40 ha, where the natural pasture for the lambs varies from grassy, dwarf shrublands (Nama-Karoo biome) to dwarf, succulent shrubs (succulent Karoo biome) (Vorster & Roux, 1983; Cloete & Olivier, 2010). Lamb produced extensively within this region is also known as “Karoo lamb”, which is known for its typical sensory characteristics attributable to the free-ranging conditions and the grazing on fragrant indigenous plants (Karoo bushes) (Estler *et al.*, 2006). Apart from the vast Karoo region, the sheep industry comprises fairly intensive enterprises in the pasture-cropping regions and intensive horticultural areas of South Africa (Cloete & Olivier, 2010). The Swartland (western seaboard) and Overberg (southern seaboard)

² Erasmus, S. W., Hoffman, L. C., Muller, M. & Van der Rijst, M. (2016). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems, *Small Ruminant Research*, **144**, 62-74. <http://dx.doi.org/10.1016/j.smallrumres.2016.07.020>.

regions of the Western Cape have a typical Mediterranean climate, where sheep production is coordinated with winter grain cropping (Cloete & Olivier, 2010). In the Overberg region, lucerne/alfalfa (*Medicago sativa*) is typically cultivated in the pasture phase and serves as feed for sheep. Small grain stubble is another characteristic feed of the region, which may also form part of the diet (Cloete & Olivier, 2010). Although less known, lamb produced within this region is known as “Rûens lamb”, where the typical diet of the sheep produced within the Rûens shale renosterveld region gives the lamb meat its unique sensory qualities. Sheep farming is also practised in the Free State grasslands region. This region consists of plains with summer rainfall and vegetation mainly consisting of Kimberley thornveld (Savanna biome) and to a lesser extent that of the Western Free State clay grassland (grassland biome) (Cloete & Olivier, 2010). Variation in the sensory profile of sheep meat is expected across South Africa based on the dietary differences linked to the variation in vegetation within the regions.

The effect of diet on the sensory characteristics of sheep meat is widely documented (Rousset-Akrim *et al.*, 1997; Fisher *et al.*, 2000; Young *et al.*, 2003; Fraser *et al.*, 2004; Almela *et al.*, 2010; Resconi *et al.*, 2010). Increased mutton and sweet-associated flavour attributes have been associated with pasture-fed lambs (Rousset-Akrim *et al.*, 1997; Young *et al.*, 2003). Priolo *et al.* (2002) and Borton *et al.* (2005) found a high lamb flavour in stall-fed lambs compared to grass-fed lambs, while the grass-fed also had more liver and less fatty flavours. Lucerne-fed lambs have received high ratings for oily or fattiness (Young *et al.*, 2003). Opposing results also exist as Fraser *et al.* (2004) found that pasture-type has no significant effect on meat flavour. It is suggested that the variation in diet can alter the composition of the meat, affecting the aroma and flavour precursors, which consist of compounds derived from the muscle tissue, fat tissue and intramuscular fat (Madruga & Mottram, 1995; Spanier *et al.*, 2004).

The effect of diet on the fatty acid composition of ruminant meat is largely responsible for the variation in sensory profiles. Sheep finished on pasture or fed linseed oil had higher concentrations of linolenic acid (C18:3 *n*-3) with high sensory scores for lamb flavour, and low scores for abnormal flavour (i.e. taints) (Fisher *et al.*, 2000; Nute *et al.*, 2007). However, sheep finished on concentrate or fed protected lipid supplement (PLS) had higher concentrations of linoleic acid (C18:2 *n*-6) with low scores for lamb flavour, and high scores for abnormal flavour. These results are consistent with other research findings (Fisher *et al.*, 2000; Sañudo *et al.*, 2000; Elmore *et al.*, 2005). The transfer of some plant secondary metabolites such as condensed tannin and terpenes from forage to meat and fat have also been studied (Priolo *et al.*, 2004; Schreurs *et al.*, 2008). The latter and other volatiles obtained from the diet directly influence the aroma and flavour of meat. Cornu *et al.* (2001) suggest the use of terpenes, from natural grasslands and subsequently found in meat, as food tracers or markers of the geographical origin.

Within South Africa, the Merino is the dominant sheep breed closely followed by the Dorper (Cloete & Olivier, 2010). Although breed has been known to influence flavour (Sink & Caporaso, 1977; Young *et al.*, 1993; Fisher *et al.*, 2000), Cloete *et al.* (2012) found no sensory differences ($P > 0.05$) in the sensory attributes of the meat from Merino, Dohne Merino, South African Mutton Merino (SAMM) and Dorper rams and ewes.

This was likely since all the sheep were raised on the same planted pasture diet, resulting in very similar fatty acid profiles and hence similar sensory characteristics. It was essential to select one breed of sheep for the purpose of this study as Brand (2000) concluded that the grazing habits between breeds (i.e. Merino and Dorper) grazing natural pastures vary. Contrary to the findings of Cloete *et al.* (2012), this could result in dietary and consequently sensory differences in the meat.

There is limited published results on the sensory profile of South African Dorper lamb from extensive grazing systems. It is essential to explore how diet, linked to the typical vegetation of the farm, affects the sensory profile of the meat and fat. Thus, the aim of this chapter was to determine the variation in sensory profiles of South African Dorper lambs from farms with different vegetation types raised under extensive grazing systems. Lambs from farms within the Northern Cape, Free State and Western Cape provinces of South Africa were included in the study.

3.2 Materials and methods

3.2.1 Experimental layout

Dorper lambs ($n = 10$) were sourced from seven different farms in South Africa (Chapter 1, Fig. 1.2), each unique in terms of its vegetation and the extensive grazing conditions. Each farm represents a treatment where ten slaughter-ready lambs were sourced. Lambs were raised extensively on natural vegetation within the vicinity of the farm. Plant samples were collected from the farms after the lambs were slaughtered. Plants were collected from the vicinity last grazed before death or, when the field was depleted, from a field similar to that of the original grazing area (less than 1 km away). The farmers were questioned upon the typical diet which the animal followed during its course of life and the provision of any additional feed. The seven treatments and plant species collected from the farms are listed in Table 3.1.

3.2.2 Study farms

Five of the seven farms selected were located within the Northern Cape province (CK, NK, HK/LO, KV, HK/CAL). CK and NK fall in the Nama-Karoo biome, HK/LO and HK/CAL in the succulent Karoo biome and KV mainly in the fynbos biome. The sheep selected from a farm within the Overberg (Western Cape) region (RU) were extensively raised on lucerne/alfalfa (*Medicago sativa*) situated within the fynbos biome and known as the Rûens shale renosterveld. The other farm selected was from the Free State grasslands region (FS). The vegetation mainly consisted of Kimberley thornveld (Savanna biome) and to a lesser extent that of the Western Free State clay grassland (grassland biome).

Table 3.1 Lamb treatments (farms) selected for this study on DSA

Treatment (Area of origin)	Code	Class (No.)	Plant species collected from area
Carnarvon (Central Karoo)	CK	A2 (n = 10)	<i>Galenia sarcophylla</i> ; <i>Lycium cinereum</i> ; <i>Pentzia incana</i> ; <i>Plinthus karrooicus</i> ; <i>Ruschia intricata</i> ; <i>Salsola glabrescens</i> ; <i>Stipagrostis ciliata</i> ; <i>Stipagrostis obtusa</i>
Prieska (Northern Karoo)	NK	A2 (n = 10)	<i>Eriocephalus ericoides</i> ; <i>Felicia muricata</i> ; <i>Fingerhuthia africana</i> ; <i>Lycium cinereum</i> ; <i>Pentzia incana</i> ; <i>Phaeoptilum spinosum</i> ; <i>Psilocalon absimile</i> ; <i>Pteronia glauca</i> ; <i>Rhigozum trichotomum</i> ; <i>Rosenia humilis</i> ; <i>Ruschia intricata</i> ; <i>Salsola aphylla</i> ; <i>Salsola calluna</i> ; <i>Salsola tuberculata</i> ; <i>Stipagrostis ciliata</i> ; <i>Stipagrostis obtusa</i> ; <i>Zygophyllum gilfillanii</i>
Loeriesfontein (Hantam Karoo)	HK/LO	A2 (n = 10)	<i>Chrysanthemoides incana</i> ; <i>Pentzia incana</i> ; <i>Pteronia sordida</i> ; <i>Salsola tuberculata</i> ; <i>Stoeberia beetzii</i> ; <i>Tetragonia fruticosa</i> ; <i>Zygophyllum lichtensteinianum</i>
Nieuwoudtville (Knersvlakte)	KV	A2 (n = 10)	<i>Chaetobromus dregeanus</i> ; <i>Ereiodium moschatum</i> ; <i>Eriocephalus punctulatus</i> ; <i>Galenia africana</i> ; <i>Lebeckia leipoldtiana</i> ; <i>Medicago polymorpha</i> ; <i>Nylandtia spinosa</i> ; <i>Pentzia incana</i> ; <i>Wiborgia monoptera</i> ; <i>Wiborgia sericea</i>
Calvinia (Hantam Karoo)	HK/CAL	A2 (n = 5) A3 (n = 5)	<i>Chrysocoma ciliata</i> ; <i>Drosanthemum hispidum</i> ; <i>Eberlanzia ferox</i> ; <i>Eriocephalus ericoides</i> ; <i>Justica orchioides</i> ; <i>Lycium spp.</i> ; <i>Mesembryanthemum vaginatum</i> (<i>Brownanthus</i> or <i>Ruschia vaginatum</i>); <i>Pentzia incana</i> ; <i>Pentzia sphaerocephala</i> ; <i>Salsola calluna</i> ; <i>Zygophyllum lichtensteinianum</i>
Swellendam (Rûens)	RU	A2 (n = 10)	<i>Cynodon dactylon</i> ; <i>Medicago sativa</i>
Boshof (Free State)	FS	A2 (n = 10)	<i>Aristida congesta subsp. congesta</i> ; <i>Cynodon dactylon</i> ; <i>Eragrostis lehmanniana</i> ; <i>Eragrostis superba</i> ; <i>Fingerhuthia africana</i> ; <i>Heteropogon contortus</i> ; <i>Schmidtia kalihariensis</i> ; <i>Themeda triandra</i>

(DSA) Descriptive sensory analysis; (No.) Number of samples; A2: (A) no permanent incisor teeth, (2) fat depth measures 1.0-4.0 mm (lean); A3: (A) no permanent incisor teeth, (3) fat depth measures 4.1-7.0 mm (medium). The fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline in sheep (DAFF, 1990).

3.2.3 Sampling and slaughtering

The day before slaughter, sheep from each farm were transported to the nearest abattoir. Slaughtering took place according to standard South African procedures and regulations (DAFF, 2000). In order to minimize variation, ten carcasses were randomly selected according to age (class A, no permanent incisor teeth), fatness (class 2 or 3) and weight (less than 20 kg). In South Africa carcasses are classified according to age, fat, conformation and damage classes as described under the Product Standards Act No.119 of 1990 and its regulations (DAFF, 1990; DAFF, 2006). The classification of fat is when the carcasses are classified into different classes according to the amount of fat measured. The fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline in sheep. The fat depth for class 2 carcasses measures 1.0-4.0 mm (lean), while class 3 measures 4.1-7.0 mm (medium).

After slaughter the carcasses were cooled at 4°C for 24 h. Carcasses were weighed and the subcutaneous fat thickness measured using an electronic handheld calliper. The *Longissimus thoracis et lumborum* (LTL) were excised from the right and left side of the carcasses. Subcutaneous fat and visible sinews were removed from both muscles. The fat was vacuum-packed and stored at -20°C until analysis. The right LTL were vacuum-packed and stored at -20°C for sensory analysis, while the left LTL were divided

so that the *Longissimus lumborum* (LL) was homogenised for chemical analyses and the *Longissimus thoracis* (LT) was vacuum-packed, stored at -20°C and used during the training phase of the sensory analyses. Two of the ten carcasses sourced were randomly selected and used for the training of the descriptive panel, while the remaining eight were used for the actual testing phase. According to Ranken (1992), at -18°C the storage life of properly packed lamb is 6-10 months. The current samples were stored for less than 6 months. Therefore, minor changes in the sensory quality of the meat is expected after it is stored under vacuum at -20°C .

3.2.4 Sample preparation

Descriptive sensory analysis was performed on the eight fat, as well as the eight meat samples per treatment (seven farms). Sample selection occurred randomly (within each farm) for each of the eight test sessions, where seven fat and seven meat treatments were rated for attribute intensity during each session. Fat and meat samples per test session were from the same carcass. The vacuum-packed and frozen fat and meat samples were thawed for 24 h in a refrigerator ($2-4^{\circ}\text{C}$) before each of the pre-determined sensory analysis sessions.

3.2.4.1 Lamb fat preparation

Each piece of subcutaneous fat strip was cut in half and placed in coded oven roasting bags (GLAD® Medium 250 mm x 400 mm). The fat samples were roasted in a preheated industrial forced convection oven (Hobart CSD 1012, France) at 160°C for 30 min. Approximately 7.5-10 mL melted fat together with two to three squares (1.5 cm^2) of solid fat, cut from the fat tissue, were transferred to glass ramekins with randomly assigned three-digit codes. Each ramekin contained fat per treatment. The ramekins were reheated in a preheated industrial convection oven (Hobart CSD 1012, France) at 100°C for 5 min before the commencement of each sensory analysis session.

3.2.4.2 Lamb meat preparation

The meat samples were placed in individual coded oven roasting bags (GLAD® Medium 250 mm x 400 mm) and positioned on a stainless steel grid which was fitted on a stainless steel oven roasting pan. A thermocouple probe, attached to a handheld digital temperature monitor (Hanna Instruments, Bellville, South Africa), was inserted into the centre of each meat sample (AMSA, 1995). The samples were roasted at 160°C in two conventional electric Defy 835 ovens connected to a computerised electronic temperature control system (Viljoen *et al.*, 2001). When the samples reached an internal temperature of 70°C (AMSA, 1995) they were immediately removed from the oven and left to cool for 15 min remaining in their roasting bags. The samples were then removed from the roasting bags, blotted dry and cut into 1.2 cm^3 cubes, perpendicular to the fibre direction. The cubes were cut from the LL of the LTL. In order to minimise variation, only the centre cubes were used with the dryer outsides being trimmed off. The cubes were individually wrapped in aluminium foil and placed into glass ramekins with randomised three-digit codes. Each ramekin contained three wrapped

meat cubes per treatment. Reheating of the samples took place in a preheated industrial forced convection oven (Hobart CSD 1012, France) at 70°C for 8 min before the start of each session. Throughout the sensory analyses no salt (NaCl) or any form of seasoning was added to the samples.

3.2.4.3 Temperature maintenance

A number of measures were taken to ensure that the temperature of the samples remained as constant as possible. The ramekins were reheated and served to the panel simultaneously. As soon as the ramekins were removed from the oven they were covered with petri-dish lids, to prevent loss of volatiles (particularly from the fat), and placed onto porcelain mugs filled halfway with warm water. Throughout the sensory analysis sessions the mugs were kept in scientific waterbaths with electronic temperature control (set to 70°C) and water circulation (Scientific Manufacturing Company, Cape Town, South Africa). The panel immediately assessed the fat and meat samples once all the ramekins were positioned in the waterbath.

3.2.5 Descriptive sensory analysis

3.2.5.1 Sensory panel

The sensory panel consisted of eight female judges, ranging from 23 to 65 years of age. They were selected based on their availability, interest and experience. The panellists had extensive experience of descriptive sensory analysis, particularly the sensory analysis of meat.

3.2.5.2 Panel training

Training of the panellists ensued according to the guidelines of the American Meat Science Association (AMSA, 1995) together with the consensus method as described by Lawless and Heymann (2010). The background and objectives of the study were explained to the panel members after which the first training sessions commenced. Eight training sessions were performed. During the first two sessions a preliminary list of sensory attributes were generated for both the meat and fat samples, while reference samples were finalised for the most recurring sensory descriptors. Reference standards are very important as they provide the panellists with a clear concept and understanding of the sensory attributes in question (Munoz & Civille, 1998). The reference samples, shown in Table 3.2, were illustrative of the aroma and flavour attributes associated with typical South African Dorper lamb meat.

Eleven plant samples, commonly eaten by sheep from the farms, were also included as reference samples (Table 3.3). These were presented to the panel in ISO wine tasting glasses covered with a plastic lid to prevent loss of volatiles. The panellists were instructed to remove the plastic lid and evaluate the aroma of the plants. Dominant aroma attributes were identified and a final list of descriptors was compiled. These descriptive words helped with the generation of relevant terminology for the sensory attributes of the various fat and meat samples, particularly with regard to the herbaceous attribute (Table 3.3).

Table 3.2 Reference standards used for DSA to illustrate specific sensory attributes

Standards	Class	Type	Diet	Aroma/flavours
Feedlot lamb	A2	Meat and fat	Intensive, Feedlot	Lamb fat, Lamb meat
Feedlot mutton	AB2	Meat and fat	Intensive, Feedlot	Mutton fat, Mutton meat
Free-range lamb from Sutherland ^a	A2/A3	Meat and fat	Extensive, Natural veld	Lamb fat, Lamb meat, Herbaceous
Free-range mutton	B2	Meat and fat	Extensive, Natural veld	Mutton fat, Mutton meat
Beef	N/A	Meat and fat	N/A	Beef
Venison/Fallow deer	N/A	Meat	Extensive, Natural veld	Gamey
Lamb liver	N/A	Organ	N/A	Livery, Metallic
Beef liver	N/A	Organ	N/A	Livery, Metallic

(DSA) Descriptive sensory analysis; ^aLamb from Sutherland fall within the Roggeveld region (Mucina & Rutherford, 2006) and produce very herbaceous lamb meat; A2: (A) no permanent incisor teeth, (2) fat depth measures 1.0-4.0 mm (lean); A3: (A) no permanent incisor teeth, (3) fat depth measures 4.1-7.0 mm (medium); AB2: (AB) at least one but not more than two permanent incisors, (2) fat depth measures 1.0-4.0 mm (lean); B2: (B) at least three but not more than six permanent incisors, (2) fat depth measures 1.0-4.0 mm (lean); The fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline in sheep (DAFF, 1990).

Table 3.3 Descriptors generated for the plant samples during the training phase of DSA

Names	Scientific names	Descriptors from literature ^a	Descriptors generated by panel
Ankerkaroo (Anchor Karoo)	<i>Pentzia incana</i>	Sage-like, Eucalyptus, Lavender, Bitter	Eucalyptus, Camphor, Pine, Thyme, Sage-like, Medicinal, Lavender, Rosemary, Floral, Minty, Sweet, Bitter
Silwerkaroo (Silver Karoo)	<i>Plinthus karrooicus</i>	Dusty, Woody, Damp, Camomile	Dry flowers, Camomile, Woody, Sweet, Sweet, Dry hay, Candle wax
Kapokbossie (Kapok bush or Wild rosemary)	<i>Eriocephalus ericoides</i>	Woody, Bitter, Eucalyptus, Lavender, Minty	Dried fruit, Dried sausage Spice, Tobacco, Thyme, Woody, Minty, Eucalyptus
Rivierganna (Ganna bush)	<i>Salsola glabrescens</i>	Dusty, Sandy, Soapy, Woody, Damp/Musty	Cayenne Pepper, Sheep wool, Tobacco, Ceylon tea, Dusty, Woody
Perdekaroo	<i>Rosenia humilis</i>	Sage, Lavender, Camomile	Spice, White pepper, Mild eucalyptus, Dry thyme, Coriander, Cumin, Fennel Seeds, Sweet
Boegoekaroo (Buchu Karoo)	<i>Pteronia mucronata</i>		Pine, Parsley, Lemon, Eucalyptus, Black pepper, Bitter
Kriedoring (Honey-thorn)	<i>Lycium cinereum</i>		Tobacco, Rooibos, Fish-like, Urine
Vyebossie	<i>Ruschia spinosa</i>		Dried/Fresh fig, Fig peel
Driedoring (Three-thorn)	<i>Rhigozum trichotomum</i>		Dusty, Hay, Musty
Lucerne (Alfalfa)	<i>Medicago sativa</i>		Cereal-like/Bran flakes, Animal feed, Sweet
Bushman grass	<i>Stipagrostis obtusa</i>		Sweet, Thatch, Grassy

(DSA) Descriptive sensory analysis; ^aLeighton *et al.* 2007.

Throughout each training session the panellists received fat and meat samples from six reference standards, cubes of liver from two reference standards, as well as fat and meat samples from the seven treatments. The fat was only assessed for aroma, whereas the meat was assessed for aroma, flavour and texture. Five of the eight treatment samples used for training, represented the samples used for the testing phase. During the course of training the panel derived several sensory attributes of which a final list was decided upon. The final list consisted of 32 terms (14 aroma descriptors, 14 flavour descriptors and 4 texture descriptors) as described in Table 3.4. Reference standards were not required for all of the derived attributes as the panel were already calibrated or had reached consensus for the sensory experience associated with each attribute.

A score sheet was then developed which the panel used to scale the intensity of each attribute on a 100-point unstructured scale. Product specific scaling was used to rate attribute intensities as this has been found to be the best scaling methods for panels that evaluate a single product category (Munoz and Civille, 1998). The panel practised intensity rating and established the maximum and minimum intensity values of the individual attributes during the final training sessions.

Table 3.4 Definition and scale of final aroma^a, flavour^b, and texture attributes used for DSA

Attribute	Definition
Lamb meat	Roasted lamb meat aroma/flavour
Mutton meat	Roasted mutton meat aroma/flavour
Lamb fat	Roasted lamb fat aroma/flavour
Mutton fat	Roasted mutton fat aroma/flavour
Oily	Oily aroma/flavour when roasting lamb/mutton
Herbaceous	Aroma/flavour typical of Karoo bushes and herbs, i.e. rosemary and thyme
Savoury/Spicy	Savoury spice aroma/flavour typical of boerewors spice, i.e. coriander, pepper, nutmeg
Sweet associated	Sweet-associated aroma/flavour
Gamey	Strong game aroma/flavour
Livery	Aroma/flavour typical of lamb liver
Metallic	Flat aroma/flavour associated with metal cans and bloody/raw meat
Sheep wool	Aroma/flavour typical of sheared sheep wool
Barnyard/Kraal	Aroma/flavour typical of livestock aromas
Rancid	Aroma/flavour typical of oxidised fats and oils
Initial juiciness	Amount of fluid exuded when pressed between thumb and forefinger
Sustained juiciness	Impression formed after first 5 chews using molar teeth
Tenderness	Impression of tenderness after the first 5 chews using molar teeth
Residue	Amount of residue left in mouth after 10 chews, using molar teeth

(DSA) Descriptive sensory analysis. ^{ab} Aroma and flavour were analysed (orthonasally and retronasally, respectively) for fat and meat samples using the following scale: 0 = None, 100 = Prominent. Texture was analysed for meat samples using the following scales: 0 = Dry, 100 = Juicy (Initial and sustained juiciness); 0 = Tough, 100 = Tender (Tenderness); 0 = None, 100 = Abundant (Residue).

3.2.5.3 Intensity rating of sensory attributes

The panel rated the intensities of the 32 attributes of the seven treatments of fat and meat samples using unstructured unipolar line scales anchored with zero and 100 (AMSA, 1995). Intensity ratings were performed in a complete randomised order. One session were conducted per day over a period of 8 days. Panellists were seated in individual tasting booths fitted with computers containing the software programme *Compusense five*® (*Compusense*, Guelph, Canada). The fat samples were analysed for aroma (orthonasally) during the first 20 min of each session. This was followed with a 10 min break, after which the meat samples were assessed for aroma (orthonasally), flavour (retronasally) and texture. The panel were instructed to assess the three meat cubes as follows: to rate the aroma of the first meat cube by taking a few short sniffs as the foil wrapping was removed. The same cube was then tested for initial juiciness by rating the amount of fluid exuded when pressed between the thumb and forefinger (0 = dry, 100 = juicy). The second cube was masticated for the assessment of flavour, while the third cube was rated for texture (Table 3.4). The sensory analysis sessions took place inside a temperature-controlled (21°C) and light-controlled (artificial daylight) room with positive airflow to ensure the removal of residual odours (AMSA, 1995). Panellists received distilled water (21°C), apple slices and water biscuits (Carr, UK) to cleanse and refresh their palates between samples.

3.2.6 Physical analyses and proximate composition

3.2.6.1 pH

The pH of the eight meat samples per treatment was measured at the 13th rib position of the right LTL muscles, after thawing the meat for 24 h at 2-4°C, using a Crison pH 25 handheld portable pH meter with an automatic temperature adjuster (Lasec Pty Ltd, Cape Town, South Africa). The pH meter was calibrated each day with standard buffers (pH 4.0 and pH 7.0) supplied by the manufacturer.

3.2.6.2 Thaw and cooking loss

Thaw loss of each muscle was calculated as the difference in sample weight before and after defrosting, expressed as a percentage of the original weight of the sample. Cooking loss was determined by weighing the muscles before and after the cooking process and expressing the difference in weight as a percentage of the original weight of the raw, defrosted samples (AMSA, 1995).

3.2.6.3 Shear force

The Warner Bratzler shear force test (WBSF) was used to analyse the instrumental shear force of the cooked meat samples (Honikel, 1998). This tenderness measurement was performed to simulate biting and mastication. Readings were taken 72 h after refrigerated storage (2-4°C) of the cooked meat samples (from the LT of the LTL). Three adjacent 1 cm x 1 cm rectangular meat strips were cut parallel to the muscle fibre direction from the centre of the samples. The meat strips were cut to obtain a total of six rectangular cubes with a length of 2 cm per cube. An Instron Universal Testing Machine (Instron UTM, Model 2519-107) attached

with a Warner-Bratzler (WB) fitting, a 1 mm thick triangular (V-notch) blade with a semi-circular cutting edge (radius of 0.508 mm), was used (Voisey, 1976). The maximum shear force values required to shear a sample of cooked muscle perpendicular to the muscle fibre longitudinal axis (at a crosshead speed of 200 mm/min) was recorded for each sample in Newton (N). The mean of the readings from the six cubes was used for statistical analyses.

3.2.6.4 Proximate composition

The moisture and ash content (g/100 g) was determined according to AOAC Official Method 934.01 and Method 942.05, respectively (AOAC, 2002). The intramuscular fat (IMF) content (g/100 g) was determined on a 5 g muscle sample using a chloroform/methanol (2:1, v/v) extraction (Lee *et al.*, 1996). The total crude protein content (g/100 g) was determined with the Dumas (Nitrogen combustion) method 992.15 (AOAC, 2002). A 0.15 g defatted, dried (at 60°C for 24 h) and finely ground meat sample encapsulated in a Leco™ foil sheet was analysed using a Leco Nitrogen/Protein Analyser (FP-528, Leco Corporation). Dumas measures total nitrogen (%) including inorganic fraction (i.e. nitrate and nitrite) (Chang, 2010). This is multiplied with a conversion factor of 6.25 (100 g protein/16 g N) since meat protein is assumed to contain 16% nitrogen. EDTA (ethylenediamine tetra acetate) (Leco Corporation, part number 502-092, lot number 1055, 3000 Lakeview Avenue, St. Joseph, USA, MI 49085-2396, USA) was used as the standard for the calibration of the nitrogen analyzer throughout the analyses to ensure accuracy and recovery rate of each sample.

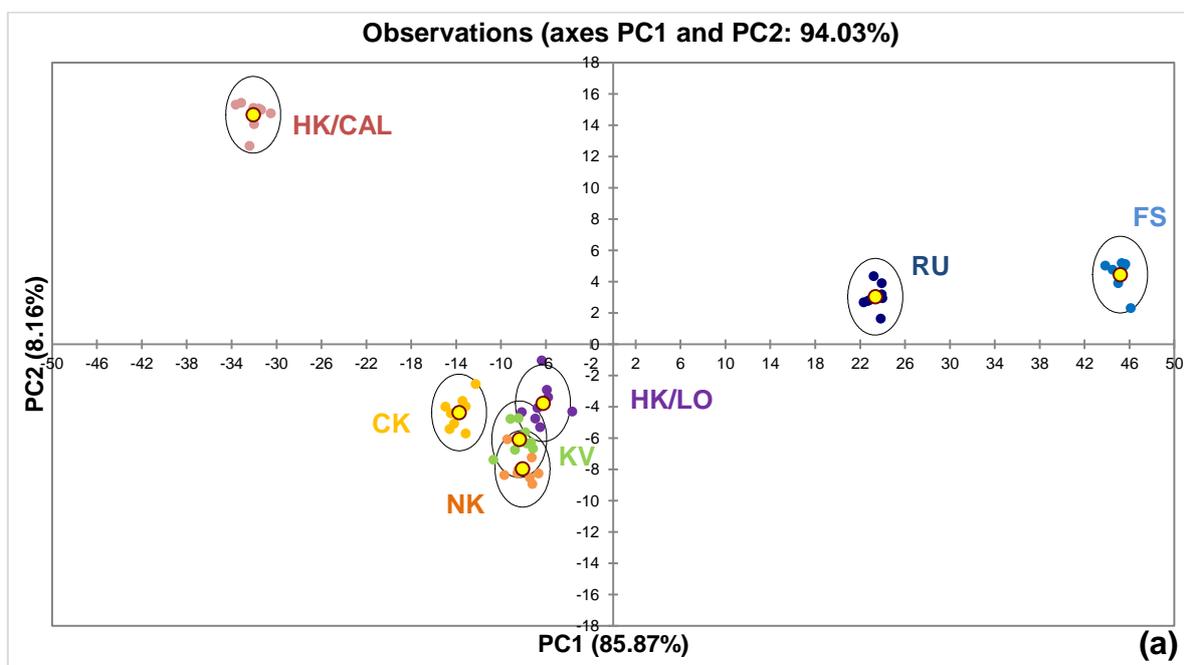
3.2.7 Statistical analysis

The experimental setup for descriptive sensory analysis as a test technique consisted of a completely randomised design with seven treatments (7 farms) and eight random replications (i.e. fat and meat samples from each carcass) per treatment. During the testing phase, the performance of the panel was monitored with *PanelCheck* Software (Version 1.4.0, www.panelcheck.com) (Næs *et al.*, 2010). Panel reliability was finally tested by subjecting the data to test-retest analysis of variance (ANOVA) using the GLM (General Linear Models) procedure of SAS™ statistical software (Statistical Analysis System, Version, 9.2, 2006, SAS Institute Inc., Cary, NC, USA). Further pre-processing of the sensory and physical data involved using the Shapiro-Wilk test to test for non-normality of residuals (Shapiro and Wilk, 1965). When non-normality was significant ($P \leq 0.05$), outliers in the data were identified and residuals greater than 3 were removed. Following the confirmation of panel reliability and normality, all data were subjected to one-way analysis of variance (ANOVA) with treatment as factor using SAS™. Fisher's Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Correlations between the sensory attributes and physical characteristics were determined by means of the Pearson's correlation coefficient (r) (Snedecor & Cochran, 1980). Associations between the sensory and physical data were illustrated with principal component analysis (PCA) and discriminant analysis (DA) (Næs *et al.*, 2010). The latter multivariate analyses were performed using XLSTAT® statistical software (Version 2014.2.03; Addinsoft, New York, USA).

3.3 Results and discussion

3.3.1 Aroma and flavour

The discriminant analysis (DA) plot of the sensory and physical data is illustrated in Figure 3.1. The DA plot was used to visualise the observations and analyse the differences and relationships between groups. The DA plot indicates that the treatments are separated. Principal component (PC) 1 and 2 explained 94.03% of the total variance of which PC1 and PC2 explained 85.87% and 8.16%, respectively. Figure 3.1a clearly shows that CK, NK, HK/LO, KV and HK/CAL are separated from RU and FS in terms of the sensory and physical characteristics. Along PC1, the former treatments (all from the Northern Cape) grouped in the left quadrants, while the latter (from the Western Cape and Free State) grouped in the right quadrants. The discriminant loadings plot (Fig. 3.1b) illustrates the discrimination of the attributes and its association with the different treatments indicated in the DA plot (Fig. 3.1a). Along PC1, RU and FS associate with oily, barnyard/kraal, sheep wool, savoury/spicy and mutton-like attributes, as well as the increase in shear force and residue. On the contrary, CK, NK, HK/LO and KV associate with herbaceous, savoury/spicy, lamb-like, as well as an increase in tenderness, with CK, NK, HK/LO and KV grouped closely (Fig. 3.1a). The overlapping of treatments (NK, HK/LO and KV) indicates that they were very similar in terms of sensory and instrumental meat quality and barely distinguishable. When the division of the left quadrants (along PC2) are taken into consideration, it is evident that the separation of HK/CAL from the other treatments is driven by its strong association with herbaceous, gamey, sheep wool and mutton-like attributes. The other treatments show less association with these and more with lamb-like attributes, tenderness, initial juiciness and sustained juiciness.



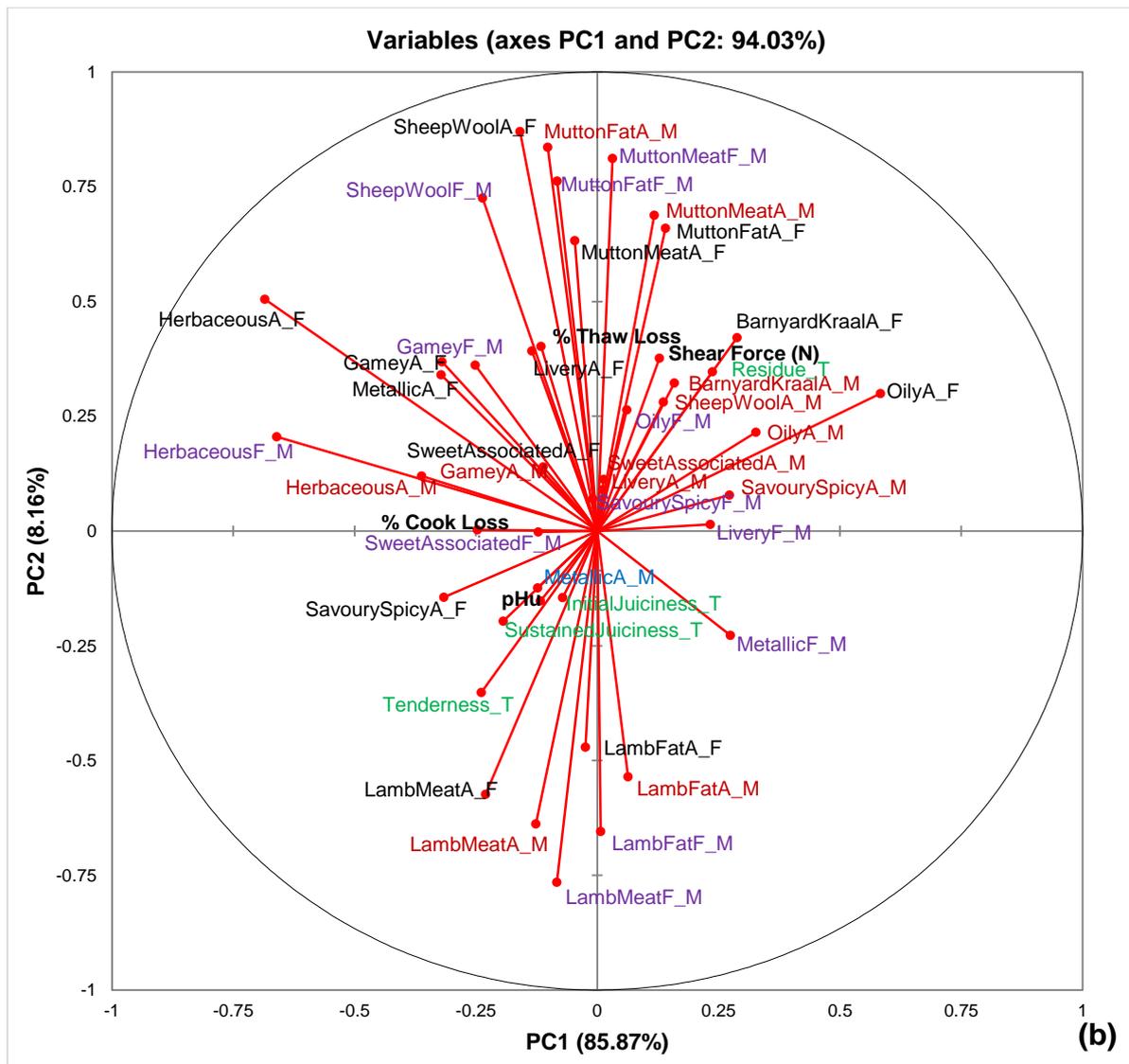


Figure 3.1 Discriminant analysis (DA) plot (a) and DA variable loadings plot (b) of the sensory attributes and physical characteristics of lamb meat and fat as affected by origin. (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) HantamKaroo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State. The letters following the attribute descriptors “A”, “F”, and “T” refer to aroma, flavour and texture, respectively and the last letter of the attribute descriptors “F” and “M” refer to fat and meat, respectively.

A principal component analysis (PCA) bi-plot which demonstrates the correlation between the different sensory attributes for the lamb meat and fat, as well as their association with other physical characteristics, is illustrated in Figure 3.2. The combination of PC1 and PC2 explained 40.55% of the total variance of which PC1 explained 28.43% and PC2 explained 12.13%. Even though only 40.55% of the variation is described, one should always bear in mind that various intrinsic and extrinsic factors (i.e., age, gender etc.) could influence the sensory profile. The PCA scores plot (Fig. 3.2a) indicates that there are definite trends between the treatments. Similarly to the DA plot, CK, NK, HK/LO and KV (except for HK/CAL) are separated from RU and FS along PC1. The same associations with the different attributes, as described for the DA plot, are also seen. The Pearson correlation coefficients (r) for lamb fat aroma, showed significant ($P = < 0.0001$) and strong

negative correlations between the following attributes: lamb meat and mutton meat ($r = -0.906$); lamb meat and mutton fat ($r = -0.873$); lamb fat and mutton meat ($r = -0.877$); lamb fat and mutton fat ($r = -0.891$). These correlations suggest that treatments with high scores for lamb-like attributes, would likely have low scores for mutton-like attributes, as seen in Figure 3.1 and 3.2 for CK, NK, HK/LO, KV and HK/CAL as opposed to RU and FS.

From both DA (Fig. 3.1) and PCA (Fig. 3.2) plots it is important to note the grouping of the farms according to the regions from which they were sourced. These groupings are likely as a result of dietary differences between the regions. Lamb from farms within the Northern Cape are of particular interest as Karoo lamb is known to originate from this region, where the meat is known for its excellent quality and unique flavour (Weissnar & Du Rand, 2012).

The sensory means with standard deviations (\pm SD) for the sensory attributes of the seven different lamb fat and meat treatments are presented in Table 3.5 and 3.6, respectively. Treatment had a significant effect ($P \leq 0.05$) on all, except six (i.e. sweet-associated and rancid aroma of the fat and meat, metallic aroma and flavour of the meat) of the attributes.

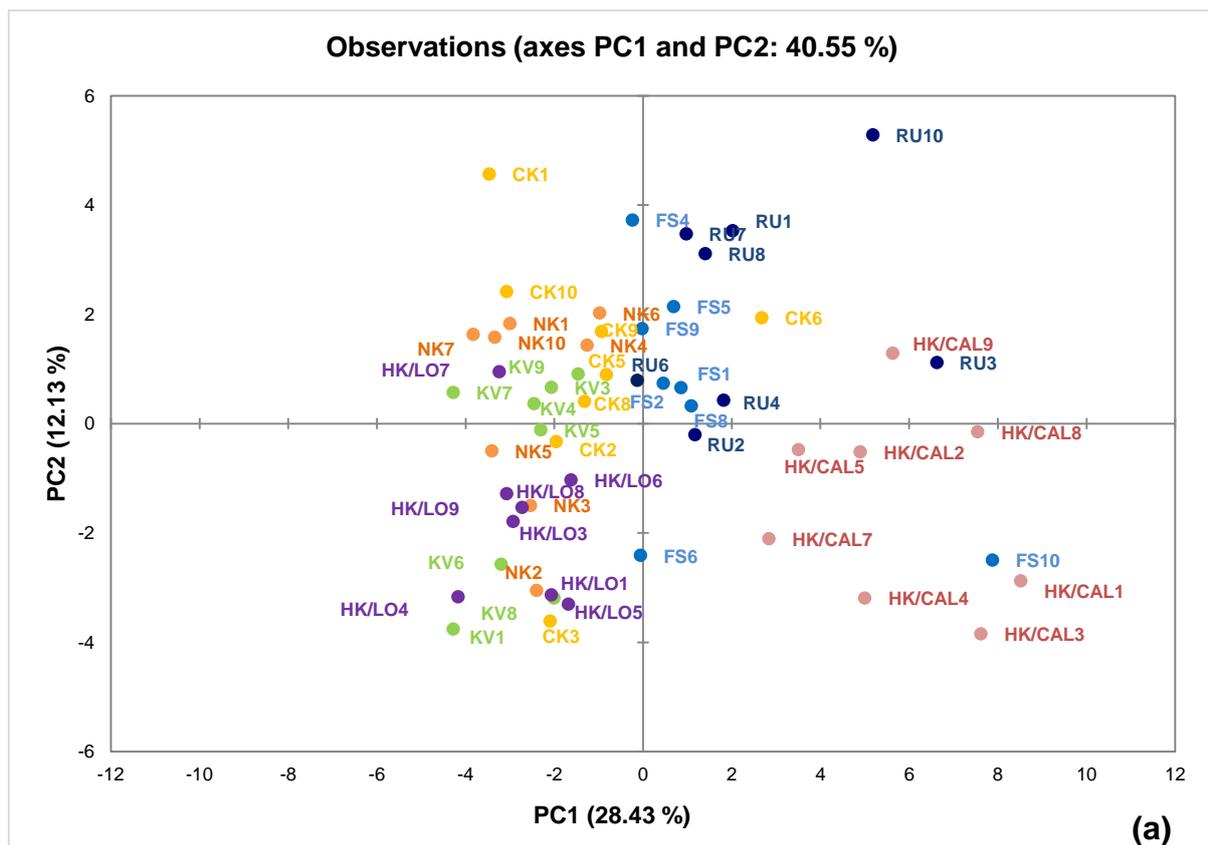


Table 3.5 Mean scores (\pm SD) for the sensory attributes of lamb fat aroma as affected by origin

Attributes	CK	NK	HK/LO	KV	HK/CAL	RU	FS	LSD
Lamb meat aroma	39.3 ^a \pm 11.47	42.9 ^a \pm 14.14	47.7 ^a \pm 11.35	43.1 ^a \pm 9.21	25.4 ^b \pm 10.72	26.5 ^b \pm 6.91	26.3 ^b \pm 10.52	10.9
Mutton meat aroma	18.8 ^{bcd} \pm 9.13	13.1 ^{de} \pm 12.76	9.0 ^e \pm 8.63	14.3 ^{cde} \pm 2.56	34.4 ^a \pm 9.39	22.8 ^{bc} \pm 8.50	24.1 ^b \pm 9.03	9.5
Lamb fat aroma	27.2 ^{abc} \pm 8.78	31.7 ^a \pm 8.26	34.6 ^a \pm 7.21	31.5 ^a \pm 7.66	19.1 ^c \pm 8.13	27.7 ^{ab} \pm 7.35	22.0 ^{bc} \pm 10.32	8.3
Mutton fat aroma	14.7 ^{cd} \pm 6.74	10.4 ^d \pm 9.86	8.8 ^d \pm 7.84	12.6 ^d \pm 6.83	31.1 ^a \pm 7.00	22.0 ^{bc} \pm 11.39	27.6 ^{ab} \pm 9.46	8.7
Oily aroma	21.8 ^d \pm 5.50	28.2 ^c \pm 6.73	30.5 ^{bc} \pm 7.61	26.0 ^{cd} \pm 3.26	28.4 ^c \pm 3.28	35.7 ^{ab} \pm 5.76	38.6 ^a \pm 6.71	5.8
Herbaceous aroma	17.4 ^b \pm 4.77	12.5 ^{cde} \pm 3.67	13.2 ^{bcd} \pm 3.64	13.9 ^{bc} \pm 5.85	28.5 ^a \pm 2.70	8.9 ^e \pm 4.15	9.2 ^{de} \pm 3.75	4.2
Savoury/Spicy aroma	34.2 ^a \pm 4.94	33.0 ^a \pm 4.50	33.6 ^a \pm 3.53	34.6 ^a \pm 4.37	33.6 ^a \pm 2.80	27.9 ^b \pm 4.10	31.4 ^{ab} \pm 5.43	4.3
Sweet-associated aroma	17.0 ^a \pm 2.00	16.1 ^a \pm 2.30	15.4 ^a \pm 2.96	17.2 ^a \pm 2.84	17.7 ^a \pm 1.87	15.7 ^a \pm 1.31	16.5 ^a \pm 3.47	2.5
Gamey aroma	0.4 ^b \pm 0.66	0.6 ^b \pm 0.90	0.9 ^b \pm 1.31	1.1 ^{ab} \pm 0.93	2.1 ^a \pm 1.54	0.8 ^b \pm 1.19	0.9 ^b \pm 1.21	1.1
Livery aroma	0.4 ^b \pm 0.66	0.5 ^b \pm 0.85	0.5 ^b \pm 0.85	0.0 ^b \pm 0.00	1.4 ^a \pm 1.08	0.9 ^{ab} \pm 1.31	0.5 ^{ab} \pm 1.06	0.9
Metallic aroma	0.6 ^{ab} \pm 0.90	0.5 ^b \pm 0.85	0.7 ^{ab} \pm 1.01	0.8 ^{ab} \pm 0.89	1.6 ^a \pm 0.92	0.6 ^{ab} \pm 0.77	1.5 ^{ab} \pm 1.86	1.1
Sheep Wool aroma	3.2 ^c \pm 3.92	3.9 ^c \pm 3.45	1.7 ^c \pm 2.13	2.3 ^c \pm 2.67	20.9 ^a \pm 2.88	8.2 ^b \pm 6.43	8.3 ^b \pm 3.54	3.8
Barnyard/Kraal aroma	1.0 ^c \pm 1.23	1.1 ^c \pm 1.05	0.8 ^c \pm 1.17	1.0 ^c \pm 1.48	3.6 ^{bc} \pm 2.22	6.9 ^a \pm 6.02	4.2 ^{ab} \pm 3.22	2.9
Rancid aroma	0.3 \pm 0.71	0.0 \pm 0.00	0.2 \pm 0.43	0.2 \pm 0.45	0.0 \pm 0.00	0.0 \pm 0.00	0.2 \pm 0.51	0.4

(SD) Standard Deviation; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State; (LSD) Least significant difference at the 5% level of significance; ^{a-e} Values in the same row with different superscripts are significantly different ($P \leq 0.05$); Means determined by a 100-point unstructured line scale (0 = low intensity; 100 = high intensity).

Table 3.6 Mean scores (\pm SD) for the sensory attributes of lamb meat aroma (A), flavour (F) and texture (T) as affected by origin

Attributes	A/F/T	CK	NK	HK/LO	KV	HK/CAL	RU	FS	LSD
Lamb meat	A	49.0 ^{ab} \pm 11.50	50.4 ^{ab} \pm 6.82	53.9 ^a \pm 3.81	51.1 ^a \pm 7.79	33.0 ^d \pm 7.25	42.5 ^{bc} \pm 10.63	37.4 ^{cd} \pm 8.75	8.46
	F	48.9 ^a \pm 8.99	54.1 ^a \pm 6.61	56.8 ^a \pm 8.35	54.9 ^a \pm 7.99	23.4 ^c \pm 10.59	32.0 ^{bc} \pm 8.46	37.2 ^b \pm 12.92	9.37
Mutton meat	A	7.5 ^{cd} \pm 9.23	4.4 ^d \pm 6.18	6.5 ^d \pm 2.49	4.0 ^d \pm 5.07	22.3 ^a \pm 6.81	13.7 ^{bc} \pm 7.05	19.9 ^{ab} \pm 9.32	6.98
	F	8.0 ^c \pm 7.08	7.1 ^c \pm 5.43	8.4 ^c \pm 3.74	6.7 ^c \pm 6.87	37.3 ^a \pm 8.80	25.3 ^b \pm 9.60	22.3 ^b \pm 12.68	8.24
Lamb fat	A	14.2 ^{bc} \pm 4.17	15.7 ^{ab} \pm 3.04	18.4 ^a \pm 1.23	15.4 ^{ab} \pm 3.21	10.1 ^d \pm 2.06	11.7 ^{cd} \pm 2.62	14.1 ^{bc} \pm 5.66	3.43
	F	14.8 ^{ab} \pm 3.80	15.5 ^{ab} \pm 3.01	15.3 ^{ab} \pm 1.41	16.5 ^a \pm 4.64	7.7 ^b \pm 4.55	11.8 ^b \pm 3.69	12.3 ^b \pm 4.41	3.81
Mutton fat	A	2.6 ^{cd} \pm 3.64	1.4 ^d \pm 2.65	1.1 ^d \pm 1.31	1.4 ^d \pm 2.18	10.7 ^a \pm 3.13	5.9 ^{bc} \pm 4.42	6.9 ^b \pm 4.65	3.35
	F	2.9 ^{cd} \pm 2.80	1.6 ^d \pm 1.59	2.8 ^{cd} \pm 2.07	2.8 ^{cd} \pm 3.06	14.7 ^a \pm 5.29	10.0 ^b \pm 5.55	6.1 ^{bc} \pm 5.46	4.03
Oily	A	1.1 ^b \pm 0.95	1.3 ^b \pm 0.92	1.5 ^b \pm 0.84	2.2 ^b \pm 1.73	2.2 ^b \pm 1.79	2.5 ^{ab} \pm 1.43	3.8 ^a \pm 1.99	1.45
	F	2.1 ^b \pm 2.38	3.5 ^{ab} \pm 3.86	3.6 ^{ab} \pm 1.67	2.9 ^{ab} \pm 2.03	4.5 ^{ab} \pm 2.07	4.6 ^a \pm 2.66	3.2 ^{ab} \pm 1.89	2.47
Herbaceous	A	13.8 ^{bc} \pm 4.70	15.4 ^{ab} \pm 3.08	18.8 ^a \pm 3.29	13.4 ^{bc} \pm 4.69	18.0 ^a \pm 3.33	10.1 ^c \pm 3.97	13.7 ^{bc} \pm 3.82	3.91
	F	17.4 ^b \pm 5.27	16.8 ^{bc} \pm 4.37	22.0 ^a \pm 2.61	18.0 ^b \pm 3.21	24.7 ^a \pm 3.80	13.7 ^{cd} \pm 1.31	11.8 ^d \pm 2.60	3.54
Savoury/Spicy	A	24.9 ^{abc} \pm 2.83	25.3 ^{abc} \pm 2.10	27.5 ^a \pm 2.05	24.1 ^{bc} \pm 3.06	24.6 ^{bc} \pm 3.19	25.6 ^{abc} \pm 1.75	27.2 ^{ab} \pm 3.87	2.80
	F	24.1 ^c \pm 2.88	26.1 ^{abc} \pm 1.92	27.6 ^a \pm 2.45	26.1 ^{ab} \pm 3.16	27.0 ^{ab} \pm 1.40	24.8 ^{bc} \pm 3.31	27.0 ^{ab} \pm 2.35	2.59
Sweet-associated	A	12.8 ^a \pm 1.19	13.6 ^a \pm 2.32	14.3 ^a \pm 2.13	12.8 ^a \pm 0.94	14.0 ^a \pm 2.56	12.8 ^a \pm 2.53	14.4 ^a \pm 1.66	2.01
	F	14.8 ^{ab} \pm 1.63	16.0 ^{ab} \pm 2.73	16.4 ^{ab} \pm 1.46	16.5 ^a \pm 2.61	16.0 ^{ab} \pm 2.33	14.4 ^b \pm 1.51	15.4 ^{ab} \pm 1.76	2.08

Table 3.6 (Continued)

Attributes	A/F/T	CK	NK	HK/LO	KV	HK/CAL	RU	FS	LSD
Gamey	A	1.4 ^{ab} ± 1.19	1.1 ^b ± 1.98	2.7 ^a ± 1.77	2.5 ^{ab} ± 1.40	2.3 ^{ab} ± 1.08	2.0 ^{ab} ± 1.56	1.3 ^{ab} ± 1.21	1.50
	F	2.1 ^{ab} ± 1.99	2.3 ^{ab} ± 2.50	3.0 ^a ± 1.59	0.9 ^b ± 1.06	3.6 ^a ± 1.48	2.6 ^{ab} ± 2.30	1.9 ^{ab} ± 1.64	1.86
Livery	A	4.1 ^a ± 2.16	2.0 ^b ± 1.50	5.0 ^a ± 2.08	3.3 ^{ab} ± 2.23	3.5 ^{ab} ± 1.91	3.5 ^{ab} ± 1.51	3.2 ^{ab} ± 2.21	1.98
	F	5.5 ^c ± 2.59	6.1 ^{bc} ± 2.14	9.0 ^a ± 3.14	5.1 ^c ± 2.31	5.7 ^{bc} ± 2.50	6.3 ^{bc} ± 2.76	8.2 ^{ab} ± 2.89	2.65
Metallic	A	3.5 ^a ± 2.33	3.6 ^a ± 1.77	3.7 ^a ± 1.81	3.9 ^a ± 1.47	3.0 ^a ± 1.95	3.4 ^a ± 2.53	3.0 ^a ± 1.30	1.93
	F	5.0 ^a ± 3.50	6.6 ^a ± 2.45	5.0 ^a ± 0.59	6.2 ^a ± 3.08	4.3 ^a ± 2.64	5.9 ^a ± 2.64	6.7 ^a ± 1.96	2.57
Sheep wool	A	1.1 ^b ± 1.35	1.1 ^b ± 1.04	2.5 ^{ab} ± 1.32	1.6 ^b ± 1.46	3.9 ^a ± 3.00	2.1 ^{ab} ± 0.85	2.3 ^{ab} ± 2.08	1.73
	F	1.7 ^c ± 1.73	1.3 ^c ± 1.19	1.9 ^c ± 1.01	1.4 ^c ± 1.42	8.0 ^a ± 3.79	4.2 ^b ± 1.98	2.6 ^{bc} ± 1.46	2.00
Barnyard/Kraal	A	0.3 ^{ab} ± 0.63	0.2 ^{ab} ± 0.51	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.8 ^{ab} ± 1.46	0.5 ^{ab} ± 0.72	1.0 ^a ± 1.66	0.93
	F	0.0 ^b ± 0.00	0.2 ^{ab} ± 0.51	0.2 ^{ab} ± 0.45	0.0 ^b ± 0.00	0.6 ^a ± 0.79	0.2 ^{ab} ± 0.51	0.5 ^{ab} ± 0.71	0.51
Rancid	A	0.0 ^a ± 0.00	0.0 ^a ± 0.00	0.0 ^a ± 0.00	0.2 ^a ± 0.43	0.0 ^a ± 0.00	0.0 ^a ± 0.00	0.0 ^a ± 0.13	0.17
	F	0.1 ^b ± 0.25	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.1 ^b ± 0.35	0.3 ^{ab} ± 0.57	0.6 ^a ± 0.64	0.0 ^b ± 0.00	0.37
Initial juiciness	T	34.4 ^{ab} ± 8.21	33.8 ^b ± 8.89	34.7 ^{ab} ± 4.47	41.7 ^a ± 7.90	33.8 ^b ± 7.32	30.4 ^b ± 8.93	35.0 ^{ab} ± 7.21	7.73
Sustained juiciness	T	41.4 ^b ± 5.21	41.4 ^b ± 11.67	44.3 ^{ab} ± 6.11	51.7 ^a ± 5.99	42.2 ^b ± 4.80	37.4 ^b ± 8.72	40.9 ^b ± 7.80	7.56
Tenderness	T	46.0 ^{bc} ± 6.69	56.1 ^{ab} ± 11.84	62.1 ^a ± 8.64	61.7 ^a ± 9.52	47.2 ^b ± 10.25	35.5 ^c ± 14.32	46.7 ^b ± 12.41	10.84
Residue	T	28.6 ^b ± 6.11	26.0 ^{bc} ± 7.85	18.4 ^c ± 4.94	19.8 ^c ± 3.44	30.3 ^{ab} ± 8.79	36.9 ^a ± 11.13	30.5 ^{ab} ± 11.71	8.26

(SD) Standard Deviation; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knervlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State; (LSD) Least significant difference at the 5% level of significance; ^{a-d} Values in the same row with different superscripts are significantly different ($P \leq 0.05$); Means determined by an 100 point unstructured line scale (0 = low intensity; 100 = high intensity).

Table 3.7 Means (\pm SD) for the carcass characteristics, physical characteristics and proximate composition (g/100 g) of lamb meat

	Treatments							LSD
	CK	NK	HK/LO	KV	HK/CAL	RU	FS	
Carcass characteristics								
Carcass weight (kg)	17.8 ^{abc} \pm 1.88	19.1 ^a \pm 0.84	18.2 ^{ab} \pm 0.65	16.3 ^d \pm 0.89	16.7 ^{cd} \pm 1.50	17.3 ^{bcd} \pm 1.26	17.7 ^{bc} \pm 1.94	1.22
Fat thickness rib ¹ (mm)	1.8 ^b \pm 0.99	1.8 ^b \pm 0.87	3.2 ^a \pm 0.87	2.0 ^b \pm 0.47	2.1 ^b \pm 0.63	2.5 ^{ab} \pm 0.93	1.8 ^b \pm 0.92	0.77
Fat thickness lumbar ² (mm)	2.5 ^c \pm 0.84	3.4 ^{bc} \pm 1.29	5.2 ^a \pm 1.49	3.7 ^b \pm 1.02	5.4 ^a \pm 1.20	3.8 ^b \pm 0.65	3.0 ^{bc} \pm 0.83	0.98
Physical characteristics								
pH	5.84 ^a \pm 0.12	5.62 ^b \pm 0.04	5.66 ^b \pm 0.06	5.61 ^b \pm 0.07	5.62 ^b \pm 0.08	5.63 ^b \pm 0.16	5.64 ^b \pm 0.10	0.10
% Thaw loss	10.1 ^{bc} \pm 1.43	10.6 ^{bc} \pm 1.05	10.9 ^{abc} \pm 1.37	9.5 ^c \pm 1.28	12.4 ^a \pm 1.65	11.6 ^{ab} \pm 1.74	10.3 ^{bc} \pm 2.31	1.60
% Cooking loss	33.3 ^{abc} \pm 3.64	36.8 ^a \pm 6.15	34.0 ^{abc} \pm 2.59	29.7 ^c \pm 4.63	35.0 ^{ab} \pm 2.56	32.1 ^{bc} \pm 5.38	30.8 ^{bc} \pm 5.38	4.55
WBSF (N)	46.6 ^b \pm 5.83	34.7 ^{de} \pm 5.88	30.0 ^e \pm 3.83	36.4 ^d \pm 4.18	44.4 ^{bc} \pm 6.43	52.7 ^a \pm 9.28	39.8 ^{cd} \pm 5.17	6.02
Proximate composition								
Moisture	76.8 ^a \pm 0.66	75.3 ^b \pm 1.38	75.1 ^b \pm 0.67	75.4 ^b \pm 0.76	76.3 ^a \pm 0.74	76.2 ^a \pm 0.63	75.3 ^b \pm 0.95	0.77
Protein	19.6 ^c \pm 0.58	20.8 ^a \pm 1.24	20.5 ^{ab} \pm 0.91	20.0 ^{bc} \pm 0.70	20.4 ^{ab} \pm 0.65	20.3 ^{abc} \pm 0.82	20.4 ^{ab} \pm 0.96	0.80
Fat	2.1 ^d \pm 0.44	2.0 ^d \pm 0.83	3.2 ^{ab} \pm 0.74	3.8 ^a \pm 0.80	2.2 ^d \pm 0.46	2.5 ^{cd} \pm 0.72	3.1 ^{bc} \pm 0.89	0.66
Ash	1.2 ^{bc} \pm 0.05	1.3 ^b \pm 0.07	1.4 ^a \pm 0.05	1.2 ^c \pm 0.04	1.1 ^d \pm 0.03	1.1 ^d \pm 0.07	1.2 ^c \pm 0.08	0.05

(SD) Standard Deviation; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State; ¹ Fat thickness measured at the 25 mm off midline of spine at 13th rib; ² Fat thickness measured between 3rd and 4th lumbar vertebrae; (LSD) Least significant difference at the 5% level of significance; (WBSF) Warner-Bratzler shear force; ^{a-e} Values in the same row with different superscripts are significantly different ($P \leq 0.05$).

According to Sink and Caporaso (1977) and Melton (1990), diet can affect the flavour intensity of sheep meat. Pasture type and condition had a significant effect on flavour as leafy and fast growing lucerne (*Medicago sativa*) produced an intense “sharp” and “sickly” aroma and flavour in meat, making it less acceptable compared to meat produced from more stem-like plants (Park *et al.*, 1972). Mutton-like attributes also tend to correlate positively with attributes such as herbaceous, sheep wool, oily and barnyard/kraal (Fig. 3.2). Pearson correlation coefficients (r) were particularly evident in the fat phase with mutton meat and mutton fat aroma having positive correlations with sheep wool aroma ($r = 0.663$; $P < 0.0001$ and $r = 0.722$; $P < 0.0001$, respectively) and barnyard/kraal aroma ($r = 0.494$; $P = 0.0001$ and $r = 0.625$; $P < 0.0001$, respectively) (Fig. 3.2). Pertaining to the negative barnyard/kraal aroma of the fat, RU and FS had the highest scores (6.9 ± 6.02 and 4.2 ± 3.22 , respectively), differing significantly ($P \leq 0.05$) from NK, CK, KV and HK/LO (Table 3.5). However, the barnyard/kraal aroma and flavour differences in the meat were very low. Scoring less than one on a 100-point scale, it is questionable whether its contribution is relevant towards the sensory profile of the meat (Table 3.6). A more distinguishing difference in scores were seen for sheep wool aroma of fat as HK/CAL scored highest (20.9 ± 2.88), significantly more than the rest, followed by FS and RU (8.3 ± 3.54 and 8.2 ± 6.43 , respectively) which did not differ from each other ($P > 0.05$) (Table 3.5). Sheep wool aroma and flavour in the meat had a similar observation to that of the fat. Medium chain length methyl-branched chain fatty acids (BCFA) and 3-methyl indole (skatole) have been linked to descriptors such as “sheep meat”, “animal” (the odour of confined livestock) and “rancid” (Rousset-Akrim *et al.*, 1997; Young *et al.*, 1997). These compounds have also been implicated in the distinctive flavours of lamb raised on lucerne (Melton, 1990; Devincenzi *et al.*, 2014) and grass (Young *et al.*, 1997), similar to that of RU and FS. Devincenzi *et al.* (2014) also found an increase in the intensity of “animal” aroma and flavour (associated with lamb chop high in skatole concentration) of lamb meat raised on a lucerne supplemented diet. Pastoral flavour is a general term associated with attributes such as “grassy”, “animal”, “barnyard”, “faecal”, “milky”, “sheepy”, “fatty”, “dairy”, “rancid” and “oily” (Schreurs *et al.*, 2008). Pastoral flavour has been attributed to the presence of specific compounds absorbed and/or metabolised from the stomach and deposited in the meat and fat of ruminants. Some consist of unaltered components of the plants (i.e. chlorophyll and secondary plant metabolites), while others (i.e. sulphur compounds, phenols, skatole and indole) originate from the fermentation of forage protein in the rumen (Schreurs *et al.*, 2008). Production of these compounds in the rumen depend on the type of dietary protein present since protein in fresh forage diets is more rapidly solubilised and degraded in the rumen than protein in grain and concentrate diets (Schreurs *et al.*, 2008). It is possible that RU, FS and HK/CAL contained higher concentrations of the aforementioned compounds, owing to its high rating for sheep wool, oily and barnyard/kraal attributes (Table 3.5 and 3.6).

Studies suggest that cooked meat from domesticated animal species (i.e. beef, pork and lamb) has a similar flavour profile, however, its species-specific flavour is derived from lipid oxidation products due to intermuscular fat (IMF) content (Berg & Walker, 2004; Pegg & Shahidi, 2004; Calkins & Hodgen, 2007; Watkins *et al.*, 2013). Rousset-Akrim *et al.* (1997) also stated that sheep fat is the true source of its species-specific

aroma and flavour. Schönfeldt *et al.* (1993) reported significant increases in the tenderness and species related flavour of cooked lamb meat with increasing carcass fatness. Similar results were seen for lamb meat and fat from the central regions of South Africa (i.e. the Karoo). HK/LO had a significantly high ($P \leq 0.05$) value for carcass weight (18.2 ± 0.65 kg) and the highest values for fat thickness at the 13th rib (3.2 ± 0.87 mm) and 3rd and 4th lumbar vertebrae (5.2 ± 1.49 mm) along with IMF content ($3.2 \pm 0.74\%$) (Table 3.7). Together with the increase in carcass fatness it had significantly high species-related (i.e. lamb-like) aroma and flavour scores for both fat and meat (Table 3.5 and 3.6). Similarly, Tshabalala *et al.* (2003) found that the overall flavour intensity of sheep meat increased because of its higher fat content. However, a different trend was seen for the latter two treatments. Although NK had the heaviest carcass weight (19.1 ± 0.84 kg), it had the lowest IMF content ($2.0 \pm 0.83\%$), while KV had the highest ($P \leq 0.05$) IMF content ($3.8 \pm 0.80\%$) with the lightest carcass weight (16.3 ± 0.89 kg) and reduced fat thickness (Table 3.7).

FS and RU lamb fat had a significantly ($P \leq 0.05$) higher oily aroma (38.6 ± 6.71 and 35.7 ± 5.76 , respectively) than HK/CAL, NK, KV and CK (Table 3.5). The oily aroma (3.8 ± 1.99) of the meat was highest for FS, significantly ($P \leq 0.05$) more than the rest except for RU (Table 3.6). Oily flavour (4.6 ± 2.66) was again the highest for RU, but not significantly more ($P > 0.05$) than the rest, except for CK ($P \leq 0.05$) (Table 3.6). The oily aroma (of fat) correlated weakly with mutton fat aroma ($r = 0.299$; $P = 0.025$), whereas oily flavour correlated positively with mutton fat flavour ($r = 0.324$; $P = 0.015$) (Fig. 3). These differences could be as a result of the fatty acid composition, since meat with a high concentration of linoleic acid has a sweet and oily flavour (Wood *et al.*, 1999). The increase in polyunsaturated fatty acid (PUFA) levels may also cause flavour changes as they are more susceptible to lipid oxidation and the generation of abnormal volatile compounds during cooking (Wood *et al.*, 1999; Geldenhuys *et al.*, 2014). The intensity of sensory attributes may also increase when linolenic acid levels are raised in meat due to grass or pasture feeding (Young *et al.*, 2003).

Even though carcasses were selected according to age, fatness and weight, variation may still occur between treatments. It is known that sheep fed concentrate-based diets have higher average daily gains than those on pasture diets (Priolo *et al.*, 2002). Therefore, when slaughtered at constant weight, different ages could exist. This makes it difficult to differentiate between the direct effects (of the diet components) and the indirect effects (caused by differences in growth rate) on the meat quality. Gender is another variable to consider. Initial studies suggest a decrease in flavour intensity from rams to ewes to wethers, especially where meat samples have a high fat content (Sink & Caporaso, 1977). Others have found that gender does not contribute to aroma differences to an age of 10 to 12 months (Park *et al.*, 1972; Claasen, 2008). At and after this age the levels of fatty acids began to vary, which could result in aroma differences. Also, the flavour intensity is likely related to the concentration of volatile compounds as Ha and Lindsay (1991) found that ram fat and lamb fat contained similar phenolic compounds, but with higher concentrations in that of ram fat. Nevertheless, the age affording differences is not definite as Cloete *et al.* (2012) found no differences ($P > 0.05$) in the sensory attributes of the meat from 20-months-old rams and ewes. For these reasons, gender was not taken into account for the purpose of this study. In general, gender starts to have an effect on aroma

after a certain physiological age (i.e. maturity/activity) is reached. This is linked with an increase in fat with increase in age causing an increase in lipid-derived aroma precursors (Ba *et al.*, 2012).

It is important to note that similar trends exist for the attribute scores of the fat and meat (Table 3.5 and 3.6). However, it is evident from the results that the fat allows for a better discrimination between treatments as the intensity ratings for the different sensory attributes increased when aroma of the fat phase was assessed (Table 3.5). The differences in mean scores were also more pronounced for the attributes of the fat aroma compared to those of the meat aroma and flavour. It is likely that if the meat was cooked and assessed with its fat a better discrimination between the meat samples would have been achieved. Also from the results one can suggest that the use of slightly older animal with increased fatness, but still classified as lamb, would provide a more prominent answer with regard to the sensory profile of the different treatments (farms).

The herbaceous aroma (28.5 ± 2.70) of the fat was significantly ($P \leq 0.05$) higher in one treatment (HK/CAL) compared to all the other treatments, while the herbaceous aroma (18.0 ± 3.33 and 18.8 ± 3.29) and flavour (24.7 ± 3.80 and 22.0 ± 2.61) of the meat was generally the highest in HK/CAL and HK/LO and lowest in RU and FS ($P \leq 0.05$) (Table 3.5 and 3.6). The higher intensity rating for flavour compared to aroma of the meat is likely as a result of the increased retention of volatile compounds in the fat tissue, which are released during mastication in the mouth. HK/CAL and HK/LO had the highest ($P \leq 0.05$) fat thickness measured between the 3rd and 4th lumbar vertebrae (Table 3.7). Fat is the main contributor towards the aroma and flavour of meat and it acts as a depot of fat soluble compounds which volatilise upon heating (Pegg & Shahidi, 2004). If volatile compounds (or unique components) from the diet are directly transferred via the intestinal tract and deposited in the fat, it is likely that the fat of HK/CAL and HK/LO contained a greater concentration of these compounds. Natural forages contain several volatile compounds that are deposited in fat tissue (Berg & Walker, 2004; Vasta & Priolo, 2006). As these compounds accumulate, they have a more profound contribution towards flavour. Hence with an increase in fat thickness, an increase in the intensity of aroma and flavour can be expected. The latter increase would make some treatments, as seen with HK/CAL and HK/LO, more distinguishable from others such as RU and FS. It is known that the distinctive aroma and flavour of Karoo lamb comes from the Karoo bushes/shrubs (indigenous plants) they consume (Estler *et al.*, 2006). Given that largest part of the Karoo is in the Northern Cape region, the sheep obtained from the farms within this region was largely exposed to Karoo veld. The farm, from which HK/CAL was sourced, was predominantly covered in Karoo bushes. Therefore, one can assume that the diet of the animals mostly consisted of herbaceous bushes, especially since Dorper sheep are more general or non-selective grazers (Du Toit, 1998). Fragrant plants collected from the farm, which did not feature in the other Northern Cape farms where: *Chrysocoma ciliata* and *Justica orchioides* (Table 3.1). It is plausible that consumption of these fragrant bushes could have contributed to the unique aroma and flavour of the HK/CAL treatment experienced by the trained panel. Typical fragrant plants species collected from the other farms located within the Karoo region were: *Pentzia spp.* [particularly *Pentzia incana* is commonly utilised by sheep (Vorster *et al.*, 1983)], *Pteronia spp.*, *Eriocephalus spp.* and *Salsola spp.* (Table 3.1). Common descriptors derived for these include

eucalyptus, camphor, thyme, minty and pine (Table 3.3). These descriptors could be linked to plant secondary metabolites, such as mono- and sesquiterpenes (Cornu *et al.*, 2001; Priolo *et al.*, 2004). The volatile compounds linked with these descriptors are expected to contribute towards the sensory profile of Karoo lamb. Considerable diversity, regarding vegetation, existed between farms, e.g. certain grass species (*Stipagrostis spp.* and *Chaetobromus dregeana*) were only present on some farms. This contributes to a variation in diet, which could result in differences in the sensory characteristics. The consumption of grass species could also exert a “diluting” effect on the sensory quality (principally towards the herbaceous attributes) of the meat obtained from the Northern Cape farms. However, further investigation is required to determine whether characteristic volatile compounds (lending the plant its unique fragrance) can be transferred from the plants via the intestinal tract to the lean and adipose tissue.

An important physical attribute contributing towards the quality of meat is ultimate pH. According to Table 3.7, the pH measured higher ($P \leq 0.05$) for CK than all the other treatments. Although CK was higher in pH (5.84 ± 0.12), the value is within the normal pH range and not high enough to be termed DFD (dark, firm and dry) meat. There were also no significant correlations between pH and the sensory attributes of the meat.

In terms of the overall sensory profile, lamb sourced from farms within the Northern Cape province grouped closely (apart from HK/CAL), where farms located near the border of the province such as HK/LO and KV, were found to be barely distinguishable from lamb of farms located near the centre (CK and NK) (Fig. 3.1 and 3.2). Lamb from farms within the Western Cape and Free State provinces grouped separate and were found to have a completely different sensory profile to lamb from farms of the Northern Cape province. These results suggest the possibility of region of origin classification for South African lamb, such as Karoo lamb. Karoo lamb is one of the first fresh meat products to be identified as having the potential to be recognised as a *Protected Geographical Indication* (PGI) in South Africa (Bramley *et al.*, 2013). The use of the words “Karoo Lamb” is included in the Merchandise Marks Act (Act 17 of 1941) (DTI, 2013), where certification under the *Karoo Meat of Origin* certification scheme of the Karoo Development Foundation (KDF), South Africa, ensures that the rules set out in the Act are complied with. Hence, the present sensory work also serves as baseline data to verify that Karoo lamb (extensively produced in the Karoo) have a unique sensory profile, characterised by its herbaceous attributes credible to a diet rich in fragrant Karoo plants.

According to the DA plot HK/LO can be considered very similar in terms of meat quality to the NK and KV treatments (Fig. 3.1). However, the plants collected from the HK/LO area, were similar to that of the HK/CAL area. Hence, one would expect the fat and meat to have similar sensory profiles based on their comparable diets, which was not the case. This complicates the certification process of region of origin products based on their geographical origin. The variation in sensory profiles could be large even if the sheep were raised on natural veld, within one set region, on different farms situated close to one another. Apart from variation between farms, internal treatment variance may also exist. As seen with the PCA scores plot (Fig. 3.2a) variation between animals within a treatment were evident. Variation within some lamb treatments (NK, HK/LO, KV and HK/CAL) were less than others (CK). RU and FS also varied with some animals (RU3, RU10,

FS6 and FS10) positioned further from the norm (Fig. 3.2a). Such variation is inevitable due to natural variation between animals, yet it is important to consider as individual animals may afford unrepresentative characteristics to the treatment group. Overall a good repeatability between animals per treatment was achieved when considering the sensory attributes analysed.

3.3.2 Physical attributes

With regard to physical attributes of the meat HK/LO, KV and NK had higher scores for juiciness and tenderness compared to RU and FS (Table 3.5). This is in agreement with the residue scores as the latter were rated overall highest for residue, but only differing significantly ($P \leq 0.05$) from the HK/LO and KV treatments (Table 3.5). According to the Pearson correlation coefficients (r) the following significant correlations were seen. Tenderness correlated strongly negative with residue ($r = -0.906$; $P = < 0.0001$) and WBSF ($r = -0.729$; $P = < 0.0001$), but moderately positive with initial juiciness ($r = 0.557$; $P = < 0.0001$) and sustained juiciness ($r = 0.754$; $P = < 0.0001$) (Fig. 3.2). Initial and sustained juiciness correlated mildly negative with cooking loss ($r = -0.444$; $P = 0.001$ and $r = -0.379$; $P = 0.004$, respectively), while residue and WBSF correlated positively with each other ($r = 0.687$; $P = < 0.0001$). As a result it is expected that treatments with a high residue score and WBSF measurement to be low in tenderness and juiciness. It is also likely that the treatment is high in cooking loss. This was seen for RU as WBSF measured significantly higher ($P \leq 0.05$) than all the other treatments and it had the lowest tenderness and highest residue scores (Table 3.5 and 3.7). Residue is the amount of meat left in mouth after 10 chews (Table 3.4). Therefore, an increase in residue score will be attributable to tougher meat as it will take longer to shear the muscle and collagen fibres. The juiciness of meat is also related to fat content (Dryden & Marchello, 1970). Initial and sustained juiciness is the ability of the muscle to release its inherent water and fat content, respectively (Hoffman *et al.*, 2003). Hence, increased content of these constituents would provide higher scores for juiciness. Subcutaneous and IMF provide insulation and prevents 'cold shortening' of muscles during refrigeration (Wood *et al.*, 1999). While, IMF accumulates in the perimysial connective tissue and lowers resistance to shear by increasing the ratio of soft fat to fibrous protein and opening the muscle structure. Intramuscular lipid deposition occurs in later stages of growth and fat deposition, being more prevalent in older animals (Wood *et al.*, 1999; Warriss, 2010). This could explain why HK/LO and NK had the highest scores for tenderness (62.1 ± 8.64 and 56.1 ± 11.84 , respectively) (Table 3.5), as their mean carcass weight was the highest (Table 3.7). However, KV was also rated most tender (61.7 ± 9.52) having the lowest ($P \leq 0.05$) mean carcass weight (16.3 ± 0.89 kg) (Table 3.5 and 3.7). The HK/LO treatment was lowest in WBSF (30.0 ± 3.83 N), while the KV treatment had the least thaw loss ($9.5 \pm 1.28\%$) and cooking loss ($29.7 \pm 4.63\%$) (Table 3.7). The reduced loss of moisture for KV during thawing and cooking and having the highest ($P \leq 0.05$) IMF content ($3.8 \pm 0.80\%$) (Table 3.7) is likely responsible for it being rated the highest for initial juiciness (41.7 ± 7.90) and sustained juiciness (51.7 ± 5.99) (Table 3.5).

3.4 Conclusions

The results serve as evidence that significant differences exist in South African Dorper lamb meat and fat from different farms. Differences were more prominent in the fat phase suggesting that fat is the main contributing factor toward the sensory profile of lamb. It can be concluded that aroma and flavour are influenced by the origin of the meat as lamb raised on grass (FS) or lucerne (RU) tend to have strong mutton-like characteristics, while lamb raised on a diet of Karoo plants and grass have more favourable lamb-like and herbaceous characteristics (CK, NK, HK/LO and KV). However, when lamb is raised on a diet rich in Karoo bushes (HK/CAL), distinctive (and in this study regarded as negative) characteristics can begin to surface such as being too herbaceous and having mutton-like, sheep wool and barnyard/kraal attributes. Therefore, diet has a significant effect on the sensory profile of extensively produced South African Dorper lamb. However, the current sample size were insufficient for comparison between regions. Therefore, for future research one should explore the regional differences taking into account the distribution of different areas of origin within each region. Future research should also explore the effects of season/diet composition, breed, gender and age, as these were outside the scope of this study.

3.5 References

- Almela, E., Jordan, M. J., Martinez, C., Sotomayor, J. A., Bedia, M. & Banon, S. (2010). Ewe's diet (pasture vs. grain-based feed) affects volatile profile of cooked meat from light lamb. *Journal of Agricultural and Food Chemistry*, **58**, 9641-9646.
- AMSA. (1995). *Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat*. Chicago, USA: National Livestock and Meat Board.
- AOAC. (2002). *Official method of analysis*. 17th ed. Virginia, USA: Association of Official Analytical Chemists.
- Ba, H. V., Hwang, I., Jeong, D. & Touseef, A. (2012). Principle of meat aroma flavors and future prospect. In: *Latest Research into Quality Control* (edited by M. S. F. Nezhad). ISBN: 978-953-51-0868-9. InTech, DOI: 10.5772/51110.
- Berg, E. P. & Walker, E. L. M. (2004). Sheep. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 1291-1295. Oxford, UK: Elsevier.
- Borton, R. J., Loerch, S. C., McClure, K. E. & Wulf, D. M. (2005). Comparison of characteristics of lambs fed concentrate or grazed on ryegrass to traditional or heavy slaughter weights. I. Production, carcass, and organoleptic characteristics. *Journal of Animal Science*, **83**(3), 679-685.
- Bramley, C., Bienabe, E. & Kirsten, J. (2009). The economics of geographical indications towards a conceptual framework for geographical indication research in developing countries. In: *The Economics of Intellectual Property*. Pp. 109-141. New York, USA: World Intellectual Property Organization (WIPO).
- Bramley, C., Bienabe, E. & Kirsten, J. (2013). *Developing Geographical Indications in the South: The Southern African Experience*. Dordrecht, the Netherlands: Springer.

- Brand, T. S. (2000). Grazing behaviour and diet selection by Dorper sheep. *Small Ruminant Research*, **36**, 147-158.
- Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, **77**, 63-80.
- Chang, S. K. C. (2010). Protein analysis. In: *Food Analysis* (edited by S. S. Nielsen). 4th ed. Pp. 133-146. New York, USA: Springer.
- Claasen, B. (2008). *The effect of agricultural production system on the meat quality of Dorper lambs*. Pp. 60-71. MSc thesis, Stellenbosch University, South Africa.
- Cloete, J. J. E., Hoffman, L. C. & Cloete, S. W. P. (2012). A comparison between slaughter traits and meat quality of various sheep breeds: wool, dual-purpose and mutton. *Meat Science*, **91**, 318-324.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Cornu, A., Carnat, A.-P., Martin, B., Coulon, J.-B., Lamaison, J.-L. & Berdague, J.-L. (2001). Solid-phase microextraction of volatile components from natural grassland plants. *Journal of Agricultural and Food Chemistry*, **49**(1), 203-209.
- Department of Agriculture, Forestry and Fisheries (DAFF). (1990). *Product Standards Act* (Act No.119 of 1990, No. R. 342). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2000). *Meat Safety Act* (Act No.40 of 2000, No. 1072). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2006). *Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa*. Government Gazette of 1 September 2006, No. R. 863. Pretoria, South Africa: Government Printer.
- Department of Trade and Industry (DTI). (2013). *Merchandise Marks Act* (Act No.17 of 1941). *Proposed prohibition on the use of certain words*. Pretoria, South Africa: Government Printer.
- Devincenzi, T., Prunier, A., Meteau, K., Nabinger, C. & Prache, S. (2014). Influence of fresh alfalfa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture. *Meat Science*, **98**, 607-614.
- Dryden, F. D. & Marchello, J. A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. *Journal of Animal Science*, **31**, 36-41.
- Du Toit, P. C. V. (1998). Diets selected by Merino and Dorper sheep in Karoo veld. *Archivos de Zootecnia*, **47**, 21-32.
- Elmore, J. S., Cooper, S. L., Enser, M., Mottram, D. S., Sinclair, L. A., Wilkinson, R. G. & Wood, J. D. (2005). Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Science*, **69**, 233-242.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.

- Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., Kurt, E., Sinclair, L. A. & Wilkinson, R. G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science*, **55**(2), 141-147.
- Fraser, M. D., Speijers, M. H. M., Theobald, V. J., Fychan, R. & Jones, R. (2004). Production performance and meat quality of grazing lamb finished on red clover, lucerne or perennial ryegrass swards. *Grass and Forage Science*, **59**, 345-356.
- Geldenhuys, G., Hoffman, L. C. & Muller, M. (2014). Sensory profiling of Egyptian goose (*Alopochen aegyptiacus*) meat. *Food Research International*, **64**, 25-33.
- Ha, J. K. & Lindsay, R. C. (1991). Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *Journal of Food Science*, **56**(5), 1197-1202.
- Hoffman, L. C., Muller, M., Cloete, S. W. P. & Schmidt, D. (2003). Comparison of six crossbred lamb types: sensory, physical and nutritional meat quality characteristics. *Meat Science*, **65**, 1265-1274.
- Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, **49**(4), 447-457.
- Lawless, H. T. & Heymann, H. (2010). Descriptive analysis. In: *Sensory Evaluation of Food: Principles and Practises*. 2nd ed. Pp. 227-258. New York, USA: Springer.
- Lee, C. M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC International*, **79**(2), 487-492.
- Leighton, C., Schönfeldt, H. C., Van Zyl, J., Van Heerden, S. M., Van Niekerk, J. M. & Morey, L. (2007). Sensory profiles of mutton from different regions in South Africa. Confidential report, Agricultural Research Council, Livestock Business Division – Animal Production, Meat Industry Centre, Irene, South Africa.
- Madruca, M. S. & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture*, **68**, 305-310.
- Melton, S. L. (1990). Effects of feeds on flavor of red meat: a review. *Journal of Animal Science*, **68**, 4421-4435.
- Mucina, L. & Rutherford, M. C. (2006). South African vegetation map. South African National Biodiversity Institute (SANBI). Available from: <http://bgis.sanbi.org/vegmap/map.asp>.
- Munoz, A. M. & Civille, G. V. (1998). Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studies*, **13**, 57-75.
- Næs, T., Brockhoff, P. B. & Tomic, O. (2010). *Statistics for Sensory and Consumer Science*. West Sussex, UK: John Wiley & Sons.
- Nute, G. R., Richardson, R. I., Wood, J. D., Hedges, S. I., Wilkinson, R. G., Cooper, S. L. & Sinclair, L. A. (2007). Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. *Meat Science*, **77**, 547-555.

- Park, R. J., Corbett, J. L. & Furnival, E. P. (1972). Flavour differences in meat from lambs grazed on lucerne (*Medicago sativa*) or phalaris (*Phalaris tuberosa*) pastures. *Journal of Agricultural Science*, **78**, 47-52.
- Pegg, R. B. & Shahidi, F. (2004). Flavour development. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 570-578. Oxford, UK: Elsevier.
- Priolo, A., Cornu, A., Prache, S., Krogmann, M., Kondjoyan, N., Micol, D. & Berdagué J.-L. (2004). Fat volatile tracers of grass feeding in sheep. *Meat Science*, **66**, 475-481.
- Priolo, A., Micol, D., Agabriel, J., Prache, S. & Dransfield, E. (2002). Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science*, **62**(2), 179-185.
- Ranken, M. D. (1992). Rancidity in meats. In: *Rancidity in Foods* (edited by J. C. Allen & R. J. Hamilton). 3rd ed. Pp. 191-202. London, UK: Blackie Academic and Professional.
- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2010). Relationship between odour-active compounds and flavour perception in meat from lamb fed different diets. *Meat Science*, **85**, 700-706.
- Rousset-Akrim, S., Young, O. A. & Berdagué, J.-L. (1997). Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Science*, **45**(2), 169-181.
- Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., Maria, G., Sierra, I. & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, **54**, 339-346.
- Schönfeldt, H. C., Naudé, R. T., Bok, W., Van Heerden, S. M. & Smit, R. (1993). Flavour- and tenderness-related quality characteristics of goat and sheep meat. *Meat Science*, **34**, 363-379.
- Schreurs, N. M., Lane, G. A., Tavendale, M. H., Barry, T. N. & McNabb, W. C. (2008). Pastoral flavour in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology*, **146**, 193-221.
- Shapiro, S. S. & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Sink, J. D. & Caporaso, F. (1977). Lamb and mutton flavour: contributing factors and chemical aspects. *Meat Science*, **1**(2), 119-127.
- Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*. 7th ed. Ames, USA: The Iowa State University Press.
- Spanier, A. M., Flores, M., Toldrá, F., Aristoy, M.-C., Bett, K. L., Bystricky, P. & Bland, J. M. (2004). Meat flavor: contribution of proteins and peptides to the flavor of beef. In: *Quality of Fresh and Processed Foods* (edited by F. Shahidi, A. M. Spanier, C.-T. Ho & T. Braggins). Pp. 33-49. Vol. 542. New York, USA: Kluwer Academic/Plenum Publishers.
- Tshabalala, P. A., Strydom, P. E., Webb, E. C. & De Kock, H. L. (2003). Meat quality of designated South African indigenous goat and sheep breeds. *Meat Science*, **65**, 563-570.
- Vasta, V. & Priolo, A. (2006). Ruminant fat volatiles as affected by diet. A review. *Meat Science*, **73**, 218-228.

- Viljoen, D. L., Muller, M., De Swart, J. B., Sadie, A. & Vosloo, M. C. (2001). Computerized electronic temperature control system for thermal efficiency during baking in food research. *International Journal of Consumer Science*, **25**, 30-42.
- Voisey, P. W. (1976). Engineering assessment and critique of instruments used for meat tenderness evaluation. *Journal of Textural Studies*, **7**, 11-48.
- Vorster, M., Botha, P. & Hobson, F. O. (1983). The utilization of Karoo veld by livestock. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa*, **18**(1), 35-39.
- Vorster, M. & Roux, P. W. (1983). Veld of the Karoo areas. *Proceedings of the Annual Congress of the Grassland Society of South Africa*, **18**, 18-24.
- Warriss, P. D. (2010). The growth and body composition of animals. In: *Meat Science: An Introductory Text*. 2nd ed. p. 11. Wallingford, UK: CABI Publishing.
- Watkins, P. J., Frank, D., Singh, T. K., Young, O. A. & Warner, R. (2013). Sheep meat flavor and the effect of different feeding systems: a review. *Journal of Agricultural and Food Chemistry*, **61**, 3561-3579.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I. & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society*, **58**, 363-370.
- Young, O. A., Berdague, J.-L., Viallon, C., Rousset-Akrim, S. & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, **45**(2), 183-200.
- Young, O. A., Lane, G. A., Priolo, A. & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food Agriculture*, **83**, 93-104.
- Young, O. A., Reid, D. H. & Scales, G. H. (1993). Effect of breed and ultimate pH on odour and flavour of sheep meat. *New Zealand Journal of Agricultural Research*, **36**, 363-370.

Chapter 4

Fatty acid profile and the volatile aroma compounds of South African Dorper lamb from extensive grazing systems

Abstract

Volatile aroma compounds and fatty acids were identified in regionally unique South African lamb meat to discriminate treatments based on their origin. Predominant volatiles and fatty acids were also determined for plant samples in order to establish a potential link between the diet and composition of the meat. Differences were observed between Karoo and Non-Karoo lamb meat, where compounds such as α -pinene, β -pinene and limonene were only detected in the meat of Karoo lamb treatments. These terpenes were particularly high in that of the meat from the Hantam Karoo/Calvinia (HK/CAL) farm. This suggests that these compounds could be markers of feeding aromatic Karoo bush-containing diets, typical of the region. The meat volatile profile allowed to discriminate between the lambs receiving Karoo bushes and those not receiving these bushes in the diet. Variation were also seen in the fatty acid profiles, where in some cases the presence of certain fatty acids could be associated with volatiles produced by lipid oxidation.

Keywords: Aroma; Extensive grazing; Fatty acids; Lamb; Volatile compounds

4.1 Introduction

Following the results of Chapter 3, it is evident that sensory differences exist in lamb from different origins and can be detected using a trained sensory panel. However, an important question is whether other analytical techniques can be used to detect differences and determine the chemical drivers for the variation in the sensory profile.

When meat is cooked, volatile and smaller non-volatile chemical compounds are produced by chemical reactions that occur between the non-volatile constituents (precursors) of lean and fat tissues in meat (Wasserman, 1972; Shahidi, 1989; Mottram, 1998; Pegg & Shahidi, 2004; Calkins & Hodgen, 2007). The precursors of meat aroma and flavour and the compounds derived from them are presented in Table 4.1 (adapted from Brennand *et al.*, 1992, Mottram, 1998 and Ba *et al.*, 2012). According to the basic theory, cooked meat from domesticated animal species has a similar flavour profile linked to protein breakdown and production of heterocyclic compounds while, species-specific flavour come from lipid oxidation products of the intramuscular fat and not water-soluble precursor compounds (Wasserman, 1972; Mottram, 1998; Berg & Walker, 2004; Pegg & Shahidi, 2004; Calkins & Hodgen, 2007; Watkins *et al.*, 2013).

Table 4.1 The precursors of meat aroma and flavour and the compounds derived from them

Precursors	Name(s) of component(s)	Flavour/Aroma compounds ^a
<i>Water-soluble compounds (muscle tissue)^b</i>		
Reducing sugars	Ribose; glucose; xylose; starch; mannose; fructose; maltose; mannose 6-phosphate, glucose 6-phosphate; fructose 6-phosphate; ribose 6-phosphate	Furanones; pyrazines; pyridines; pyrroles; oxazoles; thiazoles; thiophenes; trithiolanes; trithianes; methylfuranthio; alkanethiols; alkyl sulphides; alkyl disulphides
Free amino acids	Cystine; cysteine; glycine; lysine; alanine; valine; isoleucine; leucine; threonine; serine; proline; asparagine; aspartic acid; methionine; glutamic acid; phenylalanine; glutamine; ornithine; histidine; tyrosine; tryptophan; arginine	
Nucleotides and peptides	Glutathione; carnosine inosine; inosine monophosphate; inosine 5'-monophosphate; guanosine 5-monophosphate; creatine; creatinine; hypoxanthine	
Vitamins	Thiamine	
<i>Lipids (fat tissue and intramuscular fat)^c</i>		
Fats/Lipids	Triglycerides and phospholipids; oleic acid (C18:1 <i>n</i> -9); linoleic acid (C18:2 <i>n</i> -6); linolenic acid (C18:3 <i>n</i> -3) branched-chain fatty acids	Hydrocarbons; aldehydes; ketones; alcohols; carboxylic acids; esters; γ - and δ -lactones; alkylfurans; 4-methyl- and 4-ethyloctanoic acids; 4-methylnonanoic

^a Derived from the precursors; ^b Subject to Maillard-type reactions; ^c Subject to lipid oxidation reactions.

Essentially, the sensory characteristics of meat is linked to the presence of these volatile compounds as they mainly contribute to the flavour profile (Mottram, 1998; Calkins & Hodgen, 2007). They are transferred to the meat from the feed (diet) or arise from metabolic reactions (i.e. physiologically or from the ruminal microorganisms). Volatile aroma compounds such as aldehydes, alcohols, ketones, carboxylic acids, ethers, esters, lactones, heterocyclic compounds and hydrocarbons are produced from low-molecular weight amino acid degradation products, lipid oxidation products and reaction products of the two, while other compounds originate through Maillard (when free amino acids condense with the carbonyl groups of reducing sugars) or Strecker reactions (the degradation of amino acids by dicarbonyls formed in the Maillard reaction) (Mottram, 1998; Watkins *et al.*, 2013).

The identification of the particular chemical compounds responsible for lamb flavour have only recently been established as early studies only speculated on the chemical nature of these compounds, being carbonyl and/or sulphur-containing lipid soluble compounds (Sink & Caporaso, 1977). The volatile aroma compounds comprise aliphatic and aromatic compounds (Farmer, 1994). The latter usually contain a heteroatom such as oxygen, nitrogen or sulphur. While these compounds can be formed through various pathways, the chemical reactions caused by heating are the main source of volatile production in cooked meat.

A wide variety of volatile compounds have been identified in cooked lamb meat and other meat products (Young *et al.*, 1997; Elmore *et al.*, 2000a; Elmore *et al.*, 2000b; Madruga *et al.*, 2000; Estévez *et al.*, 2003;

Machiels & Istasse, 2003; Young *et al.*, 2003; Vasta *et al.*, 2007; Xie *et al.*, 2008; Madruga *et al.*, 2009; Almela *et al.*, 2010; Madruga *et al.*, 2010; Resconi *et al.*, 2010; Vasta *et al.*, 2010; Bueno *et al.*, 2011; Vasta *et al.*, 2011; Resconi *et al.*, 2012; Ma *et al.*, 2013; Madruga *et al.*, 2013). Furthermore, Resconi *et al.* (2010) found that aldehydes, ketones, sulphur compounds, pyrazines, phenols and a carboxylic acid produced the greatest difference in aromas between the loins of lamb finished on pasture and concentrate. The significance of carbonyl compounds (aldehydes, alkenals, alkadienals, Strecker aldehydes and ketones) in defining lamb or mutton flavour and aroma has been highlighted in several studies (Jacobson & Koehler, 1963; Sink & Caporaso, 1977; Osorio *et al.*, 2008; Wilches *et al.*, 2011; Resconi *et al.*, 2012). In grilled loins from Corriedale lambs finished on pasture or concentrate the carbonyls were the most important odour-active compounds (Resconi *et al.*, 2010). Elmore *et al.* (2005) detected a few Maillard-derived compounds such as 3-hydroxy-2-butanone and 2,3-butanedione, and the Strecker aldehydes, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal in the headspace of grilled lamb meat raised on diets high in polyunsaturated fatty acids. They also reported on the volatile compounds derived from the decomposition of two major polyunsaturated fatty acids as shown in Figure 4.1.

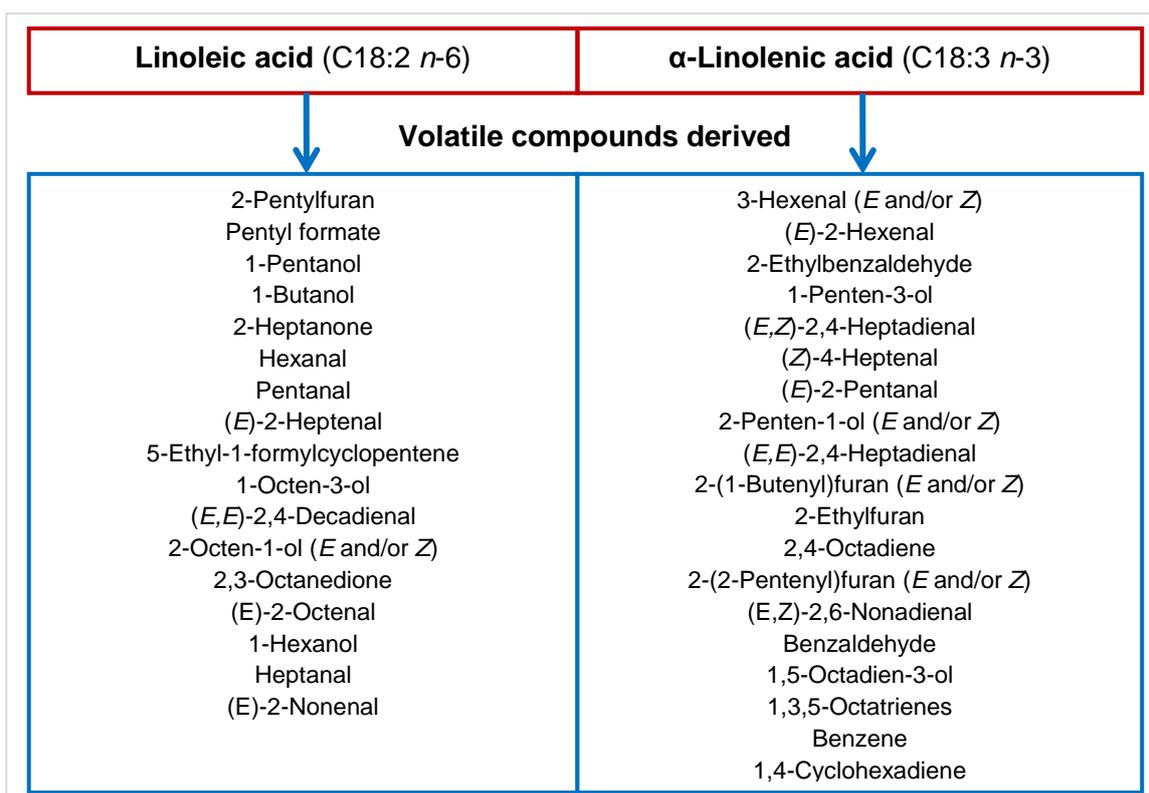


Figure 4.1 Volatile compounds derived from the decomposition of linoleic and α -linolenic acid (adapted from Elmore *et al.*, 2005)

Other important volatile compounds contributing to meat aroma are heterocyclic compounds such as thiopenes, furans, pyrazines, pyrroles, thiazoles, thiazolines and indoles. Madruga *et al.* (2013) characterised the effect of species on the volatile profile of grilled goat and lamb meat. They detected higher concentrations

of lipid-derived volatile compounds such as saturated alkanals, pyrazines and thiazoles in lamb meat, suggesting that it would also provide the meat with a stronger flavour profile.

When lipid and Maillard-type reaction products interact the flavour and aroma of meat is affected (Elmore & Mottram, 2000). These reaction products provide characteristic sensory attributes, while the overall aroma profile may be modified as lipid-derived products interact with Maillard-intermediates such as ammonia and hydrogen sulphide. Elmore and Mottram (2000) confirmed that 2-alkyl-(2H)-thiapyrans are produced when lipid-derived aldehydes interact with hydrogen sulphide from Maillard cysteine degradation. Furthermore, increased quantities of thiophenes and thiapyrans correlated with high levels of *n*-3 PUFA in the muscles as oxidation of PUFA generate lipid degradation products (i.e. 2, 4-alkadienals) (Elmore & Mottram, 2000). Thiophenes provide a sulphurous and grassy aroma to cooked meat (Sutherland & Ames, 1995), while the thiapyrans have low aroma intensities (Elmore & Mottram, 2000).

Given the important role of volatiles and fatty acids in the development of aroma and flavour of meat, the aim of this research chapter was to determine the variation in the volatile aroma compounds and fatty profile of South African lamb meat from different extensive grazing systems. In doing so, the chemical compounds responsible for the sensory variation in Chapter 3 may also be determined. It will also serve as evidence to verify the authentic nature of Karoo lamb.

4.2 Materials and methods

The reagents and chemicals used in all the chemical analysis methods were of high purity and grade, whilst bi-monthly blind sample analyses were performed to test for accuracy and repeatability as part of a National Inter-Laboratory Scheme (AgriLASA: Agricultural Laboratory Association of South Africa).

4.2.1 Experimental layout and study farms

Seven farms, each unique in terms of its vegetation and the extensive grazing conditions, were selected for the purpose of the study (Chapter 1, Fig. 1.2). Five farms were from the Northern Cape province namely, Central Karoo (CK), Northern Karoo (NK), Hantam Karoo/Loeriesfontein (HK/LO), Knersvlakte (KV) and Hantam Karoo/Calvinia (HK/CAL). One farm, the Rûens (RU), was from the Western Cape province and one from the Free State (FS). Ten slaughter ready Dorper lambs ($n = 10$) were sourced from each farm. The studied farms are the same as described in Chapter 3.

4.2.2 Meat and plant sample collection

Dorper lambs were sourced, slaughtered according to standard South African procedures and the meat samples collected from the carcasses as described in Chapter 3. The right *Longissimus thoracis et lumborum* (LTL) were vacuum-packed and stored at -20°C for sensory analysis (Chapter 3), while the left LTL were divided so that the *Longissimus lumborum* (LL) was homogenised, vacuum-packed and stored at -80°C for fatty acid analysis. After cooking the meat to an internal temperature of 70°C (Chapter 3), samples were taken from the centre of the muscles at the 3rd to 5th lumbar vertebrae for solid-phase microextraction (SPME). The

samples were finely sliced into 2-3 mm cubes. Almela *et al.* (2010) performed SPME on meat cooked to an internal temperature of 72°C to simulate the development of volatile compounds during the standard cooking procedure of meat. Also, at this temperature detectability of volatile compounds, owing to dietary changes, might increase since thermal degradation would be low. In this study, 70°C was used to simulate volatile generation during cooking. If the temperature was too high a more complex aroma profile would be formed, which could make it difficult to distinguish between samples.

Plant samples (i.e. bushes, shrubs and grasses) were collected from the different farms. These were typically eaten by the animals and represent the diet associated with the origin of the meat. The plant samples (n = 66) were dried at 60°C to a constant weight and subsequently milled to a fine powder. The time of drying varied between samples due to their different moisture contents. Therefore, during the drying step they were weighed every 1 h until a constant weight was reached. Depending on the amount of sample, the samples were milled using either a pestle and mortar or a benchtop mill (1095 Knifetec™, Höganäs, Sweden). The samples were vacuum-sealed before being stored at -80°C until analysis.

4.2.3 Determination of volatile profiles using SPME-GC/MS

A modified version of the SPME method as described by Vasta *et al.* (2011) was used for the sample preparation and determination of volatile compounds of the seven meat treatments and the various plant samples.

4.2.3.1 Sample preparation

Six gram of cooked homogenised lamb meat and 0.1 g plant sample was placed in a 20 mL SPME glass vial, closed with a screw-cap (PTFE/red silicone septa), respectively. A single SPME analysis was performed for each meat and plant sample.

4.2.3.2 Extraction procedure of volatile compounds and chromatographic analyses

Meat and plant samples were analysed on the same day the samples were placed in the vials. An internal standard (3-octanol, 1 mg/kg) of 50 µl was added to each of the sample vials. The volatile aroma compounds were then trapped and extracted from the vial headspaces by the SPME method. The vials were then allowed to equilibrate for 30 min at 70°C in the CTC (CTC Analytics CombiPAL) autosampler incubator and after this equilibration time, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/carboxen/PDMS) coated fibre (grey) was exposed to the headspace for 10 min at 70°C. After sampling, the volatile compounds adsorbed on the fibre coating were desorbed for 10 min in the GC injection port operated in pulsed splitless mode with the temperature maintained at 250°C. The separation of the volatile compounds was performed on a gas chromatograph, Agilent 6890 N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector (MSD) Agilent 5975B inert XL EI/CI MSD (Agilent, Palo Alto, CA). The GC-MS system was equipped with a DB-FFAP (60 m, 0.25 mm internal diameter, 0.5 µm film thickness) GC column. Analyses were carried out using helium as carrier gas with a flow of 1.9 mL/min. The injector temperature was maintained at 250°C.

The GC oven temperature was operated as follows: 70°C for 1 min; and then ramped up to 142°C at 3°C/min and held for 0 min followed by ramping up to 240°C at 5°C/min and held for 3 min. The total run time was 47.60 min. The MSD was operated in full scan mode and the source and quad temperatures were maintained at 230°C and 150°C, respectively. The transfer line temperature was maintained at 280°C. The electron impact energy was set at 70 eV and the data collected in the range of m/z 35-450. Compounds were tentatively identified by their mass spectra using a combination of two libraries: National Institute of Standards and Technology (NIST) 05 and Wiley (275) spectral library collection. The peak areas of each volatile organic compound detected were expressed relative to the internal standard as percentage (%) composition of the lamb meat.

4.2.4 Determination of fatty acid profiles

The fatty acids of both meat and plants samples were extracted according to the method described by Folch *et al.* (1957) using 2:1 (v/v) chloroform:methanol solution containing 0.01% butylated hydroxytoluene (BHT) (Sigma-Aldrich Inc., catalogue number B-1378, 3050 Spruce Street, St. Louis, MO 63103, USA) as antioxidant. In order to quantify the individual fatty acids present within the meat and plant samples Heptadecanoic acid (C17:0) (Sigma-Aldrich Inc., catalogue number H3500, 3050 Spruce Street, St. Louis, MO 63103, USA) was used as an internal standard. An aliquot of the extracted fatty acids were converted into corresponding methyl esters through transmethylation using a methanol:sulphuric acid (19:1; v/v) solution. Care was taken to prevent moisture contamination in the lipid extracts as derivatization reagents are moisture-sensitive (Park & Goins, 1994). The fatty acid methyl esters (FAMES) were determined by gas chromatography.

4.2.4.1 Fatty acid extraction of the meat

Homogenised meat samples, stored at -80°C, were defrosted before determination of its long-chain fatty acid content (intramuscular). A total of 2 g of meat sample was weighed off into an extraction tube (205 x 30 mm, Pyrex). This was followed with the addition of 20 mL 2:1 (v/v) chloroform:methanol solution and 500 µL internal standard (C17:0). The meat sample together with the extraction solvent was homogenised for 10 seconds by means of a polytron mixer (Kinematica AG Homogenizer, PT-500 E, serial number PF-799-0004-02-19, speed setting 7-8 x 1000 rpm). The homogenised content was then transferred to an extraction funnel (0 porosity disc) covered with glass microfibre filter paper (Whatman, GF/A, 47 mm diameter, catalogue number 1820-047) leading into a 50 mL volumetric flask. A volume of 10 mL 2:1 (v/v) chloroform:methanol solution were added to the residue, the mixture homogenised with the polytron (to rinse), filtered and the final volume made up to 50 mL. A 250 µL aliquot of the filtered phase was transferred to a Klimax tube (with Teflon lined screw cap) and dried under nitrogen in a scientific water-bath with electronic temperature control (set to 45°C) and water circulation. A volume of 2 mL of the transmethylation agent (19:1, v/v, methanol/sulphuric acid solution) was added to each sample and placed in a waterbath (set to 70°C) for 2 h. Following transmethylation the mixture was cooled to room temperature and the FAMES extracted with distilled water (1 mL) and hexane (2 mL) by transferring the top hexane phase to a clean Klimax tube and then drying it under nitrogen in a

waterbath (set to 45°C). For chromatographic analysis, 100 µL hexane was added to the dried FAMES sample and transferred into an autosampler vial with 0.1 mL insert and closed with a screw-cap (PTFE/red silicone septa) (Supelco™, 595 North Harrison Rd, Bellefonte, PA 16823-0048, USA).

4.2.4.2 Fatty acid extraction of the plants

A total of 0.4 g of homogenised and finely milled plant sample was weighed off into a 12 mL Klimax tube. This was followed with the addition of 5 mL 2:1 (v/v) chloroform:methanol solution and 100 µL internal standard (C17:0). The plant sample together with the extraction solvent was mixed (vortex) for 10 s and then transferred to an extraction funnel (0 porosity disc) covered with glass microfibre filter paper (GF/A, Whatman) leading into a 10 mL volumetric flask. A volume of 3 mL 2:1 (v/v) chloroform:methanol solution were added to the residue (to rinse), filtered and the final volume made up to 10 mL. A 250 µL aliquot of the filtered phase was transmethylated and prepared for chromatographic analysis as described for the meat samples.

4.2.4.3 Fatty acid methyl esters chromatographic analysis

The FAMES were determined by gas chromatography in an Agilent 6890 model gas chromatograph (Agilent, Palo Alto, CA), coupled with a flame-ionization detector and split injector port, set at 280°C and 240°C, respectively. Split injection was made at a 5:1 ratio. Chromatographic separation of the FAMES was performed on a DB-225MS capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness, Agilent J&W GC Columns, part number 122-2932). Hydrogen (40 mL/min flow rate) was used as carrier gas and helium as makeup gas, with a combined flow of 30 mL/min. The oven was operated as follow: 50°C for 1 min; ramped up to 175°C at 25°C/min and held for 0 min followed by ramping up to 210°C at 2°C/min and held for 6 min, then ramped up to 220°C at 1°C/min and held for 0 min followed by ramping up to 235°C at 1.5°C/min and held for 2 min. The sample volume injected was 1 µL and the run time of approximately 45 min. Fatty acids were identified by comparing their retention times to those found in a standard FAME mixture (Supelco™, 37 Component FAME mix, C₄-C₂₄, 10 mg/mL in CH₂Cl, catalogue number 47885-U, North Harrison Road, Bellefonte, PA 16823-0048, USA). The results were recorded as percentage (%) of the total fatty acids.

4.2.5 Statistical analysis

Statistical analysis of data was performed using the GLM (General Linear Models) procedure of SAS™ statistical software (Statistical Analysis System, Version, 9.2, 2006, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and XLSTAT® statistical software (Version 2016.04.33113; Addinsoft, NY, USA) for the multivariate statistical analysis. Pre-processing of the data involved using the Shapiro-Wilk test to test for deviation from normality (Shapiro & Wilk, 1965). When the deviation from normality was significant ($P \leq 0.05$) the outliers in the data were identified (with residuals greater than 3) and removed until the data was normalised or symmetrically distributed. Data were subjected to one-way ANOVA with treatment as factor. Treatment means were compared with Fisher's Least Significant Differences (LSD) at a 5% significance level.

Associations were illustrated using multivariate statistical analysis, specifically principal component analysis (PCA).

4.3 Results and discussion

The effect of diet, when linked to origin, on the volatile organic compounds (VOCs) of cooked lamb meat is shown in Table 4.2. Origin had a significant effect on the volatile profile of the cooked meat. The mean concentration of total volatile compounds detected by SPME-GC/MS was higher in the Karoo than in the Non-Karoo samples. The highest being in that of the KV and HK/CAL treatments (Table 4.2). The VOCs detected and semi-quantitatively expressed in the headspace were aldehydes, alcohols, ketones, furans, hydrocarbons, organic acids, terpenes, nitrogen- and/or sulphur-containing compounds, lactone, phenols and unidentified compounds (Table 4.2). There were significant ($P \leq 0.05$) differences in 99 of the 122 volatiles detected in all treatments. The results had high standard deviations, likely as a result of the variation in the composition of the meat and fat tissue (Almela *et al.*, 2010). Factors affecting the formation of volatiles include *ante-mortem* or *post-mortem* factors (Wasserman, 1972; Madruga & Mottram, 1995; Spanier *et al.*, 2004). These factors can alter the composition of the muscle tissue, fat tissue and intramuscular fat, affecting the volatile precursor compounds. The predominant volatiles of the plants samples collected from the different farms are presented in Table 4.3. The volatiles are listed in decreasing order of relative concentration. The means for the fatty acid composition of the lamb meat are shown in Table 4.4, while the composition of the predominant fatty acids of the plants are presented in Table 4.5. Significant differences could not be determined for the plant samples as only one sample per species were collected. However, the results give an indication of the type and concentration of fatty acids present in the plant samples.

Almela *et al.* (2010) found that aldehydes, generally predominant in the aroma of cooked meat (i.e. hexanal, nonenal, and decenal), were higher in pasture-fed compared to grain-fed lamb. Although the current study did not include feedlot lamb, differences in the aldehyde content between Karoo and Non-Karoo samples occurred (Table 4.2). Apart from CK, the other Karoo samples (NK, HK/LO, KV and HK/CAL) had higher aldehyde composition than the Non-Karoo (RU and FS) samples (Table 4.2). In fact, when the PUFA composition of the raw meat is taken into account, a similar trend is seen (Table 4.4). Though, RU had the highest ($P \leq 0.05$) PUFA composition (18.47%), FS had the lowest (11.86%) (Table 4.4), differing significantly from the rest (except for NK and HK/LO). Taking diet into account, it is evident that the grass of the FS farm had a low composition of PUFA (ranging from 19% to 34%) and high composition of SFA (ranging from 42% to 54%) (Table 4.5). Plants of the other farms typically range $>30\%$ for PUFA and $<50\%$ for SFA (Table 4.5). These differences in SFA and PUFA composition could explain the variation of PUFA in the meat as ruminal fermentation of the feed is known to decrease the PUFA content (Wood & Enser, 1997). Hence, with the diet of FS already consisting of grass with a relatively low PUFA content, the meat is bound to also contain a lower level of PUFA.

Table 4.2 Means (\pm SD) for the volatile organic compound composition (% expressed relative to the internal standard) of the lamb meat

No.	Rt	VOC	Farms							LSD P = 0.05
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
Aldehydes										
1	3.33	butanal	0.87 ^{cd} \pm 1.33	1.67 ^{abc} \pm 1.63	1.60 ^{bc} \pm 1.76	3.01 ^a \pm 1.69	2.80 ^{ab} \pm 2.03	nd	0.44 ^d \pm 0.73	1.35
5	4.90	pentanal	7.74 ^c \pm 5.25	19.41 ^{ab} \pm 8.24	14.05 ^{bc} \pm 5.20	20.68 ^a \pm 7.20	23.70 ^a \pm 10.99	13.88 ^{bc} \pm 5.19	9.91 ^c \pm 4.63	6.37
12	7.14	hexanal	78.87 ^e \pm 60.50	282.99 ^{ab} \pm 110.20	201.11 ^{cd} \pm 75.10	329.89 ^a \pm 113.57	312.21 ^a \pm 127.06	210.37 ^{bc} \pm 49.53	125.84 ^{de} \pm 75.84	81.83
20	9.52	2-methyl-2-pentenal	nd	nd	nd	nd	0.22 \pm 0.46	nd	nd	-
23	10.16	heptanal	25.45 ^d \pm 22.57	59.07 ^{bc} \pm 20.93	65.85 ^b \pm 17.68	90.25 ^a \pm 19.16	68.03 ^b \pm 16.52	65.22 ^b \pm 19.40	43.79 ^{cd} \pm 26.07	18.42
28	12.11	4-heptenal	0.83 ^b \pm 1.19	1.62 ^b \pm 1.38	1.69 ^b \pm 0.70	4.32 ^a \pm 1.56	5.09 ^a \pm 2.07	4.88 ^a \pm 1.52	0.92 ^b \pm 0.71	1.25
33	13.78	octanal	16.10 ^c \pm 16.79	40.23 ^b \pm 12.87	41.11 ^b \pm 13.70	57.60 ^a \pm 17.73	50.02 ^{ab} \pm 16.67	39.33 ^b \pm 8.56	19.86 ^c \pm 15.86	13.54
38	15.32	(E)-2-heptenal	0.53 ^c \pm 0.72	2.70 ^{ab} \pm 1.60	2.23 ^b \pm 1.42	4.04 ^a \pm 1.92	4.09 ^a \pm 2.28	2.09 ^b \pm 0.78	1.39 ^{bc} \pm 1.72	1.43
42	16.43	2-methyl-2-heptenal	nd	0.16 ^b \pm 0.34	nd	0.47 ^a \pm 0.62	0.50 ^a \pm 0.63	nd	nd	0.32
44	17.62	3-(2-thienyl)-propanal	nd	0.47 ^b \pm 0.67	nd	1.50 ^a \pm 1.17	1.22 ^a \pm 1.25	nd	nd	0.62
45	17.78	nonanal	30.17 ^c \pm 27.28	93.10 ^b \pm 49.79	84.17 ^b \pm 33.26	144.74 ^a \pm 55.73	103.46 ^b \pm 39.31	78.48 ^b \pm 20.90	42.67 ^c \pm 26.94	34.44
49	19.40	2-octenal	0.82 ^e \pm 0.95	4.79 ^{bc} \pm 2.41	3.51 ^{cd} \pm 1.59	7.72 ^a \pm 3.61	5.89 ^{ab} \pm 3.10	3.33 ^{cd} \pm 1.06	2.21 ^{de} \pm 2.13	2.09
62	21.90	decanal	1.31 ^b \pm 1.20	1.94 ^b \pm 0.79	1.85 ^b \pm 0.51	2.87 ^a \pm 1.00	2.96 ^a \pm 1.02	1.81 ^b \pm 0.37	1.41 ^b \pm 0.93	0.79
63	22.10	(E,E)-2,4-heptadienal	nd	0.07 ^b \pm 0.21	nd	0.43 ^a \pm 0.56	0.40 ^a \pm 0.42	nd	0.08 ^b \pm 0.25	0.26
70	23.51	benzaldehyde	10.22 ^d \pm 4.38	23.59 ^{abc} \pm 6.38	19.00 ^{bc} \pm 3.99	28.31 ^a \pm 7.32	23.75 ^{abc} \pm 8.70	18.12 ^c \pm 5.87	24.71 ^{ab} \pm 7.82	5.91
73	25.58	(E,E)-2,6-nonadienal	nd	nd	nd	0.67 ^a \pm 0.31	0.49 ^{ab} \pm 0.43	0.38 ^b \pm 0.32	0.10 ^c \pm 0.24	0.23
81	27.56	(E)-2-decenal	0.94 ^c \pm 1.18	2.72 ^b \pm 0.65	2.10 ^{bc} \pm 1.10	4.69 ^a \pm 1.88	4.15 ^a \pm 1.56	2.53 ^b \pm 0.88	2.18 ^{bc} \pm 2.00	1.27
83	28.21	2-butyl-2-octenal	nd	0.56 ^b \pm 0.65	nd	1.34 ^a \pm 0.99	1.34 ^a \pm 1.38	nd	0.23 ^b \pm 0.45	0.64
88	29.54	dodecanal	nd	0.33 ^c \pm 0.35	nd	1.59 ^a \pm 0.79	1.01 ^b \pm 0.72	nd	nd	0.39
89	29.61	2,4-nonadienal	nd	0.17 ^b \pm 0.26	nd	nd	0.73 ^a \pm 0.52	nd	nd	0.20
90	30.02	4-ethyl-benzaldehyde	1.23 ^c \pm 1.64	2.52 ^{bc} \pm 1.36	3.82 ^b \pm 2.10	8.53 ^a \pm 4.24	7.29 ^a \pm 3.65	4.39 ^b \pm 1.77	1.23 ^c \pm 1.01	2.31
93	30.95	2-undecenal	0.85 ^c \pm 0.99	2.24 ^b \pm 0.46	1.78 ^{bc} \pm 0.83	4.32 ^a \pm 1.75	3.57 ^a \pm 1.26	2.27 ^b \pm 0.72	1.36 ^{bc} \pm 0.83	0.97
96	31.40	(E,E)-2,4-decadienal	nd	0.21 \pm 0.25	nd	nd	0.28 \pm 0.24	nd	nd	-
100	32.58	tetradecanal	0.38 ^e \pm 0.54	1.45 ^{bc} \pm 1.03	1.11 ^{cde} \pm 0.42	2.44 ^a \pm 1.09	2.14 ^{ab} \pm 1.54	1.33 ^{bcd} \pm 0.49	0.57 ^{de} \pm 0.72	0.82
101	32.73	(E,E)-2,4-decadienal	nd	1.02 ^{ab} \pm 0.69	0.59 ^{bc} \pm 0.40	1.39 ^a \pm 0.69	1.13 ^a \pm 0.88	nd	0.22 ^c \pm 0.39	0.48

Table 4.2 (Continued)

No.	Rt	VOC	Farms							LSD
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
114	37.66	hexadecanal or pentadecanal	1.33 ^c ± 1.04	3.10 ^b ± 1.46	2.90 ^{bc} ± 0.97	5.54 ^a ± 2.24	5.19 ^a ± 3.22	3.32 ^b ± 1.50	2.00 ^{bc} ± 1.75	1.72
119	39.88	hexadecanal	1.90 ^c ± 0.87	4.01 ^a ± 2.04	3.90 ^{ab} ± 1.73	2.87 ^{abc} ± 1.31	2.80 ^{abc} ± 1.15	2.57 ^{bc} ± 1.57	2.52 ^c ± 1.52	1.34
Alcohols										
18	8.96	1-penten-3-ol	0.37 ^d ± 0.46	0.94 ^{cd} ± 0.37	1.55 ^{bc} ± 1.05	2.45 ^a ± 1.36	2.13 ^{ab} ± 0.92	1.46 ^{bc} ± 0.45	0.78 ^{cd} ± 0.98	0.79
27	11.95	1-pentanol	6.22 ^{cd} ± 3.42	6.15 ^{cd} ± 2.19	7.66 ^c ± 3.75	11.01 ^{ab} ± 4.05	11.70 ^a ± 4.99	7.91 ^{bc} ± 2.57	3.96 ^d ± 2.93	3.21
36	14.62	(Z)-2-penten-1-ol	nd	nd	0.26 ^b ± 0.35	0.60 ^a ± 0.49	0.55 ^a ± 0.47	nd	nd	0.26
40	15.78	1-hexanol	1.44 ^d ± 1.30	2.82 ^{bc} ± 0.86	4.01 ^b ± 1.76	5.63 ^a ± 2.02	4.07 ^b ± 1.41	3.67 ^b ± 1.30	1.91 ^{cd} ± 1.01	1.33
41	16.19	2-octen-1-ol	nd	0.15 ± 0.32	nd	0.40 ± 0.57	0.21 ± 0.32	nd	nd	-
43	17.28	3-octanol (Internal standard)	100	100	100	100	100	100	100	-
46	17.97	2-butoxy-ethanol	1.84 ^a ± 1.88	nd	nd	nd	0.84 ^b ± 1.56	nd	nd	0.80
50	19.56	1-octen-3-ol	7.18 ^e ± 7.01	27.12 ^{bc} ± 16.14	22.80 ^{cd} ± 12.42	52.14 ^a ± 27.05	39.77 ^{ab} ± 23.18	21.22 ^{cde} ± 7.30	9.56 ^{de} ± 10.20	14.81
52	19.80	1-heptanol	2.52 ^c ± 2.53	4.23 ^{bc} ± 1.08	5.06 ^b ± 2.44	7.74 ^a ± 3.30	7.77 ^a ± 3.20	5.25 ^b ± 1.77	2.35 ^c ± 1.37	2.19
54	20.14	3-ethyl-3-octanol	nd	1.29 ^b ± 0.26	nd	1.45 ^{ab} ± 0.19	1.53 ^a ± 0.25	1.54 ^a ± 0.39	nd	0.19
71	23.86	1-octanol	6.55 ^{cd} ± 5.91	6.94 ^{cd} ± 2.44	8.53 ^{bc} ± 4.02	14.86 ^a ± 6.95	12.21 ^{ab} ± 4.76	8.34 ^{bcd} ± 2.77	4.25 ^d ± 2.36	4.13
75	26.08	2-nonen-1-ol or 2-decen-1-ol	nd	0.79 ^{bc} ± 0.58	0.64 ^{bc} ± 0.46	1.66 ^a ± 0.95	1.09 ^b ± 0.74	0.74 ^{bc} ± 0.29	0.31 ^c ± 0.45	0.52
76	26.18	(Z)-2-octen-1-ol	0.75 ^c ± 0.923	2.28 ^{bc} ± 1.48	2.35 ^b ± 1.71	4.79 ^a ± 2.43	4.10 ^a ± 2.65	2.21 ^{bc} ± 0.97	0.84 ^{bc} ± 0.98	1.55
92	30.51	1-nonen-3-ol	0.41 ^c ± 0.54	0.85 ^{bc} ± 0.70	0.93 ^{bc} ± 0.61	2.18 ^a ± 1.29	1.29 ^b ± 0.86	0.83 ^{bc} ± 0.35	0.44 ^c ± 0.66	0.69
95	31.31	3,7,11-trimethyl-1-dodecanol	nd	0.12 ^b ± 0.16	nd	0.79 ^a ± 0.33	0.74 ^a ± 0.39	nd	0.09 ^b ± 0.16	0.18
103	33.11	(Z,E)-9,12-tetradecadien-1-ol	0.20 ± 0.40	nd	nd	nd	nd	nd	nd	-
109	34.41	benzyl alcohol	0.22 ^a ± 0.22	0.03 ^b ± 0.09	0.11 ^{ab} ± 0.20	0.08 ^{ab} ± 0.18	0.07 ^b ± 0.14	nd	0.05 ^b ± 0.16	0.14
111	35.25	tetradecanal or (Z)-2-dodecenol	0.52 ^d ± 0.58	1.80 ^{bc} ± 1.29	1.39 ^{cd} ± 0.47	2.84 ^a ± 1.27	2.59 ^{ab} ± 1.67	1.64 ^{bc} ± 0.73	0.87 ^{cd} ± 0.93	0.96
112	36.09	1-decene or 1-tetradecanol	nd	0.10 ^b ± 0.20	nd	0.39 ^a ± 0.23	0.34 ^a ± 0.34	nd	0.10 ^b ± 0.21	0.17
116	38.35	1-hexadecanol or pentadecanol	0.09 ^d ± 0.20	0.47 ^{bc} ± 0.43	0.40 ^{cd} ± 0.26	0.93 ^a ± 0.42	0.79 ^{ab} ± 0.57	nd	0.18 ^{cd} ± 0.32	0.32
Ketones										
4	4.75	2,3-butanedione	nd	nd	nd	nd	nd	nd	0.70 ^a ± 0.79	0.25

Table 4.2 (Continued)

No.	Rt	VOC	Farms							LSD P = 0.05
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
10	6.42	3-methyl-2-pentanone	nd	1.05 ^b ± 1.00	nd	1.60 ^a ± 0.90	1.98 ^a ± 0.88	nd	nd	0.54
22	10.05	2-heptanone	nd	0.49 ± 1.48	nd	nd	0.44 ± 1.32	nd	nd	-
26	11.87	6-methyl-2-heptanone	nd	0.41 ^{bc} ± 0.51	0.37 ^{bc} ± 0.53	0.96 ^{ab} ± 1.04	1.40 ^a ± 1.32	nd	0.49 ^{bc} ± 0.46	0.65
29	12.47	3-octanone	9.76 ^b ± 1.15	10.32 ^{ab} ± 1.94	9.52 ^b ± 1.18	10.61 ^{ab} ± 0.56	11.67 ^a ± 1.50	10.82 ^{ab} ± 1.69	10.02 ^b ± 2.07	1.41
32	13.60	2-octanone	nd	0.10 ^b ± 0.20	0.13 ^b ± 0.22	0.46 ^a ± 0.41	0.08 ^b ± 0.17	nd	nd	0.18
34	13.85	3-hydroxy-2-butanone	3.50 ^a ± 4.61	nd	nd	nd	1.16 ^b ± 0.88	nd	3.42 ^a ± 4.78	2.24
37	14.91	2,3-octanedione	1.90 ^e ± 2.29	33.41 ^{bc} ± 24.08	23.75 ^{cd} ± 16.15	54.13 ^a ± 29.79	46.61 ^{ab} ± 33.57	20.63 ^{cde} ± 9.07	9.68 ^{de} ± 15.11	19.13
39	15.63	5-methyl-3-hepten-2-one	0.88 ^c ± 0.75	2.06 ^{bc} ± 1.22	2.16 ^{bc} ± 0.97	4.12 ^a ± 2.49	3.48 ^{ab} ± 2.20	1.75 ^c ± 0.68	1.55 ^c ± 1.83	1.46
55	20.45	5-isopropyl-1,3-cyclohexanedione	nd	0.82 ^b ± 0.92	nd	2.69 ^a ± 1.83	2.41 ^a ± 2.60	nd	nd	1.11
60	21.64	2-decanone	nd	0.61 ^b ± 0.61	0.51 ^{bc} ± 0.59	1.36 ^a ± 0.83	0.76 ^b ± 0.44	nd	0.11 ^c ± 0.23	0.44
65	22.95	3,5-octadien-2-one	nd	nd	nd	0.07 ± 0.21	0.17 ± 0.27	nd	nd	-
72	25.03	3,5-octadien-2-one	0.32 ^b ± 0.49	0.79 ^b ± 0.73	0.82 ^b ± 0.58	2.18 ^a ± 1.39	1.87 ^a ± 1.35	0.97 ^b ± 0.59	0.39 ^b ± 0.73	0.82
85	28.88	5-ethenyldihydro-5-methyl-2(3H)-furanone	nd	0.04 ^b ± 0.11	nd	nd	0.18 ^a ± 0.25	nd	nd	0.09
91	30.34	[1,1'-bicyclopentyl]-2-one	0.11 ± 0.18	0.04 ± 0.13	nd	nd	0.05 ± 0.14	nd	nd	-
97	31.53	1-(4,5-dihydro-2-thiazolyl)-ethanone	nd	nd	nd	0.03 ^b ± 0.06	nd	nd	0.20 ^a ± 0.18	0.06
98	31.62	5-ethyl-3-hydroxy-2(5H)-furanone	nd	nd	nd	0.05 ± 0.15	nd	nd	0.06 ± 0.17	-
106	33.64	(E)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone)	0.52 ^{ab} ± 0.51	0.26 ^{bc} ± 0.37	0.49 ^{ab} ± 0.30	0.73 ^a ± 0.31	0.54 ^{ab} ± 0.57	nd	0.12 ^c ± 0.29	0.34
Furans										
2	4.36	2-ethyl-furan	nd	3.65 ^{ab} ± 2.46	3.26 ^b ± 1.67	5.18 ^a ± 2.68	4.63 ^{ab} ± 1.28	3.54 ^{ab} ± 1.93	1.00 ^c ± 1.34	1.71
15	8.36	2-n-butyl-furan	nd	0.23 ^b ± 0.35	nd	0.77 ^a ± 1.42	0.24 ^{ab} ± 0.50	nd	nd	0.54
25	11.56	2-pentyl-furan	3.10 ^e ± 3.61	18.28 ^{abc} ± 13.83	11.32 ^{cde} ± 5.69	26.22 ^a ± 13.70	19.90 ^{ab} ± 11.35	11.90 ^{bcd} ± 5.58	5.40 ^{de} ± 5.57	8.45
35	14.16	cis-2-(2-pentenyl)furan	nd	0.36 ^b ± 0.63	nd	1.45 ^a ± 0.89	nd	nd	nd	0.35
67	23.19	2-n-octylfuran	nd	0.77 ^b ± 0.69	0.78 ^b ± 0.38	1.24 ^a ± 0.68	0.78 ^b ± 0.67	nd	0.29 ^c ± 0.54	0.46

Table 4.2 (Continued)

No.	Rt	VOC	Farms							LSD P = 0.05
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
<i>Hydrocarbons</i>										
3	4.64	3,3,6-trimethyl-1,5-heptadiene	1.02 ^a ± 1.08	nd	nd	nd	nd	nd	nd	0.33
6	5.28	3,7-dimethyl-, (Z)-2-octene	2.46 ^b ± 3.83	2.14 ^b ± 3.46	3.39 ^b ± 3.16	1.68 ^b ± 1.60	11.27 ^a ± 11.23	nd	nd	4.09
8	5.80	1-decene	nd	1.29 ^a ± 0.88	nd	1.49 ^a ± 1.12	1.27 ^a ± 2.12	nd	0.16 ^b ± 0.33	0.91
9	6.21	toluene	1.08 ^c ± 0.38	2.08 ^a ± 0.77	1.50 ^{bc} ± 0.58	1.62 ^{ab} ± 0.55	1.99 ^{ab} ± 0.67	1.09 ^c ± 0.46	1.58 ^{abc} ± 0.52	0.52
11	6.76	cyclodecene	nd	1.75 ± 1.57	nd	2.47 ± 2.11	1.83 ± 1.58	nd	nd	-
13	7.57	4-methyl-1-(1-methylethyl)-cyclohexene	nd	nd	nd	nd	1.62 ± 2.86	nd	nd	-
21	9.82	dodecane	nd	0.09 ± 0.28	0.03 ± 0.10	0.22 ± 0.37	0.24 ± 0.48	nd	nd	-
30	13.18	1-methyl-2-(1-methylethyl)-benzene	0.93 ± 1.85	nd	nd	nd	1.09 ± 1.00	nd	nd	-
31	13.39	tridecane	nd	1.08 ^b ± 0.83	1.25 ^b ± 0.87	2.19 ^a ± 1.11	2.39 ^a ± 1.85	nd	0.39 ^b ± 0.61	0.89
47	18.53	2,6,10,14-tetramethyl-hexadecane	nd	nd	nd	nd	0.30 ^b ± 0.33	nd	0.93 ^a ± 0.28	0.14
48	19.08	(Z)-3-ethyl-2-methyl-1,3-hexadiene	nd	1.77 ^{bc} ± 1.22	1.21 ^{bcd} ± 0.70	2.79 ^a ± 1.32	1.93 ^b ± 1.36	1.03 ^{cd} ± 0.57	0.45 ^d ± 0.57	0.84
51	19.70	1-methyl-4-(1-methylethenyl)-benzene	nd	nd	nd	nd	0.22 ± 0.62	nd	nd	-
57	21.07	3,5,5-trimethyl-2-hexene	nd	0.23 ^b ± 0.35	0.64 ^b ± 0.59	0.48 ^b ± 0.87	1.14 ^a ± 0.79	nd	nd	0.46
58	21.14	pentadecane	1.23 ^b ± 0.75	1.72 ^b ± 0.98	1.17 ^b ± 0.39	2.46 ^a ± 1.12	1.79 ^{ab} ± 1.09	nd	1.41 ^b ± 0.75	0.73
64	22.60	2,3,5,8-tetramethyl-1,5,9-decatriene	1.29 ^b ± 0.79	0.57 ^d ± 0.70	0.73 ^{bcd} ± 0.54	1.14 ^{bc} ± 0.44	2.93 ^a ± 0.83	nd	0.72 ^{cd} ± 0.70	0.56
66	23.11	1-pentadecene	nd	0.20 ^b ± 0.35	nd	0.24 ^b ± 0.34	0.56 ^a ± 0.53	nd	nd	0.24
84	28.60	1,3-cyclooctadiene	0.84 ^c ± 0.72	0.60 ^c ± 0.53	1.58 ^b ± 0.66	1.78 ^b ± 0.90	2.46 ^a ± 1.12	nd	0.90 ^c ± 0.12	0.63
86	29.36	(Z)-3-heptadecene	0.09 ^a ± 0.27	nd	nd	nd	nd	nd	nd	0.08
94	31.21	2-ethyl-1-hexanol or 3,4-dimethyl-1-decene	nd	0.41 ^{bc} ± 0.38	0.40 ^{bc} ± 0.38	1.01 ^a ± 0.64	0.60 ^b ± 0.61	0.36 ^{bc} ± 0.26	0.09 ^c ± 0.19	0.37
102	33.00	1,4-octadiene	nd	0.12 ^c ± 0.26	nd	0.71 ^a ± 0.36	0.45 ^b ± 0.45	nd	nd	0.21
105	33.46	[R-[R*,R*-(E)]]-3,7,11,15-tetramethyl-2-hexadecene	nd	nd	nd	0.23 ^b ± 0.24	nd	nd	0.50 ^a ± 0.13	0.10

Table 4.2 (Continued)

No.	Rt	VOC	Farms							LSD P = 0.05
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
110	34.72	1-methyl-3-(1-methylethyl)-cyclohexene	nd	0.41 ^b ± 0.44	nd	0.84 ^a ± 0.71	0.47 ^b ± 0.60	nd	nd	0.35
113	36.34	(3E,5Z)-1,3,5-undecatriene	nd	0.28 ^b ± 0.30	0.17 ^b ± 0.19	0.71 ^a ± 0.46	0.40 ^b ± 0.39	nd	nd	0.24
118	39.54	nonane	nd	0.04 ^b ± 0.12	nd	0.21 ^a ± 0.28	nd	nd	nd	0.11
121	40.43	cyclododecane	nd	0.30 ^b ± 0.55	nd	1.00 ^a ± 0.78	0.94 ^a ± 0.91	nd	nd	0.45
Organic acids										
53	20.02	acetic acid	0.49 ^b ± 0.13	0.91 ^a ± 0.51	0.88 ^a ± 0.25	1.04 ^a ± 0.37	1.17 ^a ± 0.53	nd	0.85 ^a ± 0.43	0.33
78	26.74	butanoic acid	nd	0.50 ^{ab} ± 0.36	0.70 ^a ± 0.16	0.16 ^{cd} ± 0.27	0.29 ^{bc} ± 0.39	nd	0.05 ^d ± 0.15	0.21
80	27.27	(E)-2-hexenyl ester hexanoic acid	nd	0.24 ± 0.47	nd	0.16 ± 0.40	0.30 ± 0.51	nd	nd	-
104	33.24	hexanoic acid	0.80 ^b ± 0.32	1.57 ^a ± 0.78	1.50 ^a ± 0.39	1.76 ^a ± 0.72	1.60 ^a ± 0.67	1.00 ^b ± 0.19	0.68 ^d ± 0.41	0.48
108	34.05	1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester-2-methyl-propanoic acid	nd	0.12 ± 0.24	nd	0.17 ± 0.23	0.14 ± 0.23	nd	0.04 ± 0.11	0.14
115	38.22	octanoic acid	0.51 ^c ± 0.12	0.67 ^{bc} ± 0.27	0.97 ^a ± 0.18	0.82 ^{ab} ± 0.24	0.68 ^{bc} ± 0.17	0.64 ^{bc} ± 0.10	0.25 ^d ± 0.23	0.17
120	40.40	nonanoic acid	0.64 ^{abc} ± 0.21	0.68 ^{abc} ± 0.46	0.74 ^{ab} ± 0.34	0.42 ^{bc} ± 0.46	0.40 ^c ± 0.49	nd	0.76 ^a ± 0.37	0.33
123	42.42	decanoic acid	nd	0.44 ^a ± 0.35	0.57 ^a ± 0.29	0.50 ^a ± 0.23	0.63 ^a ± 0.28	nd	0.11 ^b ± 0.20	0.21
Terpenes										
7	5.70	α-pinene	2.68 ^b ± 3.94	nd	0.26 ^b ± 0.43	nd	13.60 ^a ± 15.23	nd	1.29 ^b ± 1.43	4.92
14	7.74	β-pinene	1.38 ^b ± 2.19	0.77 ^b ± 2.44	nd	0.05 ^b ± 0.15	22.71 ^a ± 4.01	nd	nd	1.60
16	8.66	trans-carane	nd	nd	nd	nd	0.82 ± 1.01	nd	nd	-
19	9.18	β-myrcene	nd	nd	nd	nd	0.03 ± 0.08	nd	nd	-
24	10.56	limonene	nd	0.71 ^b ± 1.76	nd	0.42 ^b ± 1.34	4.06 ^a ± 4.08	nd	nd	1.52
56	20.73	cis-linaloloxide	nd	nd	0.04 ± 0.12	0.13 ± 0.25	nd	nd	0.04 ± 0.14	-
61	21.69	α-copaene	0.42 ± 0.46	nd	nd	nd	0.63 ± 0.48	nd	nd	-
68	23.22	α-gurjunene	0.10 ± 0.20	nd	nd	nd	nd	nd	nd	-
74	25.95	trans-caryophyllene	1.04 ^b ± 0.75	0.85 ^b ± 0.69	1.06 ^b ± 0.41	1.18 ^b ± 0.48	3.69 ^a ± 1.77	nd	0.78 ^b ± 0.74	0.73
Nitrogen- and/or sulphur-containing compounds										

Table 4.2 (Continued)

No.	Rt	VOC	Farms							LSD P = 0.05
			CK	NK	HK/LO	KV	HK/CAL	RU	FS	
69	23.38	N-ethyl-2-methylallylamine	nd	0.31 ^b ± 0.38	nd	0.89 ^a ± 0.71	0.60 ^{ab} ± 0.76	nd	nd	0.37
77	26.64	2-ethyl-4,5-dimethyl-thiazole (solan, herbicide)	nd	0.61 ^b ± 0.61	nd	1.39 ^a ± 1.11	0.70 ^b ± 0.74	nd	0.10 ^c ± 0.22	0.50
87	29.45	3-mercaptohexyl hexanoate	nd	0.08 ^c ± 0.13	nd	0.58 ^a ± 0.19	0.42 ^b ± 0.20	nd	0.19 ^c ± 0.24	0.13
Lactones										
82	27.65	butyrolactone	0.93 ^{ab} ± 0.62	0.68 ^b ± 0.53	0.77 ^{ab} ± 0.44	1.21 ^a ± 0.49	1.18 ^a ± 0.74	nd	0.76 ^{ab} ± 0.51	0.47
Phenolic compounds										
99	32.17	2,3,4,6-tetramethyl-phenol	nd	0.08 ^b ± 0.16	nd	0.28 ^a ± 0.27	0.31 ^a ± 0.30	nd	nd	0.15
107	33.82	3-methyl-6-propyl-phenol	nd	0.16 ± 0.29	nd	0.18 ± 0.26	0.22 ± 0.37	nd	nd	-
Other and unidentified compounds										
17	8.86	pentyl-oxirane	nd	0.24 ± 0.30	nd	0.47 ± 0.57	0.50 ± 0.73	nd	nd	-
59	21.32	2-anthracenamine	nd	1.32 ^b ± 0.19	nd	1.59 ^a ± 0.36	1.61 ^a ± 0.51	nd	nd	0.21
79	26.92	unidentified	nd	0.23 ± 0.48	nd	0.44 ± 0.54	0.49 ± 0.57	nd	nd	-
117	39.27	unidentified	nd	0.15 ^b ± 0.23	nd	0.57 ^a ± 0.35	0.46 ^a ± 0.45	nd	nd	0.21
122	41.95	unidentified	nd	0.37 ^b ± 0.25	nd	0.56 ^a ± 0.21	0.43 ^{ab} ± 0.31	nd	0.29 ^b ± 0.25	0.18

(No.) Number; (Rt) Retention time in min; (VOC) Volatile organic compound; (SD) Standard Deviation; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rùens; (FS) Free State; (LSD) Least significant difference at the 5% level of significance; ^{a-e} Values in the same row with different superscripts are significantly different (P ≤ 0.05); (nd) None detected.

Table 4.3 Ten predominant volatile organic compounds of the plants samples values in brackets expressed as percentage (%) relative to the internal standard

Code	Species name	VOC detected in decreasing order of relative concentration
RU1	<i>Medicago sativa</i>	3-octanone (48); ethyl ester-benzoic acid (34), phenylethyl alcohol (21); benzaldehyde (16); benzeneacetaldehyde (11); ethyl ester-hexadecanoic acid (7); 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (7); benzyl alcohol (5); nonanal (5); ethyl ester-hexanoic acid (4)
RU2	<i>Medicago sativa</i>	3-octanone (31); ethyl ester-benzoic acid (23), benzaldehyde (17); phenylethyl alcohol (13); benzeneacetaldehyde (11); ethyl ester-hexadecanoic acid (5); benzyl alcohol (5); 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (4); nonanal (3); 1-octen-3-ol (2)
RU3	<i>Medicago sativa</i>	3-octanone (45); phenylethyl alcohol (14); benzaldehyde (10); 6,10,14-trimethyl-2-pentadecanone (8); methyl ester-2-hydroxy-benzoic acid (7); hexanoic acid (6); 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (6); ethyl ester-hexanoic acid (6); dodecane (5); benzyl alcohol (5)
RU4	<i>Cynodon dactylon</i>	3-octanone (19); ethyl ester-decanoic acid (7); benzaldehyde (5); benzeneacetaldehyde (4); ethyl ester-benzoic acid (4), ethyl ester-hexadecanoic acid (3); ethyl ester-dodecanoic acid (3); phenylethyl alcohol (3); ethyl ester-nonanoic acid (2); nonanal (2)
FS1	<i>Themeda triandra</i>	benzeneacetaldehyde (25); benzaldehyde (18); caprolactam (17); benzyl alcohol (17); 3-octanone (15); phenylethyl alcohol (10); 2-methyl-phenol (10); 1-chloro-pentane (8); nonanal (8); 2-ethyl-1-hexanol (7)
FS2	<i>Cynodon dactylon</i>	benzaldehyde (53); caprolactam (52); benzeneacetaldehyde (36); 6,10,14-trimethyl-2-pentadecanone (34); nonanal (33); 2-hexenal (23); 2-pentyl-furan (22); hexanal (20); 3-ethyl-3-octanol (20); 2-ethyl-furan (19)
FS3	<i>Eragrostis superba</i>	2-heptanol (88); 2-heptanone (84); benzaldehyde (48); 2-undecanone (47); 6-methyl-3-heptanone (42); ethyl ester-dodecanoic acid (30); 2-nonanone (19); phenylethyl alcohol (18); 1-undecene (17); 2-nonanol (16)
FS4	<i>Fingerhuthia africana</i>	caprolactam (20); nonanal (11); decanal (9); 1-pentanol (8); 6-methyl-3-heptanone (8); 2-ethyl-1-hexanol (8); benzaldehyde (6); octanal (6); pentadecane (4); 6,10,14-trimethyl-2-pentadecanone (4)
FS5	<i>Aristida congesta subsp. congesta</i>	6-methyl-3-heptanone (26); nonanal (13); caprolactam (11); 1-hexanol (9); benzaldehyde (7); octanal (6); 1-octanol (5); decanal (5); 2-ethyl-1-hexanol (5); pentadecane (4)
FS6	<i>Schmidtia kalahariensis</i>	caprolactam (23); 6-methyl-3-heptanone (9); 2-ethyl-1-hexanol (8); nonanal (8); 1-pentanol (8); 3-ethyl-3-octanol (7); benzaldehyde (5); 1-hexanol (5); 6,10,14-trimethyl-2-pentadecanone (5); acetic acid (4)
FS7	<i>Eragrostis lehmanniana</i>	3-octanone (36); caprolactam (21); hexyl ester-formic acid (10); nonanal (9); benzaldehyde (8); benzeneacetaldehyde (7); benzyl alcohol (6); phenylethyl alcohol (6); 6-methyl-3,5-heptadiene-2-one (6); 1-octanol (5)
FS8	<i>Heteropogon contortus</i>	3-octanone (21); 6-methyl-5-hepten-2-ol (15); caprolactam (14); 1-hexanol (11); benzeneacetaldehyde (9); nonanal (7); 2-ethyl-1-hexanol (6); benzaldehyde (6); ethyl ester-nonanoic acid (5); ethyl ester-benzeneacetic acid (5)
CK1	<i>Pentzia incana</i>*	eucalyptol (523); 1,5-dimethyl-1-vinyl-4-hexenyl butyrate (223); (1R)-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (92); 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (84); 1,5,5-trimethyl-6-methylene-cyclohexene (66); α -cubebene (53); β -pinene (41); copaene (34); β -myrcene (30); α -pinene (23)
CK2	<i>Plinthus karrooicus</i>*	eucalyptol (618); α -cubebene (208); 1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone (188); spathulenol (149); β -pinene (136); α -pinene (122); 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene (99); 1-methyl-2-(1-methylethyl)-benzene (58), α -cadinol (56); β -terpineol (36)
CK3	<i>Lycium cinereum</i>	spathulenol (29); benzeneacetaldehyde (11); nonanal (10); benzyl alcohol (10); phenylethyl alcohol (7); eucalyptol (6); 3-allyl-6-methoxyphenol (6); benzaldehyde (6); 2-methoxy-phenol (6); 3-hexen-1-ol (5)
CK4	<i>Ruschia intricata</i>	styrene (34); 3-ethyl-3-octanol (4); nonanal (4); 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (3); benzeneacetaldehyde (3); benzaldehyde (2); 3-octanone (2); acetic acid (2); 2-heptenal (1); eucalyptol (1)

Table 4.3 (Continued)

Code	Species name	VOC detected in decreasing order of relative concentration
CK5	<i>Galenia sarcophylla</i>	nonanal (8); 2-hexenal (6); 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (5); 2-methyl-1-penten-3-one (4); benzyl alcohol (4); 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (4); benzaldehyde (3); ethyl ester-nonanoic acid (3); eucalyptol (3); 1-methyl-1H-1,2,4-triazole (3)
CK6	<i>Stipagrostis ciliata</i>	3-octanone (8); nonanal (7); decanal (6); 1-pentanol (5); benzeneacetaldehyde (5); benzaldehyde (5); eucalyptol (4); pentadecane (3); octanal (2); 6,10,14-trimethyl-2-pentadecanone (2)
CK7	<i>Stipagrostis obtusa</i>	hexadecane (17); 2-pentyl-furan (11); 6,10,14-trimethyl-2-pentadecanone (10); 3-octanone (10); benzeneacetaldehyde (9); decanal (8); nonanal (8); benzaldehyde (6); ethyl ester-tetradecanoic acid (5); 6,10-dimethyl-2-undecanone (5)
CK8	<i>Salsola glabrescens</i>	2-methyl-butanoic acid (32); 3-octanone (27); ethyl ester-benzeneacetic acid (9); ethyl ester-hexanoic acid (8); butanoic acid (8); eucalyptol (8); benzaldehyde (5); ethyl ester-butanoic acid (5); phenylethyl alcohol (4)
KV1	<i>Lebeckia leipoldtiana</i>	ethyl ester-benzoic acid (108); methyl ester-benzoic acid (26); benzeneacetaldehyde (18); butanoic acid (15); 3-allyl-6-methoxyphenol (12); phenylethyl alcohol (11); 4-hexen-3-one (9); ethyl ester-butanoic acid (9); 2-pentyl-furan (8); 3-octanone (8)
KV2	<i>Wiborgia monoptera</i>	2-pentyl-furan (28); benzeneacetaldehyde (21); 2-methyl-butanoic acid (17); ethyl ester-benzoic acid (14); nonanal (13); 1-hexanol (13); hexanoic acid (12); 3-octanone (12); benzaldehyde (11); acetic acid (10)
KV3	<i>Wiborgia sericea</i>	benzeneacetaldehyde (35); benzaldehyde (21); 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (13); 3-octanone (10); 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (9); acetic acid (7); hexyl ester-formic acid (7); phenylethyl alcohol (7); 2-hexenal (6); 2-methyl-hexanoic acid (6)
KV4	<i>Eriocephalus punctulatus</i>*	α -cubebene (155); 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (137); 1-methyl-2-(1-methylethyl)-benzene (122); α -pinene (111); 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene (101); eucalyptol (100); pinen-3-one (69); 3,7,7-trimethyl-bicyclo[4.1.0]hept-2-ene (55); α -phellandrene (11); D-limonene (11)
KV5	<i>Galenia africana</i>	benzaldehyde (57); phenol (36); styrene (29); ethyl ester-benzenepropanoic acid (25); 2-hydroxy-benzaldehyde (13); ethyl ester-benzoic acid (8); eucalyptol (6); benzyl alcohol (6); 2-hexenal (6); benzeneacetaldehyde (5)
KV6	<i>Pentzia incana</i>*	2-methyl-1-penten-3-one (580); copaene (238); eucalyptol (234); 3,3,6-trimethyl-1,5-heptadien-4-one (210); γ -elemene (199); caryophyllene (191); isocyclocitral (147); α -cubebene (135); estragole (103); 2,5,5-trimethyl-1,3,6-heptatriene (95)
KV7	<i>Nylandtia spinosa</i>	1-octanol (41); phenylethyl alcohol (40); 2-hexenal (34); 2-methyl-1-penten-3-one (32); dihydro-3-methylene-2(3H)-furanone (28); ethyl ester-hexanoic acid (25); benzyl alcohol (17); eucalyptol (11); ethyl ester-3-hexenoic acid (11); cyclohexanol (10)
KV8	<i>Chaetobromus dregeanus</i>	3-octanone (19); benzaldehyde (5); benzeneacetaldehyde (5); 2-ethyl-1-hexanol (4); 1-pentanol (3); 3-methyl-1-butanol (3); phenylethyl alcohol (3); acetic acid (2); azulene (2); ethyl ester-hexadecanoic acid (2)
KV9	<i>Ereiodium moschatum</i>	9-octadecyne (104); benzeneacetaldehyde (35); benzaldehyde (24); cis-2-(2-pentenyl)furan (24); α -ethylidene-benzeneacetaldehyde (17); ethyl ester-undecanoic acid (11); ethyl ester-benzeneacetic acid (10); caryophyllene (10); 2-methyl-1-penten-3-one (10); 2-pentyl-furan (6)
KV10	<i>Medicago polymorpha</i>	3-octanone (107); ethyl ester-octanoic acid (40); benzaldehyde (37); ethyl ester-hexanoic acid (32); phenylethyl alcohol (30); 3-allyl-6-methoxyphenol (25); benzeneacetaldehyde (19); 4-hexen-3-one (17); ethyl ester-dodecanoic acid (14); benzyl alcohol (12)
NK1	<i>Stipagrostis obtusa and ciliata</i>	(isothiocyanatomethyl)-benzene (36); 3-octanone (31); nonanal (21); acetic acid (13); decanal (12); octanal (11); benzaldehyde (10); 3-ethyl-3-octanol (10); tridecane (10); thymol (7)
NK2	<i>Fingerhuthia africana</i>	3-octanone (40); 1-tetradecene (33); 10-undecen-1-ol (11); benzaldehyde (10); 6,10-dimethyl-2-undecanone (8); acetic acid (8); decanal (7); nonanal (7); caprolactam (7); 1-nonadecene (7)

Table 4.3 (Continued)

Code	Species name	VOC detected in decreasing order of relative concentration
NK3	<i>Felicia muricata</i>	3-hexen-1-ol (92); benzyl alcohol (90); 3-octanone (40); benzaldehyde (22); n-valeric acid cis-3-hexenyl ester (17); nonanal (16); benzeneacetaldehyde (16); hexyl ester-formic acid (15); ethyl ester-hexanoic acid (13); phenylethyl alcohol (13)
NK4	<i>Pteronia glauca*</i>	1,2,3-trimethoxy-5-(2-propenyl)-benzene (927); 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene (921); caryophyllene (711); D-limonene (455); β -pinene (204); 1-methyl-2-(1-methylethyl)-benzene (194); α -pinene (161); α -phellandrene (92); α -caryophyllene (87); 3,7-dimethyl-1,3,6-octatriene (71)
NK5	<i>Salsola calluna</i>	3-octanone (46); ethyl ester-benzoic acid (24); 2-hexenal (23); dl-6-methyl-5-hepten-2-ol (21); hexyl ester-formic acid (18); benzyl alcohol (11); methyl ester-benzoic acid (10); acetic acid (8); benzaldehyde (6); 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (6)
NK6	<i>Phaeoptilum spinosum</i>	2-methyl-5-(1-methylethyl)-phenol (121); caryophyllene (16); 1-methyl-2-(1-methylethyl)-benzene (15); 3-octanone (13); benzaldehyde (12); 6,10-dimethyl-5,9-undecadien-2-one (9); 2,6,6-trimethyl-2-cyclohexene-1,4-dione (8); 2,6-nonadienal (5); naphthalene (4); α -phellandrene (4)
NK7	<i>Psilocaulon absimile</i>	2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (39); 1-piperidinecarboxaldehyde (33); 1-acetyl-piperidine (16); 2-methoxy-3-(2-propenyl)-phenol (15); acetic acid (12); ethyl ester-decanoic acid (12); 2,6,6-trimethyl-2,4-cycloheptadien-1-one (10); benzaldehyde (10); phenylethyl alcohol (9); 1-hexanol (8)
NK8	<i>Salsola tuberculata</i>	ethyl ester-benzoic acid (55); 3-hexen-1-ol (17); 3-octanone (16); benzyl alcohol (10); ethyl ester-hexanoic acid (9); butanoic acid (7); phenylethyl alcohol (6); 1-hexanol (6); benzaldehyde (5); hexanoic acid (4)
NK9	<i>Eriocephalus ericoides*</i>	eucalyptol (439); 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (122); α -cubebene (114); tridecyl ester-3-methyl-2-butenic acid (50); 1-methyl-2-(1-methylethyl)-benzene (27); 2-pentyl ester-pentanoic acid (23); 3-methyl-4-isopropylphenol (18); benzaldehyde (18); α -pinene (16); 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol (15)
NK10	<i>Rhigozum trichotomum</i>	caryophyllene (555); 1-Octen-3-ol (365); α -pinene (79); 1,7,7-trimethyl-tricyclo[2.2.1.0(2,6)]heptane (22); α -cubebene (18); camphene (16); benzyl alcohol (13); 1,Z-5,E-7-dodecatriene (13); 3-octanone (12); 3-methyl-1-butanol (9)
NK11	<i>Unidentified</i>	acetate-5-methyl-2-(1-methylethyl)-phenol (3093); thymol (1392); 1-methyl-3-(1-methylethyl)-benzene (1106); 5,9,9-trimethyl-spiro[3.5]non-5-en-1-one (418); caryophyllene (255); β -myrcene (192); 3,7-dimethyl-1,3,6-octatriene (182); spathulenol (174); α -phellandrene (136); α -pinene (135); β -pinene (119); limonene (109)
NK12	<i>Pentzia incana*</i>	1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (144); eucalyptol (136); 1-methyl-2-(1-methylethyl)-benzene (36); acetate-2,6,6-trimethyl-bicyclo[3.1.1]hept-2-en-4-ol (32); 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (27); methyl ester-2-hydroxy-benzoic acid (22); 2,5,5-trimethyl-1,3,6-heptatriene (17); acetate-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol (15); 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene (13); copaene (13)
NK13	<i>Rosenia humilis*</i>	1-methyl-4-(1-methylethyl)-benzene (114); spathulenol (110); α -pinene (82); 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (33); benzyl alcohol (25); 3-octanone (18); 3-hexen-1-ol (12); 2-hexenal (10); γ -elemene (10); D-limonene (9)
NK14	<i>Salsola aphylla</i>	1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone (50); 3-octanone (44); benzyl alcohol (28); ethyl ester-benzoic acid (27); ethyl ester-hexanoic acid (18); phenylethyl alcohol (18); 3-hexen-1-ol (14); butanoic acid (10); benzaldehyde (9); 6,10-dimethyl-5,9-undecadien-2-one (9)
NK15	<i>Zygophyllum gilfillanii</i>	ethyl ester-benzeneacetic acid (45); 3-methyl-4-isopropylphenol (25); benzyl alcohol (14); 3-octanone (13); 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (7); 2-methyl-1-propanol (5); phenylethyl alcohol (5); 1-(2-hydroxy-6-methoxyphenyl)-ethanone (4); 6-methyl-5-hepten-2-ol (3); 2-methyl-1-butanol (3)
NK16	<i>Ruschia intricata</i>	styrene (121); 2,6-dimethyl-4-heptanol (13); benzyl alcohol (11); 3-octanone (10); 1-methyl-3-(1-methylethyl)-benzene (7); benzeneacetaldehyde (5); phenylethyl alcohol (5); β -phellandrene (4); 2-heptanol (3); ethyl ester-benzeneacetic acid (2)
NK17	<i>Lycium cinereum</i>	benzyl alcohol (50); 3,7-dimethyl-1,6-octadien-3-ol (21); 3-octanone (10); benzeneacetaldehyde (6); phenylethyl alcohol (5); nonanal (5); eugenol (4); 1-octen-3-ol (4); 2-methoxy-phenol (3); ethyl ester-hexanoic acid (3)

Table 4.3 (Continued)

Code	Species name	VOC detected in decreasing order of relative concentration
HK/CAL1	<i>Pentzia incana</i> *	5,9,9-trimethyl-spiro[3.5]non-5-en-1-one (626); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (404); eucalyptol (281); 2-aminobenzoate-3,7-dimethyl-1,6-octadien-3-ol (115); 1-methyl-4-(1-methylethyl)-Benzene (87); caryophyllene (59); α -cubebene (57); copaene (51); α -pinene (44); 1,5,5-trimethyl-6-methylene-cyclohexene (42); α -cadinol (38)
HK/CAL2	<i>Pentzia sphaerocephala</i> *	α -pinene (144); (+)-epi-bicyclosesquiphellandrene (70); α -cubebene (59); 1-(1,4-dimethyl-3-cyclohexen-1-yl)-ethanone (46); α -cadinol (33); pinen-3-one (32); 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol (32); 1-methyl-2-(1-methylethyl)-benzene (25); eucalyptol (17); γ -elemene (16)
HK/CAL3	<i>Chrysocoma ciliata</i> *	benzaldehyde (181); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (178); 2-aminobenzoate-3,7-dimethyl-1,6-octadien-3-ol (172); caryophyllene (162); α -pinene (117); ethyl ester-benzoic acid (112); γ -elemene (76); eucalyptol (64); β -pinene (61); 1-methyl-2-(1-methylethyl)-benzene (33)
HK/CAL4	<i>Eriocephalus ericoides</i> *	2-aminobenzoate-3,7-dimethyl-1,6-octadien-3-ol (227); eucalyptol (218); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (193); γ -elemene (65); 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (53); α -pinene (35); 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (33); caryophyllene (32); 3,7-dimethyl-1,6-octadien-3-ol (30); 1-methyl-2-(1-methylethyl)-benzene (20)
HK/CAL5	<i>Salsola calluna</i>	1-(2-hydroxy-4,6-dimethoxyphenyl)-ethanone (41); 3-octanone (34); ethyl ester-benzeneacetic acid (26); phenylethyl alcohol (21); 2,2,4,6,6-pentamethyl-heptane (18); ethyl ester-benzoic acid (11); acetic acid (7); benzaldehyde (7); naphthalene (7); benzyl alcohol (6)
HK/CAL6	<i>Justica orchiioides</i> *	limonene (496); 1-methyl-2-(1-methylethyl)-benzene (367); 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (326); eucalyptol (318); 3,7-dimethyl-1,3,6-octatriene (311); caryophyllene (310); acetate-3,7-dimethyl-2,6-octadien-1-ol (279); α -pinene (201); 3,7-dimethyl-1,3,6-octatriene (155); 1-undecene (143)
HK/CAL7	<i>Zygophyllum lichtensteinianum</i>	3-octanone (32); 2-hexenal (18); 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (12); naphthalene (9); 1-pentanol (5); 2-ethyl-furan (4); 1-(2,4-dimethylphenyl)-ethanone (4); cyclohexanol (3); ethyl ester-benzeneacetic acid (3); 2-ethyl-1-hexanol (3)
HK/CAL8	<i>Lycium spp.</i>	γ -elemene (44); caryophyllene (41); 10s,11s-himachala-3(12),4-diene (37); 3-octanone (17); 3-hexen-1-ol (15); 2,2,4,6,6-pentamethyl-heptane (15); benzyl alcohol (14); 2-hexenal (13); naphthalene (11); benzeneacetaldehyde (10)
HK/CAL9	<i>Eberlanzia ferox</i>	styrene (47); 3-octanone (25); 2,2,4,6,6-pentamethyl-heptane (10); acetic acid (6); azulene (4); 2-ethyl-1-hexanol (3); benzaldehyde (3); benzeneacetaldehyde (3); nonanal (2); ethyl ester-benzoic acid (2)
HK/CAL10	<i>Drosanthemum hispidum</i>	3-octanone (26); 2,2,4,6,6-pentamethyl-heptane (13); benzyl alcohol (12); phenylethyl alcohol (9); benzaldehyde (6); benzeneacetaldehyde (5); 1-hexanol (5); 2-hexenal (4); dimethyl trisulfide (3); 3-methyl-1-butanol (3)
HK/CAL11	<i>Mesembryanthemum vaginatum</i>	3-octanone (30); ethyl ester-benzoic acid (19); benzaldehyde (12); benzyl alcohol (12); phenylethyl alcohol (7); benzeneacetaldehyde (7); 2-methoxy-phenol (7); acetic acid (6); 2-methoxy-4-vinylphenol (5); 3-methyl-1-butanol (5)
HK/LO1	<i>Pentzia incana</i> *	5,9,9-trimethyl-spiro[3.5]non-5-en-1-one (847); 4-methyl-1,6-heptadien-4-ol (532); eucalyptol (509); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (432); 3,3,6-trimethyl-1,5-heptadien-4-ol (286); 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene (278); caryophyllene (167); 2-aminobenzoate-3,7-dimethyl-1,6-octadien-3-ol (107); γ -elemene (84); α -pinene (82)
HK/LO2	<i>Tetragonia fruticosa</i>	3-octanone (27); benzaldehyde (9); phenylethyl alcohol (6); 2-hexenal (5); 2-ethyl-1-hexanol (3); benzyl alcohol (2); 6-methyl-3,5-heptadiene-2-one (2); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (2); 3,3,6-trimethyl-1,5-heptadien-4-ol (2); 1-pentanol (1)
HK/LO3	<i>Chrysanthemoides incana</i>	caryophyllene (53); 3-octanone (27); 2,2,4,6,6-pentamethyl-heptane (22); benzaldehyde (12); ethyl ester-benzoic acid (9); α -cubebene (8); phenylethyl alcohol (5); azulene (5); 2-hydroxy-benzaldehyde (4); benzyl alcohol (3)
HK/LO4	<i>Salsola tuberculata</i>	3-octanone (34); 2,2,4,6,6-pentamethyl-heptane (20); benzaldehyde (17); phenylethyl alcohol (16); 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (14); 3,3,6-trimethyl-1,5-heptadien-4-ol (13); ethyl ester-decanoic acid (12); benzyl alcohol (10); 1-methyl-2-(1-methylethyl)-benzene (9); 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene (9)

Table 4.3 (Continued)

Code	Species name	VOC detected in decreasing order of relative concentration
HK/LO5	<i>Stoeberia beetzii</i>	3-octanone (125); ethyl ester-benzoic acid (58); 2-butenal (57); benzaldehyde (30); 2,2,4,6,6-pentamethyl-heptane (29); camphor (18); benzyl alcohol (16); 1-butanol (9); naphthalene (8); phenylethyl alcohol (7)
HK/LO6	<i>Pteronia sordida</i> *	5,9,9-trimethyl-spiro[3.5]non-5-en-1-one (329); 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene (267); 1-methyl-4-(1-methylethyl)-benzene (254); 3,7-dimethyl-1,3,6-octatriene (185); caryophyllene (120); 6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (53); 1-undecene (37); α -pinene (34); D-limonene (33); pinocarveol (27)
HK/LO7	<i>Zygophyllum lichtensteinianum</i>	3-octanone (24); 2-ethyl-1-hexanol (2); benzeneacetaldehyde (2); 6,6-dimethyl-2-methylene-bicyclo[2.2.1]heptan-3-one (1); benzaldehyde (1); azulene (1); acetic acid (1); benzyl alcohol (1); ethyl ester-benzoic acid (1); 2,4-heptadienal (1)
HK/LO8	<i>Unidentified</i>	2,2,4,6,6-pentamethyl-heptane (36); 3-octanone (34); 2-hexenal (19); benzeneacetaldehyde (14); naphthalene (12); 1,7,7-trimethyl-Bicyclo[2.2.1]heptan-2-one (10); eucalyptol (10); phenylethyl alcohol (9); benzaldehyde (5); benzyl alcohol (5)

(CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State; ***Fragrant/Herbaceous bushes indicated in bold.**

Table 4.4 Means (\pm SD) for the fatty acid composition (% of total fatty acids) of the lamb meat

Fatty acid	Farms							LSD
	CK	NK	HK/LO	KV	HK/CAL	RU	FS	P = 0.05
SFA								
C14:0	3.44 ^{cd} \pm 0.91	5.01 ^{ab} \pm 0.91	5.26 ^a \pm 1.22	4.28 ^{bc} \pm 0.82	3.78 ^{cd} \pm 0.79	2.94 ^d \pm 0.83	3.63 ^{cd} \pm 1.06	0.85
C15:0	1.02 ^a \pm 0.19	0.93 ^{ab} \pm 0.10	0.90 ^b \pm 0.14	0.78 ^c \pm 0.14	0.74 ^c \pm 0.09	0.93 ^{ab} \pm 0.10	0.93 ^{ab} \pm 0.15	0.12
C16:0	21.51 ^c \pm 2.95	26.25 ^a \pm 2.10	25.73 ^{ab} \pm 1.95	22.14 ^c \pm 0.87	24.12 ^b \pm 1.72	22.08 ^c \pm 1.70	24.87 ^{ab} \pm 3.15	1.95
C18:0	17.81 ^a \pm 1.99	14.17 ^{cd} \pm 1.34	13.37 ^d \pm 0.84	15.62 ^b \pm 0.42	17.50 ^a \pm 1.96	15.54 ^{bc} \pm 1.71	15.91 ^b \pm 1.82	1.40
C20:0	0.97 ^a \pm 0.51	0.43 ^{bc} \pm 0.35	0.27 ^c \pm 0.37	0.54 ^{bc} \pm 0.31	0.32 ^c \pm 0.34	0.43 ^{bc} \pm 0.57	0.73 ^{ab} \pm 0.48	0.38
C21:0	0.74 ^{ab} \pm 0.24	0.42 ^d \pm 0.13	0.43 ^d \pm 0.14	0.46 ^{cd} \pm 0.15	0.45 ^{cd} \pm 0.08	0.84 ^a \pm 0.17	0.60 ^{bc} \pm 0.21	0.15
C22:0	0.62 ^{ab} \pm 0.61	0.63 ^{ab} \pm 0.22	0.44 ^{ab} \pm 0.28	nd	0.73 ^a \pm 0.12	0.73 ^a \pm 0.67	0.31 ^b \pm 0.44	0.37
C23:0	0.08 ^b \pm 0.15	0.04 ^b \pm 0.12	0.03 ^b \pm 0.11	0.33 ^a \pm 0.24	0.29 ^a \pm 0.20	nd	0.13 ^b \pm 0.19	0.15
C24:0	1.27 ^{bc} \pm 0.54	1.14 ^c \pm 0.55	1.54 ^{bc} \pm 0.47	1.70 ^{ab} \pm 0.45	1.35 ^{bc} \pm 0.26	2.08 ^a \pm 0.61	1.43 ^{bc} \pm 0.40	0.43
MUFA								
C14:1	0.96 ^a \pm 0.27	0.54 ^{cd} \pm 0.16	0.56 ^{cd} \pm 0.16	0.41 ^d \pm 0.19	0.64 ^{bc} \pm 0.18	0.78 ^b \pm 0.19	0.59 ^c \pm 0.16	0.17
C15:1	0.75 ^a \pm 0.26	0.36 ^b \pm 0.13	0.41 ^b \pm 0.14	0.45 ^b \pm 0.16	0.43 ^b \pm 0.09	0.45 ^b \pm 0.32	0.45 ^b \pm 0.28	0.19
C16:1	1.73 ^c \pm 0.28	2.20 ^b \pm 0.26	2.48 ^a \pm 0.34	1.97 ^b \pm 0.19	1.66 ^c \pm 0.19	1.72 ^c \pm 0.20	1.71 ^c \pm 0.36	0.24
C18:1 <i>n</i> -9c	31.55 ^c \pm 3.32	32.97 ^{abc} \pm 3.48	34.25 ^{ab} \pm 1.07	35.49 ^a \pm 1.97	31.97 ^{bc} \pm 2.65	33.11 ^{abc} \pm 3.30	35.06 ^a \pm 3.77	2.66
C20:1	0.57 ^a \pm 0.20	0.23 ^{bc} \pm 0.18	0.19 ^c \pm 0.18	0.40 ^{ab} \pm 0.15	0.17 ^c \pm 0.18	0.49 ^a \pm 0.30	0.53 ^a \pm 0.17	0.18
PUFA								
C18:2 <i>n</i> -6c	7.15 ^a \pm 1.99	7.59 ^a \pm 1.52	6.47 ^a \pm 1.34	6.66 ^a \pm 0.82	7.41 ^a \pm 1.35	7.59 ^a \pm 1.95	4.55 ^b \pm 1.81	1.42
C18:2 <i>n</i> -6t	0.71 ^b \pm 0.18	0.45 ^c \pm 0.11	0.71 ^b \pm 0.12	0.71 ^b \pm 0.15	0.68 ^b \pm 0.09	0.89 ^a \pm 0.13	0.67 ^b \pm 0.19	0.13
C18:3 <i>n</i> -6	0.61 ^a \pm 0.23	0.30 ^b \pm 0.14	0.42 ^b \pm 0.20	0.31 ^b \pm 0.13	0.36 ^b \pm 0.07	0.68 ^a \pm 0.12	0.57 ^a \pm 0.19	0.15
C18:3 <i>n</i> -3	2.57 ^b \pm 0.63	1.55 ^d \pm 0.26	2.22 ^{bc} \pm 0.25	2.29 ^b \pm 0.32	2.54 ^b \pm 0.33	3.06 ^a \pm 0.36	1.88 ^{cd} \pm 0.47	0.36
C20:2	0.86 ^a \pm 0.19	0.54 ^c \pm 0.18	0.60 ^c \pm 0.20	0.66 ^{bc} \pm 0.19	0.49 ^c \pm 0.13	0.93 ^a \pm 0.19	0.78 ^{ab} \pm 0.25	0.17
C20:3 <i>n</i> -6	3.82 ^a \pm 0.92	3.36 ^{ab} \pm 0.76	2.93 ^{bc} \pm 0.82	3.77 ^a \pm 0.61	3.38 ^{ab} \pm 0.74	2.25 ^{ab} \pm 0.52	2.36 ^c \pm 0.81	0.70
C20:3 <i>n</i> -3	0.82 ^b \pm 0.23	0.45 ^c \pm 0.20	0.80 ^b \pm 0.23	0.70 ^{bc} \pm 0.25	0.77 ^b \pm 0.33	1.45 ^a \pm 0.41	0.77 ^b \pm 0.41	0.27
SFA	47.85 ^{ab} \pm 3.10	49.08 ^a \pm 2.15	48.28 ^a \pm 1.90	45.83 ^{bc} \pm 1.21	49.53 ^a \pm 1.48	45.16 ^c \pm 2.63	47.56 ^{ab} \pm 2.74	2.05
MUFA	35.55 ^c \pm 3.24	36.29 ^{bc} \pm 3.30	37.25 ^{abc} \pm 2.29	38.73 ^{ab} \pm 1.97	34.86 ^c \pm 2.43	36.38 ^{bc} \pm 2.79	39.23 ^a \pm 3.05	2.49
PUFA	16.61 ^{ab} \pm 3.86	14.63 ^{bc} \pm 3.56	14.47 ^{bc} \pm 3.01	15.44 ^{ab} \pm 1.97	15.62 ^{ab} \pm 2.48	18.47 ^a \pm 4.31	11.86 ^c \pm 4.00	3.04
PUFA/SFA	0.35 ^{ab} \pm 0.10	0.30 ^{bc} \pm 0.08	0.30 ^{bc} \pm 0.07	0.34 ^b \pm 0.05	0.32 ^{bc} \pm 0.06	0.41 ^a \pm 0.12	0.25 ^c \pm 0.10	0.08
<i>n</i> -6/ <i>n</i> -3	3.67 ^{bc} \pm 0.42	6.04 ^a \pm 0.71	3.34 ^c \pm 0.43	3.88 ^b \pm 0.58	3.60 ^{bc} \pm 0.44	2.87 ^d \pm 0.43	3.21 ^{cd} \pm 0.52	0.46

(SD) Standard Deviation; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rùens; (FS) Free State; (LSD) Least significant difference at the 5% level of significance; (SFA) Saturated fatty acids; (MUFA) Mono-unsaturated fatty acids; (PUFA) Polyunsaturated fatty acids; (PUFA/SFA) Polyunsaturated fatty acid/Saturated fatty acid ratio; (*n*-6/*n*-3) Omega 6/Omega 3 ratio; ^{a-d} Values in the same row with different superscripts are significantly different ($P \leq 0.05$); (nd) None detected.

Table 4.5 Values for the fatty acid composition (% of total fatty acids) of the predominant fatty acids of the plants samples

Code	Species name	Predominant fatty acids								Fatty acid ratios						
		C16:0	C18:0	C20:0	C16:1	C18:1 n-9c	C20:1	C18:2 n-6c	C18:3 n-3	SFA	MUFA	PUFA	PUFA/SFA	n-6	n-3	n-6/n-3
RU1	<i>Medicago sativa</i>	16.46	4.15	1.54	1.96	3.43	0.00	14.14	51.53	26.16	6.89	66.95	2.56	14.49	52.46	0.28
RU2	<i>Medicago sativa</i>	17.77	4.44	1.37	1.37	3.61	0.49	14.62	47.13	28.22	7.91	63.87	2.26	15.83	47.68	0.33
RU3	<i>Medicago sativa</i>	30.04	9.38	3.81	2.86	6.43	1.44	11.76	22.50	50.23	13.51	36.26	0.72	11.76	24.50	0.48
RU4	<i>Cynodon dactylon</i>	21.06	9.23	4.37	2.50	12.06	1.63	19.43	17.85	41.66	19.17	39.17	0.94	19.43	19.75	0.98
FS1	<i>Themeda triandra</i>	13.40	5.81	18.61	1.39	4.24	16.26	7.18	4.88	54.05	27.13	18.83	0.35	9.75	9.07	1.08
FS2	<i>Cynodon dactylon</i>	22.70	9.47	4.98	3.13	12.96	5.40	13.54	6.26	46.78	30.92	22.30	0.48	16.04	6.26	2.56
FS3	<i>Eragrostis superba</i>	23.32	9.67	4.43	2.45	15.27	3.69	13.68	7.72	48.30	28.73	22.97	0.48	15.25	7.72	1.98
FS4	<i>Fingerhuthia africana</i>	23.20	8.75	7.55	2.19	10.49	3.18	11.69	10.54	47.03	24.02	28.96	0.62	13.63	15.33	0.89
FS5	<i>Aristida congesta subsp. congesta</i>	19.75	7.87	5.74	2.79	9.93	5.54	10.80	4.89	42.68	24.48	32.84	0.77	13.29	19.55	0.68
FS6	<i>Schmidtia kalahariensis</i>	17.98	5.74	8.48	1.63	11.06	6.98	20.11	6.37	41.80	30.21	278.00	0.67	21.62	6.37	3.39
FS7	<i>Eragrostis lehmanniana</i>	22.67	7.12	4.98	2.02	9.84	4.44	12.80	11.75	43.47	26.32	30.21	0.70	14.69	15.53	0.95
FS8	<i>Heteropogon contortus</i>	26.47	6.34	3.90	1.73	6.05	4.86	16.67	11.27	44.00	22.29	33.71	0.77	18.13	15.58	1.16
CK1	<i>Pentzia incana</i>*	18.78	3.97	2.82	0.00	10.96	1.05	22.27	24.70	32.25	19.64	48.11	1.49	23.41	24.70	0.95
CK2	<i>Plinthus karrooicus</i>*	13.11	2.55	1.48	1.17	2.78	1.30	19.81	27.04	26.39	11.00	62.61	2.37	32.03	27.97	1.15
CK3	<i>Lycium cinereum</i>	17.98	3.26	1.12	0.00	5.95	0.98	18.85	33.57	26.14	10.86	623.00	2.41	23.91	38.65	0.62
CK4	<i>Ruschia intricata</i>	15.06	2.93	1.70	1.31	12.19	2.77	21.30	31.75	24.45	20.64	54.92	2.25	22.10	32.81	0.67
CK5	<i>Galenia sarcophylla</i>	16.29	3.46	5.03	1.65	9.60	5.52	18.04	22.82	30.28	20.50	49.21	1.63	19.49	29.72	0.66
CK6	<i>Stipagrostis ciliata</i>	18.91	9.29	4.59	2.16	16.40	3.57	15.33	8.21	40.10	29.92	29.98	0.75	16.83	13.15	1.28
CK7	<i>Stipagrostis obtusa</i>	25.26	8.87	3.30	2.47	12.22	2.99	12.14	15.76	46.98	23.96	29.06	0.62	13.30	15.76	0.84
CK8	<i>Salsola glabrescens</i>	17.85	5.52	4.39	2.63	10.69	1.87	13.81	16.62	35.85	21.80	42.34	1.18	17.91	24.44	0.73
KV1	<i>Lebeckia leipoldtiana</i>	16.26	5.82	2.51	1.77	4.53	2.58	14.63	43.33	28.19	13.19	58.62	2.08	15.28	43.33	0.35
KV2	<i>Wiborgia monoptera</i>	18.84	6.17	6.19	1.45	4.73	8.29	14.00	26.98	35.66	22.30	42.04	1.18	15.06	26.98	0.56
KV3	<i>Wiborgia sericea</i>	20.16	9.41	3.43	1.85	7.38	2.84	11.70	31.63	38.32	17.52	44.16	1.15	12.53	31.63	0.40
KV4	<i>Eriocephalus punctulatus</i>*	7.46	17.32	0.00	6.33	3.05	0.00	5.60	7.84	30.33	9.84	59.83	1.97	12.34	47.49	0.26
KV5	<i>Galenia africana</i>	28.69	7.55	4.67	1.56	16.37	4.25	12.38	14.46	47.00	24.31	28.69	0.61	14.23	14.46	0.98

Table 4.5 (Continued)

Code	Species name	Predominant fatty acids							Fatty acid ratios							
		C16:0	C18:0	C20:0	C16:1	C18:1 n-9c	C20:1	C18:2 n-6c	C18:3 n-3	SFA	MUFA	PUFA	PUFA/SFA	n-6	n-3	n-6/n-3
KV6	<i>Pentzia incana*</i>	16.39	8.74	1.86	1.88	8.77	2.21	15.82	18.03	32.41	19.34	48.25	1.49	18.39	29.86	0.62
KV7	<i>Nylandtia spinosa</i>	17.70	6.68	2.53	12.35	18.58	2.59	16.35	10.29	32.21	40.24	27.55	0.86	17.25	10.29	1.68
KV8	<i>Chaetobromus dregeanus</i>	21.65	5.40	2.63	1.60	11.28	1.13	18.69	19.48	37.68	17.75	44.57	1.18	20.31	24.26	0.84
KV9	<i>Ereiodium moschatum</i>	26.37	5.05	2.57	1.71	7.43	1.79	6.69	34.54	41.14	16.68	42.18	1.03	7.64	34.54	0.22
KV10	<i>Medicago polymorpha</i>	21.40	5.65	1.71	1.91	5.37	0.72	10.60	42.62	34.13	10.79	55.08	1.61	11.28	43.80	0.26
NK1	<i>Stipagrostis obtusa and ciliata</i>	28.47	13.08	8.89	1.49	19.78	3.49	3.88	3.14	58.69	30.05	11.26	0.19	6.89	4.37	1.57
NK2	<i>Fingerhuthia africana</i>	23.45	7.98	4.51	2.24	7.95	4.71	11.89	15.67	44.47	25.76	29.78	0.67	14.11	15.67	0.90
NK3	<i>Felicia muricata</i>	22.97	4.86	1.62	2.05	7.66	0.63	14.38	37.60	33.42	12.95	53.62	1.60	15.10	38.53	0.39
NK4	<i>Pteronia glauca*</i>	31.06	8.47	5.03	1.60	7.05	1.71	11.43	19.53	52.84	13.33	33.83	0.64	12.58	21.25	0.59
NK5	<i>Salsola calluna</i>	19.34	4.41	4.03	1.38	7.01	1.45	21.39	27.04	35.17	13.78	51.05	1.45	21.39	29.66	0.72
NK6	<i>Phaeoptilum spinosum</i>	19.12	4.76	2.70	2.21	8.27	0.00	8.51	34.84	34.48	13.64	51.88	1.50	8.51	43.37	0.20
NK7	<i>Psilocaulon absimile</i>	21.70	6.54	4.26	1.35	12.80	1.47	17.21	18.19	40.25	18.97	40.77	1.01	18.97	21.81	0.87
NK8	<i>Salsola tuberculata</i>	22.74	6.54	3.93	2.96	12.33	1.80	14.17	14.75	41.75	20.97	37.28	0.89	16.36	20.92	0.78
NK9	<i>Eriocephalus ericoides*</i>	13.77	5.02	2.90	1.60	7.36	0.89	27.15	15.83	27.34	12.17	60.49	2.21	42.07	17.46	2.41
NK10	<i>Rhigozum trichotomum</i>	19.96	4.04	2.67	1.64	5.24	1.06	10.05	41.10	32.45	9.70	57.85	1.78	10.05	47.80	0.21
NK11	<i>Unidentified</i>	20.49	4.65	2.39	1.53	8.06	1.08	29.16	22.15	33.44	11.92	54.64	1.63	30.52	24.12	1.27
NK12	<i>Pentzia incana*</i>	21.30	5.69	3.93	4.69	7.20	1.22	23.32	12.04	35.44	15.74	48.82	1.38	27.47	21.35	1.29
NK13	<i>Rosenia humilis*</i>	19.10	4.59	2.24	1.22	7.13	0.87	24.90	14.43	36.24	12.20	51.56	1.42	28.11	23.44	1.20
NK14	<i>Salsola aphylla</i>	26.71	6.61	2.33	1.55	14.67	1.22	13.76	11.95	42.14	24.57	33.29	0.79	15.61	17.68	0.88
NK15	<i>Zygophyllum gilfillanii</i>	22.29	7.09	3.83	1.54	9.61	1.32	10.77	28.60	43.30	15.08	41.62	0.96	10.77	30.85	0.35
NK16	<i>Ruschia intricata</i>	18.73	6.91	11.65	1.36	12.15	0.00	16.83	16.75	45.16	15.06	39.79	0.88	17.99	21.80	0.83
NK17	<i>Lycium cinereum</i>	13.17	3.69	1.91	2.12	4.86	2.39	9.02	16.86	23.50	10.99	65.51	2.79	13.07	52.45	0.25
HK/CAL1	<i>Pentzia incana*</i>	13.34	3.79	2.38	1.35	3.72	1.01	20.89	35.03	24.28	12.01	63.72	2.63	22.08	41.63	0.53
HK/CAL2	<i>Pentzia sphaerocephala*</i>	17.79	10.33	2.66	15.20	2.32	0.63	16.50	24.29	32.80	20.48	46.73	1.43	18.54	27.70	0.67
HK/CAL3	<i>Chrysocoma ciliata*</i>	19.87	4.48	3.34	11.39	1.94	1.08	12.70	27.14	34.09	19.90	46.01	1.35	16.02	29.99	0.53

Table 4.5 (Continued)

Code	Species name	Predominant fatty acids								Fatty acid ratios						
		C16:0	C18:0	C20:0	C16:1	C18:1 <i>n-9c</i>	C20:1	C18:2 <i>n-6c</i>	C18:3 <i>n-3</i>	SFA	MUFA	PUFA	PUFA/SFA	<i>n-6</i>	<i>n-3</i>	<i>n-6/n-3</i>
HK/CAL4	<i>Eriocephalus ericoides</i> *	13.64	3.03	4.86	1.81	2.86	2.89	18.37	26.97	28.13	15.81	56.06	1.99	20.97	33.91	0.62
HK/CAL5	<i>Salsola calluna</i>	15.74	2.46	1.89	1.53	5.46	0.89	23.50	37.42	23.54	12.75	63.72	2.71	24.84	38.88	0.64
HK/CAL6	<i>Justica orchioides</i> *	13.57	2.46	0.99	1.70	3.49	1.30	19.63	47.80	20.87	10.72	68.41	3.28	20.62	47.80	0.43
HK/CAL7	<i>Zygophyllum lichtensteinianum</i>	15.80	5.52	3.74	1.97	3.99	3.82	10.89	31.51	30.77	19.91	49.32	1.60	12.22	37.10	0.33
HK/CAL8	<i>Lycium spp.</i>	16.93	1.08	2.01	2.86	4.73	1.69	0.00	38.63	24.92	18.05	57.03	2.29	13.77	43.26	0.32
HK/CAL9	<i>Eberlanzia ferox</i>	16.69	3.19	0.00	1.56	3.51	0.00	26.99	36.75	24.47	10.33	65.20	2.67	28.45	36.75	0.77
HK/CAL10	<i>Drosanthemum hispidum</i>	15.02	3.09	4.05	1.72	4.84	2.81	20.96	37.04	26.79	14.01	59.20	2.21	22.16	37.04	0.60
HK/CAL11	<i>Mesembryanthemum vaginatum</i>	15.55	3.69	2.71	1.51	7.73	2.14	19.59	30.57	27.58	18.42	54.00	1.96	20.71	33.30	0.62
HK/LO1	<i>Pentzia incana</i> *	15.09	3.27	1.29	1.50	3.81	1.24	20.90	37.37	23.96	13.05	62.99	2.63	21.85	41.14	0.53
HK/LO2	<i>Tetragonia fruticosa</i>	16.21	2.82	2.90	2.18	5.07	2.70	23.36	36.23	25.74	12.97	61.29	2.38	25.06	36.23	0.69
HK/LO3	<i>Chrysanthemoides incana</i>	16.62	2.44	0.98	1.41	2.21	1.01	21.48	45.87	24.05	7.87	68.08	2.83	22.21	45.87	0.48
HK/LO4	<i>Salsola tuberculata</i>	15.43	2.90	1.53	2.05	9.52	1.46	23.24	32.25	23.49	17.50	59.00	2.51	24.55	34.46	0.71
HK/LO5	<i>Stoeberia beetzii</i>	15.49	3.72	2.54	2.60	8.33	2.47	20.08	20.26	26.22	23.59	50.19	1.91	21.06	29.13	0.72
HK/LO6	<i>Pteronia sordida</i> *	16.55	5.59	3.48	1.97	6.00	4.74	19.07	21.05	33.06	21.83	45.11	1.37	21.14	23.97	0.88
HK/LO7	<i>Zygophyllum lichtensteinianum</i>	16.88	3.55	4.25	2.05	4.19	1.27	19.64	34.53	31.39	13.31	55.30	1.76	20.77	34.53	0.60
HK/LO8	<i>Unidentified</i>	18.59	5.50	2.56	2.29	5.57	2.70	9.00	40.37	33.00	16.36	50.64	1.54	10.27	40.37	0.25

(SFA) Saturated fatty acids; (MUFA) Mono-unsaturated fatty acids; (PUFA) Polyunsaturated fatty acids; (PUFA/SFA) Polyunsaturated fatty acid/Saturated fatty acid ratio; (*n-6/n-3*) Omega 6/Omega 3 ratio; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State; ***Fragrant/Herbaceous bushes indicated in bold.**

Some plant secondary metabolites, such as mono- and sesquiterpenes, can be transferred from forage to meat (Larick *et al.*, 1987). Priolo *et al.* (2004) evaluated the possibility of using 2,3-octanedione, skatole, mono- and sesquiterpenes as indicators of grass feeding. Similarly, Cornu *et al.* (2001) suggests that in natural grasslands a variety of terpenes can be found in meat, which might be used as food tracers or markers of the geographical origin. Terpenes have characteristic aromas. For example, pinene has the typical aroma associated with pine trees and is found in the essential oil of rosemary (*Rosmarinus officinalis*), while limonene is described as having pleasant lemon-like, turpentine, citrus and fruity attributes (Calkins & Hodgen, 2007). The presence of these compounds in the meat and fat are likely to contribute toward the sensory quality of the product. According to Table 4.2 the Karoo samples contained more terpenes than the Non-Karoo samples. In fact, no terpenes were detected for RU whereas only two were detected for FS. HK/CAL had the highest concentrations of α -pinene, β -pinene and limonene, being significantly ($P \leq 0.05$) more than the rest. When the predominant VOCs of the plants samples are studied, it is evident that the herbaceous Karoo bushes contained a greater concentration of volatile compounds typically found in the essential oil of common herbs and spices, such as oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and cloves (*Syzygium aromaticum*) (Viuda-Martos *et al.*, 2007) (Table 4.3). The characteristic compounds detected were eucalyptol, α -cubebene, β -pinene, copaene, β -myrcene, α -pinene, spathulenol, α -cadinol, β -terpineol, pinen-3-one, α -phellandrene, D-limonene, γ -elemene, α -caryophyllene, isocyclocitral, α -cadinol, (+)-epi-bicyclosquiphellandrene and pinocarveol (Table 4.3).

Priolo *et al.* (2004) found that β -caryophyllene was absent in the fat of animals raised and finished on concentrates. Its concentration also varied between animals raised and finished on grass, and those raised on grass and finished on concentrates (Priolo *et al.*, 2004). It is suggested that this compound is metabolised or eliminated during the period of stall-feeding, diluted with new fat formation or only deposited in the period before slaughtering. Hence, the latter research group proposed the use of this sesquiterpene as a biomarker due to its widespread occurrence in nature. Trans-caryophyllene was detected in all the samples, except for RU (Table 4.2). The highest concentration were in the HK/CAL meat ($P \leq 0.05$), followed by the rest of the samples which did not differ significantly from each other. The reason for this variation could be again linked to diet, as caryophyllene was detected as a predominant volatile in some of the plants samples collected from the farms, excluding the RU farm, where it was not a main volatile for lucerne (*Medicago sativa*) (Table 4.3).

Phenolic compounds may also be incorporated into meat through the animal's diet and ruminal fermentation in the digestive tract (Ha & Lindsay, 1991). Animals raised on phenolic rich feed are expected to have high levels of phenolic compounds in their depot fats (Ha & Lindsay, 1991). A possible precursor for some phenols is the polymer lignin. The functioning of enzymes such as ligninase in the rumen enables its degradation to monomeric phenols (Chen *et al.*, 1985). Green herbage has a higher content of lignin compared to that of grain-based diets and could explain why pastoral flavour have been linked to high concentrations of indoles and methylphenols in pasture-fed lamb fat (Young *et al.*, 2003; Schreurs *et al.*, 2008). Also,

alkylphenols may be produced from diterpenes derived from ruminal degradation of chlorophyll (Ha & Lindsay, 1991). While, microbial fermentation of tyrosine (amino acid) in the rumen and lower intestinal tract may also produce phenolic compounds (Ha & Lindsay, 1991). However, in the current study only two phenols were detected (i.e. 2,3,4,6-tetramethyl-phenol and 3-methyl-6-propyl-phenol) in the lamb meat of NK, KV and HK/CAL (Table 4.2). Similarly, Vasta *et al.* (2013) did not report any phenolic compounds found in fresh lamb meat supplemented with rosemary or artemisia oil. Given that the meat of the current study were cooked prior to analysis, an explanation could be that the phenolic compounds were lost during the cooking process of the sampling procedure and the concentration left in the meat were too low to be detected using SPME. Heptanal, 2-nonenal, 4-heptenal and 3-hydroxy-2-butanone arise from lipid oxidation and was present in similar amounts in the meat of lambs fed grape seed extract and grape seed extract plus an oil blend (Vasta *et al.*, 2010). The latter propose that, in the presence of grape seed extract, oil supplementation does not enhance the production of these volatile compounds. This is possibly due to the presence of polyphenols in the grape seed extract. Similarly, the phenolic compounds present in the raw meat samples of the current study could have acted as antioxidants, thereby lowering the final concentration detected in the meat.

The oxidation product of linoleic acid, 2,3-octanedione, and indicator for pasture diet (Young *et al.*, 1997; Priolo *et al.*, 2004) was dominant in KV and HK/CAL meat (Table 4.2). The concentrations were significantly more and approximately double that detected in NK, HK/LO and RU meat. FS and CK meat contained the lowest concentrations, with that of CK being the lowest overall ($P \leq 0.05$). This was rather unexpected as CK lamb grazed on similar Karoo veld conditions to that of NK. However, the fatty acid composition of linoleic acid (C18:2 *n*-6c) for CK, NK, HK/LO, KV, HK/CAL and RU were the highest and did not differ significantly from each other (Table 4.4). Whereas, that of FS meat correlated with the lower concentration detected for 2,3-octanedione (Table 4.2). These results can be explained based on the diet of the animals. The FS lamb grazed predominantly on savannah-type grasses (Erasmus *et al.*, 2016), which are known to be C₄ plants, similar to that of maize and millet (Capuano *et al.*, 2013). Given that 2,3-octanedione is generated through the enzymatic activity of lipoxygenase, which is absent in maize but present in green herbage (Young *et al.*, 1997; Vasta & Priolo, 2006), one can expect low or no enzymatic activity in the grass. Hence, producing a low concentration of 2,3-octanedione in FS meat. Another factor that could influence the concentration of 2,3-octanedione is temperature (either cooking, equilibration and/or extraction). The concentration have been found to increase with an increase in the SPME extraction temperature notwithstanding the animal's diet (Vasta *et al.*, 2007).

Vasta *et al.* (2010) detected volatile compounds (specifically C₇ to C₁₀ saturated and monounsaturated aldehydes, 3-hydroxy-2-butanone, 1-penten-3-ol and 2-octen-1-ol) which are, according to Elmore *et al.* (2000a), characteristic of fat-enriched diets. Correspondingly, Almela *et al.* (2010) stated that a high concentration of 3-hydroxy-2-butanone in meat is produced by grain-based feeding. However, these compounds were not affected by diet in the study by Vasta *et al.* (2010). The reason being that it might be produced at high cooking temperatures (140°C) which activate the oxidation of PUFA and produce the volatiles

derived from lipid degradation. Interestingly, CK and FS had the highest content of 3-hydroxy-2-butanone [described as having a lardy, sweet, caramel or creamy odour (Almela *et al.*, 2010)], while none were detected for the other treatments, except for HK/CAL (Table 4.2). This could suggest that these treatments might have been exposed to higher temperatures (>100°C), yet this was strictly controlled, or they could have been more prone to lipid oxidation. Therefore, Vasta *et al.* (2010) states that the extraction of volatile compounds at milder conditions (60°C) enables a better evaluation of the effect of diet on muscle metabolism as heat-produced volatiles are less. On the other hand, the extraction of volatiles at decreased temperatures may prevent the production of heat-induced volatiles from Maillard's and Strecker's reactions. These compounds are more likely to contribute to the flavour of cooked meat (Mottram, 1998), making their assessment important when dietary effect linked to the flavour perception of cooked meat is investigated. Furans, heterocyclic compounds, are such volatiles known to arise from Maillard and Strecker reactions (Vasta *et al.*, 2010). The results show that only five furans were detected of which the Karoo samples (KV and HK/CAL) had the highest ($P \leq 0.05$) concentrations (Table 4.2).

According to Almela *et al.* (2010), grasses are richer in linolenic acid, the precursor of various unsaturated aldehydes such as 4-heptenal, 2,4-heptadienal, and 2,6-nonadienal. Although results show that CK, FS and RU had significantly higher concentrations of linolenic acid, they did not contain the highest concentrations of 4-heptenal, 2,4-heptadienal, and 2,6-nonadienal, except for RU. The RU samples had a significantly high concentration of 4-heptenal ($4.89 \pm 1.52\%$) and the third highest concentration for 2,6-nonadienal ($0.38 \pm 0.32\%$) (Table 4.2). These findings could be related to the type of linolenic acid namely, α -linolenic acid (C18:3 *n*-3) and γ -linolenic acid (C18:3 *n*-6). In Figure 4.1, Elmore *et al.* (2005) illustrates that the compounds mentioned above are derived from α -linolenic acid. Table 4.4 show that the fatty acid composition of the α -linolenic acid and γ -linolenic acid varies for CK, RU and FS. For both fatty acids, RU had significantly highest values, whereas CK and FS only had greater values for the γ -linolenic acid compared to NK, HK/LO, KV and HK/CAL ($P \leq 0.05$) (Table 4.4). In addition, two of the three lucerne plant samples of the RU farm had the highest α -linolenic acid composition (Table 4.5). Yet it is difficult to relate the fatty acid composition of the plants to that of the meat as the hydrogenating action of the rumen bacteria may lower the proportion of linoleic acid deposited in the meat (Wood & Enser, 1997). As dietary intake of the lambs were not controlled and monitored, one can only speculate regarding the intake of typical plants collected from the farms.

Grain-based diets, richer in oleic and linoleic acids, increase the levels of certain aldehydes, such as hexanal, 2-heptenal, and 2-4-decadienal (Vasta & Priolo, 2006; Almela *et al.*, 2010). Seventeen typical volatile compounds derived from linoleic acid is shown in Figure 4.1 of which four were not detected in the lamb meat (Table 4.2). The composition of the *cis* linoleic acid (C18:2 *n*-6c) were significantly lower for FS, but did not differ for the other samples (Table 4.4). While the composition of the *trans* linoleic acid (C18:2 *n*-6t) were significantly highest for RU, lowest for NK and did not differ between CK, HK/LO, KV, HK/CAL and FS ($P > 0.05$) (Table 4.4). Overall, KV and HK/CAL had the highest and CK and FS the lowest volatile composition for

2-pentyl-furan, 1-pentanol, 2-heptanone, hexanal, pentanal, (E)-2-heptenal, 1-octen-3-ol, (E,E)-2,4-decadienal, (Z)-2-octen-1-ol, 2,3-octanedione, (E)-2-octenal, 1-hexanol and heptanal likely derived from linoleic acid as shown in Figure 4.1 (Table 4.2).

4.4 Conclusions

The volatile and fatty acid profiles of the meat and plants revealed reasonable variation between farms from different regions. The results show that diet, which is origin-specific, affected the fatty acid composition of the meat. Consequently, the fatty acid profile influenced the type of volatile compounds of the cooked meat. The results show the important role of linoleic and linolenic acid on the volatile profile. By determining the volatiles present in typical plant samples from the different farms, it was possible to link the presence of certain terpenes to that of the meat. Compounds such as α -pinene, β -pinene and limonene were only detected in the meat of Karoo lamb treatments, being particularly high in that of the meat from the HK/CAL farm. Linking these results to Chapter 3, the herbaceous aroma and flavour attributes of the Hantam Karoo meat and fat can be validated. Also, the potential exists to use these terpene compounds as markers of feeding regime for detecting the authenticity of Karoo lamb (that is whether or not they consumed fragrant Karoo bushes). However, other analytical techniques should also be explored as the current methods are time-consuming and unprofitable in the long run. To link the sensory data with that of the volatile compounds, a technique such as gas chromatography with olfactometric detection (GC–O) can be used. GC–O is based on the sensory analysis of the eluate from the chromatographic column. As volatile compounds are eluted from the column they are smelt by panellists and assigned descriptors. The benefits of using such a method is a faster throughput of results, while it also provides the ability to identify key odour notes in the product.

4.5 References

- Almela, E., Jordan, M. J., Martinez, C., Sotomayor, J. A., Bedia, M. & Banon, S. (2010). Ewe's diet (pasture vs. grain-based feed) affects volatile profile of cooked meat from light lamb. *Journal of Agricultural and Food Chemistry*, **58**, 9641-9646.
- Ba, H. V., Hwang, I., Jeong, D. & Touseef, A. (2012). Principle of meat aroma flavors and future prospect. In: *Latest Research into Quality Control* (edited by M. S. F. Nezhad). ISBN: 978-953-51-0868-9. InTech, DOI: 10.5772/51110.
- Berg, E. P. & Walker, E. L. M. (2004). Sheep. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 1291-1295. Oxford, UK: Elsevier.
- Brennand, C. P. & Lindsay, R. C. (1992). Distribution of volatile branched-chain fatty acids in various lamb tissues. *Meat Science*, **31**, 411-421.
- Bueno, M., Resconi, V. C., Campo, M. M., Cacho, J., Ferreira, V. & Escudero, A. (2011). Gas chromatographic-olfactometric characterisation of headspace and mouthspace key aroma compounds in fresh and frozen lamb meat. *Food Chemistry*, **129**, 1909-1918.

- Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, **77**, 63-80.
- Capuano, E., Boerrigter-Eenling, R., Van der Veer, G. & Van Ruth, S. M. (2013). Analytical authentication of organic products: an overview of markers. *Journal of the Science of Food and Agriculture*, **93**, 12-28.
- Chen, W., Supanwong, K., Ohmiya, K., Shimizu, S. & Kawakami, H. (1985). Anaerobic degradation of veratrylglycerol- β -guaiacyl ether and guaiacoxycetic acid by mixed rumen bacteria. *Applied and Environmental Microbiology*, **50**(6), 1451-1456.
- Cornu, A., Carnat, A.-P., Martin, B., Coulon, J.-B., Lamaison, J.-L. & Berdague, J.-L. (2001). Solid-phase microextraction of volatile components from natural grassland plants. *Journal of Agricultural and Food Chemistry*, **49**(1), 203-209.
- Elmore, J. S., Cooper, S. L., Enser, M., Mottram, D. S., Sinclair, L. A., Wilkinson, R. G. & Wood, J. D. (2005). Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Science*, **69**, 233-242.
- Elmore, J. S. & Mottram, D. S. (2000). Formation of 2-Alkyl-(2H)-thiapyrans and 2-Alkylthiophenes in cooked beef and lamb. *Journal of Agricultural and Food Chemistry*, **48**, 2420-2424.
- Elmore, J. S., Mottram, D. S., Enser, M. & Wood, J. D. (2000a). The effects of diet and breed on the volatile compounds of cooked lamb. *Meat Science*, **55**, 149-159.
- Elmore, J. S., Mottram, D. S. & Hierro, E. (2000b). Two-fibre solid-phase microextraction combined with gas chromatography-mass spectrometry for the analysis of volatile aroma compounds in cooked pork. *Journal of Chromatography A*, **905**, 233-240.
- Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016b). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.
- Estévez, M., Morcuende, D., Ventanas, S. & Cava, R. (2003). Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS. *Journal of Agricultural and Food Chemistry*, **51**, 3429-3435.
- Farmer, L. J. (1994). The role of nutrients in meat flavour formation. *Proceedings of the Nutrition Society*, **53**, 327-333.
- Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497-509.
- Ha, J. K. & Lindsay, R. C. (1991). Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *Journal of Food Science*, **56**(5), 1197-1202.
- Jacobson, M. & Koehler, H. H. (1963). Meat flavor, components of the flavor of lamb. *Journal of Agricultural and Food Chemistry*, **11**(4), 336-339.
- Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., Garner, G. B. & Morrow, R. E. (1987). Flavor constituents of beef as influenced by forage- and grain-feeding. *Journal of Food Science*, **52**(2), 245-251.

- Ma, Q. L., Hamid, N., Bekhit, A. E. D., Robertson, J. & Law, T. F. (2013). Optimization of headspace solid-phase microextraction (HS-SPME) for gas chromatography mass spectrometry (GC-MS) analysis of aroma compounds in cooked beef using response surface methodology. *Microchemical Journal*, **111**, 16-24.
- Machiels, D. & Istasse, L. (2003). Evaluation of two commercial solid-phase microextraction fibres for the analysis of target aroma compounds in cooked beef meat. *Talanta*, **61**, 529-537.
- Madruga, M. S., Arruda, S. G. B., Narain, N. & Souza, J. G. (2000). Castration and slaughter age effects on panel assessment and aroma compounds of the "mestico" goat meat. *Meat Science*, **56**, 117-125.
- Madruga, M. S., Dantas, I., Queiroz, A., Brasil, L. & Ishihara, Y. (2013). Volatiles and water- and fat-soluble precursors of Saanen goat and cross Suffolk lamb flavour. *Molecules*, **18**, 2150-2165.
- Madruga, M. S., Elmore, J. S., Dodson, A. T. & Mottram, D. S. (2009). Volatile flavour profile of goat meat extracted by three widely used techniques. *Food Chemistry*, **115**, 1081-1087.
- Madruga, M. S., Elmore, J. S., Oruna-Concha, M. J., Balagiannis, D. & Mottram, D. S. (2010). Determination of some water-soluble aroma precursors in goat meat and their enrolment on flavour profile of goat meat. *Food Chemistry*, **123**, 513-520.
- Madruga, M. S. & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture*, **68**, 305-310.
- Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, **62**(4), 415-424.
- Osorio, M. T., Zumalacarregui, J. M., Cabeza, E. A., Figueira, A. & Mateo, J. (2008). Effect of rearing system on some meat quality traits and volatile compounds of suckling lamb meat. *Small Ruminant Research*, **78**(1-3), 1-12.
- Park, P. W. & Goins, R. E. (1994). *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, **59**(6), 1262-1266.
- Pegg, R. B. & Shahidi, F. (2004). Flavour development. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 570-578. Oxford, UK: Elsevier.
- Priolo, A., Cornu, A., Prache, S., Krogmann, M., Kondjoyan, N., Micol, D. & Berdagué J.-L. (2004). Fat volatile tracers of grass feeding in sheep. *Meat Science*, **66**, 475-481.
- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2010). Relationship between odour-active compounds and flavour perception in meat from lamb fed different diets. *Meat Science*, **85**, 700-706.
- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2012). Gas chromatographic-olfactometric aroma profile and quantitative analysis of volatile carbonyls of grilled beef from different finishing feed systems. *Meat Science*, **77**(6), S240-S246.

- Schreurs, N. M., Lane, G. A., Tavendale, M. H., Barry, T. N. & McNabb, W. C. (2008). Pastoral flavour in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology*, **146**, 193-221.
- Shahidi, F. (1989). Flavor of cooked meats. In: *Flavor Chemistry Trends and Developments* (edited by R. Teranishi, R. G. Buttery & F. Shahidi). Pp. 188-201. Washington, USA: American Chemical Society.
- Shapiro, S. S. & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Sink, J. D. & Caporaso, F. (1977). Lamb and mutton flavour: contributing factors and chemical aspects. *Meat Science*, **1**(2), 119-127.
- Spanier, A. M., Flores, M., Toldrá, F., Aristoy, M.-C., Bett, K. L., Bystricky, P. & Bland, J. M. (2004). Meat flavor: contribution of proteins and peptides to the flavor of beef. In: *Quality of Fresh and Processed Foods* (edited by F. Shahidi, A. M. Spanier, C.-T. Ho & T. Braggins). Pp. 33-49. Vol. 542. New York, USA: Kluwer Academic/Plenum Publishers.
- Stahnke, L. H., Holck, A., Jensen, A., Nilsen, A. & Zanardi, E. (2002). Maturity acceleration of Italian dried sausage by *Staphylococcus carnosus* – Relationship between maturity and flavor compounds. *Journal of Food Science*, **67**(5), 1914-1921.
- Sutherland, M. M. & Ames, J. M. (1995). The effect of castration on the headspace aroma components of cooked lamb. *Journal of the Science of Food and Agriculture*, **69**, 403-413.
- Vasta, V., Aouadi, D., Brogna, D. M. R., Scerra, M., Luciano, G., Priolo, A. & Salem, H.B. (2013). Effect of the dietary supplementation of essential oils from rosemary and artemisia on muscle fatty acids and volatile compound profiles in Barbarine lambs. *Meat Science*, **95**, 235-241.
- Vasta, V., Jeronimo, E., Brogna, D. M. R., Dentinho, M. T. P., Biondi, L., Santos-Silva, J., Priolo, A. & Bessa, R. J. B. (2010). The effect of grape seed extract or *Cistus ladanifer* L. on muscle volatile compounds of lambs fed dehydrated lucerne supplemented with oil. *Food Chemistry*, **119**, 1339-1345.
- Vasta, V., Luciano, G., Dimauro, C., Röhrle, F., Priolo, A., Monahan, F. J. & Moloney, A. P. (2011). The volatile profile of *Longissimus dorsi* muscle of heifers fed pasture, pasture silage or cereal concentrate: Implication for dietary discrimination. *Meat Science*, **87**, 282-289.
- Vasta, V. & Priolo, A. (2006). Ruminant fat volatiles as affected by diet. A review. *Meat Science*, **73**, 218-228.
- Vasta, V., Ratel, J. & Engel, E. (2007). Mass spectrometry analysis of volatile compounds in raw meat for the authentication of the feeding background of farm animals. *Journal of Agricultural and Food Chemistry*, **55**, 4630-4639.
- Viuda-Martos, M., Ruíz-Navajas, Y., Fernández-López, J. & Pérez-Álvarez, J. A. (2007). Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. *Acta Chimica Slovenica*, **54**, 921-926.
- Wasserman, A. E. (1972). Thermally produced flavor components in the aroma of meat and poultry. *Journal of Agricultural and Food Chemistry*, **20**(4), 737-741.

- Watkins, P. J., Frank, D., Singh, T. K., Young, O. A. & Warner, R. (2013). Sheep meat flavor and the effect of different feeding systems: a review. *Journal of Agricultural and Food Chemistry*, **61**, 3561-3579.
- Wettasinghe, M., Vasanthan, T., Temelli, F. & Swallow, K. (2001). Volatile flavour composition of cooked by-product blends of chicken, beef and pork: a quantitative GC-MS investigation. *Food Research International*, **34**, 149-158.
- Wilches, D., Rovira, J., Jaime, I., Palacios, C., Luruena-Martinez, M. A., Vivar-Quintana, A. M. & Revilla, I. (2011). Evaluation of the effect of a maternal rearing system on the odour profile of meat from suckling lamb. *Meat Science*, **88**, 415-423.
- Wood, J. D. & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, **78** (Suppl. 1), S49-S60.
- Xie, J., Sun, B., Zheng, F. & Wang, S. (2008). Volatile flavor constituents in roasted pork of Mini-pig. *Food Chemistry*, **109**, 506-514.
- Young, O. A., Berdague, J.-L., Viallon, C., Rousset-Akrim, S. & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, **45**(2), 183-200.
- Young, O. A., Lane, G. A., Priolo, A. & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food Agriculture*, **83**, 93-104.

Chapter 5

Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat³

Abstract

Stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of South African Dorper lambs from farms with different vegetation types were measured by isotope ratio mass spectrometry (IRMS) to evaluate it as a tool for the authentication of origin and feeding regime. Homogenised and defatted meat of the *Longissimus lumborum* (LL) muscle of lambs from seven different farms was assessed. The $\delta^{13}\text{C}$ values were affected by the origin of the meat, mainly reflecting the diet. The Rûens and Free State farms had the lowest ($P \leq 0.05$) $\delta^{15}\text{N}$ values, followed by the Northern Cape farms, with Hantam Karoo/Calvinia having the highest $\delta^{15}\text{N}$ values. Discriminant analysis showed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences as promising results for the use of IRMS as a reliable analytical tool for lamb meat authentication. The results suggest that diet, linked to origin, is an important factor to consider regarding region of origin classification for South African lamb.

Keywords: Extensive grazing; Geographical origin; Lamb meat; Stable isotope ratios

5.1 Introduction

Stable isotope ratio analysis can be an important analytical tool when determining geographic origin. It's a well-known and accurate method than can be used for the authentication of meat products (Franke *et al.*, 2005; Camin *et al.*, 2007). Recent studies have focussed on the use of isotopic measurements to determine the geographical origin of beef and lamb (Kelly *et al.*, 2005). Piasentier *et al.* (2003) draws attention to the use of stable isotope ratio analysis as a tool for the characterisation of animal diet by tracing the feeding system used in lamb meat production. The determination of stable isotope ratios are used as an analytical tool to confirm the origin of meat as specific isotopic patterns may subsist in the region of origin (Franke *et al.*, 2005; Crawford *et al.*, 2008). Isotopes are incorporated in local feeds and consequently taken up through the diet of animals during their lifetime (DeNiro & Epstein, 1978). Carbon (^{13}C) and nitrogen (^{15}N) isotope enrichment of animal products depend largely on the diet (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981; Codron *et al.*, 2005a; Franke *et al.*, 2005; Kelly *et al.*, 2005; Camin *et al.*, 2007; Sandberg *et al.*, 2012). Hence, through isotope enrichment from one trophic level to another it is possible to link meat to its diet and if the diet is regionally unique, to its geographic origin (Sandberg *et al.*, 2012).

By examining the $^{13}\text{C}/^{12}\text{C}$ isotope ratio it is possible to determine whether animals predominantly ate C_3 , C_4 or crassulacean acid metabolism (CAM) plants (Sandberg *et al.*, 2012; Capuano *et al.*, 2013). The C_4

³ Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.

pathway enables the plant to concentrate atmospheric CO₂ in such a way to avoid photorespiration due to the specialized Kranz anatomy (bundle sheath cells) of the leaves, which is absent in the C₃ pathway (Vogel *et al.*, 1978; Gibson, 2009). Essentially, C₄ and C₃ plants are distinguished based on Kranz and non-Kranz anatomy, whereas CAM plants have the ability to utilize both C₃ and C₄ modes of carbon fixation (Vogel *et al.*, 1978). C₄ plants result in ¹³C-enrichment (i.e. elevated carbon isotope ratios) compared to C₃ and CAM plants (Vogel *et al.*, 1978; Kelly *et al.*, 2005; Gibson, 2009). Previously published data for South Africa revealed average δ¹³C values of -26.5‰ for C₃ plants and -12.6‰ for C₄ grasses (Vogel *et al.*, 1978). C₃ plants consist of trees, bushes/shrubs (including their leaves and fruits), non-grassy herbs/forbs, most vegetables, cool-season grasses and grains such as lucerne (alfalfa), wheat, oats, barley and rice (Vogel *et al.*, 1978; Capuano *et al.*, 2013). C₄ plants include warm-season or tropical grasses and sedges and their seeds, leaves or storage organs such as roots and tubers (Vogel *et al.*, 1978; Capuano *et al.*, 2013). C₄ grains include maize and millet. Succulents are typical CAM plants (Vogel *et al.*, 1978).

Nitrogen isotopes also provide some information about the animal's diet as the consumption of leguminous plants may decrease the δ¹⁵N values, while the application of organic fertilizers, domestic grazing and cultivation may result in an increase of δ¹⁵N values (DeNiro & Epstein, 1981; Piasentier *et al.*, 2003; Perini *et al.*, 2009; Sandberg *et al.*, 2012; Devincenzi *et al.*, 2014). Not all leguminous plants may result in low δ¹⁵N values since *Senegalia nigrescens* and *Colophospermum mopane* (common leguminous tree taxa of the savanna) have high δ¹⁵N values (Codron *et al.*, 2005b). Ratios within soils and plants also increase with decreasing rainfall (i.e. arid conditions) (Perini *et al.*, 2009; Sandberg *et al.*, 2012). The protein fraction of young and light lamb meat, raised on ewe's milk, may have the highest nitrogen isotope ratios as a result of ¹⁵N-enrichment of the milk (Perini *et al.*, 2009). However, such trophic level enrichment can vary and depend on the combination of the specific plant species, habitat and feeding regime at hand. An important aspect of the measurement of nitrogen isotopes is that different isotope signatures may exist in sheep fed the same diet in varying spatial scales (DeNiro & Epstein, 1981; Piasentier *et al.*, 2003; Perini *et al.*, 2009). This enables the characterisation of meat obtained from different regions, although raised on the same diet.

In South Africa, several extensive sheep grazing systems, varying on account of diet, exist. Karoo lamb, produced in the Karoo region and known for its specific regional qualities (Du Plessis & Du Rand, 2012; Weissnar & Du Rand, 2012), is the most well-known. Consumers appreciate Karoo lamb for its quality and unique sensory characteristics (i.e. herbaceous aroma and flavour), which are believed to be attributed to the free-range conditions and the grazing on fragrant Karoo plants (Estler *et al.*, 2006; Weissnar & Du Rand, 2012). The labelling of certified sheep meat and the associated premium price also authenticate to consumers a superior quality. Sheep meat from the Karoo may be certified as *Karoo Meat of Origin* through the certification scheme of the Karoo Development Foundation (KDF), South Africa. Certified meat is recognised by a mark placed on the packaging of the meat (*Certified Karoo Meat of Origin*). Other typical sheep grazing systems include sheep from the Swartland and Overberg regions in the Western Cape, sheep from the Kalahari, sheep raised on either the grasslands of the Free State or planted kikuyu/clover pastures of the South-Western Cape.

Evaluating plant type intake is an important aspect of this research study, given the widely documented effect of diet on the sensory characteristics of sheep meat (Young *et al.*, 2003; Almela *et al.*, 2010; Resconi *et al.*, 2010). The effect of diet is commonly being used in current marketing strategies as more emphasis is being placed on product qualities linked to the origin of meat. In South Africa, sheep meat are particularly promoted through labelling according to production practice, such as “free-range lamb”, “certified natural lamb” or origin, such as “Karoo lamb”. However, with these developments, the probability for opportunistic behaviour and false labelling increases. To ensure enforcement and better policing, the need exists to establish analytical tools for authentication. In keeping with current methods, a reasonable approach could be through the application of stable isotope ratio analysis; although there is currently no official methods in the field of food control for multi-element stable isotope analysis of animal products (Camin *et al.*, 2007).

There are currently no published results regarding the use of stable isotope ratios for the purpose of authenticating South African lamb. Hence, the stable isotope ratios of carbon and nitrogen were measured to provide an evaluation of the effectiveness of stable isotope ratio analysis as a potential tool for the authentication of origin and feeding regime of South African Dorper lamb from extensive grazing systems. It was important to determine whether lamb from different farms can be distinguished from one another based on its isotopic profile as dietary differences linked to the variation in vegetation within the regions is expected. It is also essential to establish the discriminative power of the isotope results. Lambs from farms within the Northern Cape, Western Cape and Free State provinces of South Africa were included in the study.

5.2 Materials and methods

5.2.1 Experimental layout and study farms

Seven farms, each unique in terms of its vegetation and the extensive grazing conditions, were selected for the purpose of the study (Table 5.1). Five farms were from the Northern Cape (CK, NK, HK/LO, KV, HK/CAL), one from the Western Cape (RU) and one from the Free State (FS). Ten slaughter ready Dorper lambs ($n = 10$) were sourced from each farm. See General introduction (Chapter 1, Fig. 1.2) for the selected farms.

5.2.2 Northern Cape province

The Northern Cape covers the vast Karoo ecotype and is described as arid to hyper-arid with limited cropping potential (Cloete & Olivier, 2010). Sheep farming is practised in 82.0% of the province due to the limitation on alternative farming ventures. The Karoo constitutes the largest area of the province and features a variety of different vegetation types (Vorster & Roux, 1983; Bramley *et al.*, 2009). The rainfall is low and varies from less than 200 mm or 201-400 mm to 401-600 mm per annum in some places, while droughts may also occur for several years on end (Palmer & Ainslie, 2005). During these periods of drought, the region's plant growth is greatly affected. The region has a low carrying capacity of less than one large stock unit per 40 ha, where the natural pasture for the lambs varies from grassy, dwarf shrublands (Nama-Karoo biome) to dwarf, succulent shrubs (succulent Karoo biome) (Vorster & Roux, 1983; Acocks, 1988; Cloete & Olivier, 2010; Du

Plessis & Du Rand, 2012). From the five farms selected within the Northern Cape: CK and NK fall in the Nama-Karoo biome, HK/LO and HK/CAL in the succulent Karoo biome and KV mainly in the fynbos biome.

5.2.3 Western Cape province

The South African sheep industry comprises of either extensive or fairly intensive enterprises in the pasture-cropping regions and intensive horticultural areas of South Africa (Cloete & Olivier, 2010). The Swartland (western seaboard) and Overberg (southern seaboard) regions of the Western Cape has a typical Mediterranean climate, where sheep production is coordinated with winter grain cropping (Cloete & Olivier, 2010). In the Overberg region, lucerne/alfalfa (*Medicago sativa*) is typically cultivated in the pasture phase and serves as feed for sheep. Small grain stubble is another characteristic feed of the region, which may also form part of the diet (Cloete & Olivier, 2010). The lamb produced within this region is known as “Rûens lamb” (RU), where the typical diet of the sheep associated with the region and traditional farming practises gives the lamb meat its unique sensory qualities. The sheep selected from a farm within the Overberg region was extensively raised on lucerne situated within the fynbos biome and known as the Rûens shale renosterveld.

5.2.4 Free State province

Sheep farming is also practised extensively in the Free State grasslands region. This region consists of plains with summer rainfall (Cloete & Olivier, 2010). The vegetation of the farm selected (FS) mainly consisted of Kimberley thornveld (Savanna biome) and to a lesser extent that of the Western Free State clay grassland (grassland biome) (Cloete & Olivier, 2010).

5.2.5 Plants

Lambs were raised extensively on natural vegetation within the vicinity of the farm. Plant samples were collected from the farms after the lambs were slaughtered. Plants were either collected from the field last grazed before slaughter or, in cases where the field was depleted, from a similar field (less than 1 km away). Sampling of plants from the vicinity grazed provides an indication of the diet of the animal as the latter is largely dependent on animal behaviour and the availability of forage (Radloff *et al.*, 2013). The farmers confirmed the typical diet of the sheep. Within all farms the lambs were born and raised extensively within the borders of the farm. Plant species collected from the farms are listed in Table 5.1 and a more detailed description are provided as Supplementary data (Table S5.1). The number of species from the group with a specific pathway (i.e. C₃, CAM or C₄) is also shown in Table 5.1.

Table 5.1 Lamb farms selected for this study and the plant species collected from the farms

Farm	Area of origin	Code	Carcass classification (No.)	Plant species collected from area	Number of species with pathway ^a		
					C ₃	CAM	C ₄
Carnarvon	Central Karoo	CK	A2 (n = 10)	<i>Galenia sarcophylla</i> ; <i>Lycium cinereum</i> ; <i>Pentzia incana</i> ; <i>Plinthus karrooicus</i> ; <i>Ruschia intricata</i> ; <i>Salsola glabrescens</i> ; <i>Stipagrostis ciliata</i> ; <i>Stipagrostis obtusa</i>	4	1	3
Prieska	Northern Karoo	NK	A2 (n = 10)	<i>Eriocephalus ericoides</i> ; <i>Felicia muricata</i> ; <i>Fingerhuthia africana</i> ; <i>Lycium cinereum</i> ; <i>Pentzia incana</i> ; <i>Phaeoptilum spinosum</i> ; <i>Psilocaulon absimile</i> ; <i>Pteronia glauca</i> ; <i>Rhigozum trichotomum</i> ; <i>Rosenia humilis</i> ; <i>Ruschia intricata</i> ; <i>Salsola aphylla</i> ; <i>Salsola calluna</i> ; <i>Salsola tuberculata</i> ; <i>Stipagrostis ciliata</i> ; <i>Stipagrostis obtusa</i> ; <i>Zygophyllum gilfillanii</i>	8	2	7
Loeriesfontein	Hantam Karoo	HK/LO	A2 (n = 10)	<i>Chrysanthemoides incana</i> ; <i>Pentzia incana</i> ; <i>Pteronia sordida</i> ; <i>Salsola tuberculata</i> ; <i>Stoeberia beetzii</i> ; <i>Tetragonia fruticosa</i> ; <i>Zygophyllum lichtensteinianum</i>	3	2	2
Nieuwoudtville	Knersvlakte	KV	A2 (n = 10)	<i>Chaetobromus dregeanus</i> ; <i>Ereiodium moschatum</i> ; <i>Eriocephalus punctulatus</i> ; <i>Galenia africana</i> ; <i>Lebeckia leipoldtiana</i> ; <i>Medicago polymorpha</i> ; <i>Nylandtia spinosa</i> ; <i>Pentzia incana</i> ; <i>Wiborgia monoptera</i> ; <i>Wiborgia sericea</i>	9	0	1
Calvinia	Hantam Karoo	HK/CAL	A2 (n = 5) A3 (n = 5)	<i>Chrysocoma ciliata</i> ; <i>Drosanthemum hispidum</i> ; <i>Eberlanzia ferox</i> ; <i>Eriocephalus ericoides</i> ; <i>Justica orchioides</i> ; <i>Lycium spp.</i> ; <i>Mesembryanthemum vaginatum</i> (<i>Brownanthus</i> or <i>Ruschia vaginatum</i>); <i>Pentzia incana</i> ; <i>Pentzia sphaerocephala</i> ; <i>Salsola calluna</i> ; <i>Zygophyllum lichtensteinianum</i>	6	3	2
Swellendam	Rûens	RU	A2 (n = 10)	<i>Cynodon dactylon</i> ; <i>Medicago sativa</i>	1	0	1
Boshof	Free State	FS	A2 (n = 10)	<i>Aristida congesta subsp. congesta</i> ; <i>Cynodon dactylon</i> ; <i>Eragrostis lehmanniana</i> ; <i>Eragrostis superba</i> ; <i>Fingerhuthia africana</i> ; <i>Heteropogon contortus</i> ; <i>Schmidtia kalihariensis</i> ; <i>Themeda triandra</i>	0	0	8

(No.) Number of samples; A2: (A) no permanent incisor teeth, (2) fat depth measures 1.0-4.0 mm (lean); A3: (A) no permanent incisor teeth, (3) fat depth measures 4.1-7.0 mm (medium); The fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline in sheep (NDA, 1990); ^a See Table S5.1 (Supplementary data) for specific details; (CAM) crassulacean acid metabolism.

5.2.6 Sample collection

The day before slaughter, lambs from each farm were transported to the nearest abattoir. Animals were slaughtered according to standard South African procedures and regulations (DAFF, 2000). Variation among animals was reduced by randomly selecting ten carcasses according to age (class A, no permanent incisor teeth), fatness (class 2 or 3) and weight (less than 20 kg). In South Africa carcasses are classified according to age, fat, conformation and damage classes as described under the Product Standards Act No.119 of 1990 and its regulations (DAFF, 1990; DAFF, 2006). The degree of fatness is classified according to the amount of fat measured between the 3rd and 4th lumbar vertebrae, where class 2 carcasses measures 1.0-4.0 mm (lean) and class 3 measures 4.1-7.0 mm (medium fatness). Following slaughtering, the carcasses were cooled at 4°C for 24 h. The *Longissimus thoracis et lumborum* (LTL) muscle were excised from the left side of the carcasses. Subcutaneous fat and visible sinews were removed and the LTL divided so that the *Longissimus lumborum* (LL) was homogenised for stable isotope ratio analysis.

5.2.7 Sample preparation

The fat of a 5 g homogenised meat sample were extracted (Lee *et al.*, 1996). The meat was defatted to correct for variation in isotopic ratios between proteins and lipids due to the effect of the biochemical isotopic fractionation (Camin *et al.*, 2007). As the lipid fraction contains little nitrogen, stable isotope analysis of meat was only conducted on the protein fraction (Camin *et al.*, 2007). The protein residue was freeze-dried and finely ground into a homogenous powder using a pestle and mortar. Powdered meat samples were then vacuum-sealed and stored at -20°C until analysis.

5.2.8 Isotope ratio analysis

Powdered meat samples were weighed into tin cups to an accuracy of 1 microgram and combusted individually in a Flash 2000 organic elemental analyser, and the resultant CO₂ and N₂ gas introduced to a isotopic mass spectrometer (Delta V Plus) using a ConFlo IV gas control unit (Thermo Scientific, Bremen, Germany). Isotope ratios are expressed in the conventional delta (δ) notation in parts per mil (‰) and correspond to Vienna PeeDee Belemnite (PDB) and nitrogen air (N₂) (internal standards) according to the following general formula:

$$\delta\text{‰} = \frac{\mathbf{R} \text{ sample} - \mathbf{R} \text{ standard}}{\mathbf{R} \text{ standard}} \times 1000$$

where **R** represents the ratio between the abundant isotopes i.e. ¹³C/¹²C and ¹⁵N/¹⁴N. Standard deviations of repeated measurements of in-house standards were less than 0.25‰. The in-house standards used were: Choc, a commercial chocolate/egg mixture; Sucrose, Australian National University (ANU) sucrose; Valine, DL Valine purchased from Sigma; MG (Merck Gel), a proteinaceous gel produced by Merck and Seal which is a seal bone crushed, demineralised and dissolved in acid, and then reconstituted in gel form. All in-house standards were calibrated against the International Atomic Energy Agency (IAEA) standards. PDB has

relatively more ^{13}C than most of the terrestrial biosphere (Sandberg *et al.*, 2012). Accordingly, the tissues of plants and animals have negative $\delta^{13}\text{C}$ values

5.2.9 Statistical analysis

Statistical analysis of data was performed using GLM (General Linear Models) procedure of SAS[™] statistical software (Statistical Analysis System, Version, 9.2, 2006, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and XLSTAT[®] statistical software (Version 2014.2.03; Addinsoft, New York, USA) for the multivariate statistical analysis. Pre-processing of the data involved using the Shapiro-Wilk test to test for deviation from normality (Shapiro & Wilk, 1965). When the deviation from normality was significant ($P \leq 0.05$) the outliers in the data were identified and removed until the data was normal or symmetrically distributed. Following the confirmation of normality of the data, one-way ANOVA was carried out for each stable isotope ratio with farm as factor. Fisher's Least Significant Differences (LSD) was calculated at a 5% significance level to compare farm means. A probability level of 5% was considered significant for all the significance tests. Multivariate statistical techniques were used to find significant patterns and associations in the collected data. Discriminant analysis (DA) was carried out to determine whether lamb meat from the different farms could be mathematically distinguished on the basis of its stable isotope ratios. Farm separation was described and elucidated by means of linear functions of the variables (discriminant functions) that best separated farms, using the first six observations from each farm as a training set. The model was then validated using leave-N-out (LNO) cross-validation by using the last four observations from each farm as a test set. The grouping of the farms in separate clusters, on the basis of their meat stable isotope ratios, was performed applying Ward's hierarchical clustering technique (Ward, 1963). This technique clusters animals into smaller numbers of mutually exclusive groups, each having members that are similar with respect to specified characteristics.

5.3 Results and discussion

On the basis of production, seven groups (farms) of different geographic origin can be identified. Farms can further be divided in three groups based on diet (Table 5.2). Lamb from the Northern Cape were mainly raised on Karoo bushes/shrubs and grasses (CK, NK, HK/LO, KV and HK/CAL), while lamb from the Western Cape and Free State were raised on lucerne/alfalfa (*Medicago sativa*) (RU) and grass (FS), respectively (Table 5.2). It is important to note that although one would expect the isotopic differences of the plants to be exactly replicated in the tissues of animals living on these plants; this is not the case as isotope fractionation takes place during metabolism and results in varying isotope ratios between different tissues of the animal (Vogel, 1978). Therefore, for the purpose of this study only the protein fraction was used (i.e. defatted meat).

Table 5.2 The means (\pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in ‰, relative to the PeeDee Belemnite and atmospheric N_2 standards, respectively) of lamb meat from different farms

Code	Diet	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
CK	Shrubs/bushes and grass	10	$-19.6^c \pm 1.07$	$8.9^c \pm 0.41$
NK	Shrubs/bushes and grass	10	$-20.1^c \pm 0.82$	$12.1^f \pm 0.80$
HK/LO	Shrubs/bushes and grass	10	$-18.7^d \pm 0.47$	$10.3^e \pm 0.22$
KV	Shrubs/bushes and grass	10	$-24.3^a \pm 0.24$	$9.5^d \pm 0.26$
HK/CAL	Shrubs/bushes and grass	10	$-22.9^b \pm 0.41$	$12.9^g \pm 0.56$
RU	Lucerne/alfalfa	10	$-22.7^b \pm 0.20$	$6.7^a \pm 0.36$
FS	Grass	10	$-15.8^e \pm 1.16$	$8.2^b \pm 0.45$
LSD (P = 0.05)			0.65	0.43

(SD) Standard Deviation; (n) number of samples (animals); (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rùens; (FS) Free State; (LSD) Least significant difference; ^{a-e} Values in the same column for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with different superscripts are significantly different ($P \leq 0.05$).

5.3.1 Variability of meat stable isotope ratios

Mean values of the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios of meat protein are shown in Table 5.2. The $\delta^{13}\text{C}$ values are affected by the origin of the meat, mainly reflecting the diet. KV had the lowest and significant ($P \leq 0.05$) $\delta^{13}\text{C}$ value ($-24.3 \pm 0.24\text{‰}$), followed by HK/CAL ($-22.9 \pm 0.41\text{‰}$) and RU ($-22.7 \pm 0.20\text{‰}$), which did not differ from each other (Table 5.2). The Free State lamb (FS) had the least negative $\delta^{13}\text{C}$ value ($-15.8 \pm 1.16\text{‰}$) (Table 5.2). It is known that the carbon isotope ratio is largely influenced by the amount of C_3 and C_4 plant material incorporated into the animal's diet. The C_3 -pathway (typical of temperate pasture plants) causes a large change in the carbon isotope proportions relative to atmospheric carbon dioxide (Piasentier *et al.*, 2003). However, the C_4 -pathway (typical of tropical plants) incorporates more ^{13}C and results in a smaller change i.e. less negative $\delta^{13}\text{C}$ values. Hence, C_4 plants have higher $^{13}\text{C}/^{12}\text{C}$ ratios in their tissues compared to C_3 plants (Vogel *et al.*, 1978; Gibson, 2009). The same difference in $^{13}\text{C}/^{12}\text{C}$ ratios is recorded in the tissues of the animals raised on C_3 and C_4 plants, respectively (Codron *et al.*, 2005c). Perini *et al.* (2009) found that lambs predominantly fed forage have the lowest (most negative) $\delta^{13}\text{C}$ values, while those which had concentrates included in their diets had less negative values. This is attributed to the use of grains and by-products of maize (a C_4 plant) in the concentrates. A similar ^{13}C -enrichment was reported for lambs fed milk (Camin *et al.*, 2007).

The $\delta^{13}\text{C}$ values reported in Table 5.2 for the Western Cape (RU) and Free State farms (FS) are similar to the $\delta^{13}\text{C}$ values reported by Codron *et al.* (2005b) for lucerne/alfalfa (C_3 plant) diet (-26.8‰) and Bermuda grass (C_4 plant) diet (-13.6‰), respectively. Given that the RU lambs were raised on lucerne/alfalfa and the FS lamb on grass, the $\delta^{13}\text{C}$ values of the meat are similar to that of the diet. Hence, one can assume that the

carbon isotope ratios expressed in the meat is representative of the diet of the animal. Although Codron *et al.* (2005b) determined the carbon isotope ratios of the faeces and not of the meat, they also established a direct link between diet and the faeces with some discrimination resulting in lower faecal $\delta^{13}\text{C}$ values. Typically, pure grazer animals consuming C_4 plants had the highest $\delta^{13}\text{C}$ values (-14.7 to -13.5‰), while browsers consuming C_3 plants had the lowest (-27.2 to -26.2‰) and mixed-feeders consuming both C_4 and C_3 plants had intermediate values (-23.0 to -19.0‰) (Codron *et al.*, 2005c). Similarly, Vogel *et al.* (1978) found C_3 plants to have $\delta^{13}\text{C}$ values more negative than -20‰ and C_4 plants more positive than -16‰ . Following these findings and the results presented in Table 5.2 in combination with the plants collected from the different areas (Table 5.1), one can interpret the results in a similar manner.

The CK and NK farms had intermediate $\delta^{13}\text{C}$ values (not significantly different) (Table 5.2), which is expected as the plants collected from the areas included both C_3 and C_4 plants (Table 5.1). The Dorper is known to be a more general or non-selective grazer, focussing on the woody plant types of the veld, such as Karoo bushes, trees and shrubs (Du Toit, 1998). However, they do graze sparingly on the latter if there are still soft forages available in the veld. Soft forage may consist of perennial grasses known to be very palatable grazing plants (Acocks, 1988). Hence, the main contributor towards the increase in $\delta^{13}\text{C}$ values of CK and NK is likely to be the C_4 grass species such as *Fingerhuthia africana*, *Stipagrostis ciliata* and *Stipagrostis obtusa* (Table 5.1). Karoo bushes are mostly consumed during winter and times of drought when the perennial grasses are depleted (Acocks, 1988). Similarly, Radloff *et al.* (2013) reported that the dietary intake of cattle is largely dependent on the forage conditions to which they are exposed to. Cattle, typically considered grazers, would increase their browse intake to compensate for the decrease in grass quality during the late dry season.

HK/LO had slightly higher $\delta^{13}\text{C}$ values than that of CK and NK, differing significantly from the rest ($P \leq 0.05$) (Table 5.2). Although the plants collected from the HK/LO area did not include C_4 grass species, CAM plants (*Stoeberia beetzii* and *Tetragonia fruticosa*) were prevalent in the area and these are known to have intermediate $\delta^{13}\text{C}$ values (Table 5.1) (Codron *et al.*, 2005c; Capuano *et al.*, 2013). The plants collected from the HK/CAL farm mainly included C_3 Karoo bushes/shrubs (*Chrysocoma ciliata*, *Eriocephalus ericoides*, *Justica orchioides*, *Lycium spp.*, *Pentzia incana* and *Pentzia sphaerocephala*) and some succulents (*Drosanthemum hispidum*, *Eberlanzia ferox* and *Mesembryanthemum vaginatum*) known to provide negative and intermediate $\delta^{13}\text{C}$ values, respectively (Table 5.1) (Capuano *et al.*, 2013). As a result, the HK/CAL lamb had low $\delta^{13}\text{C}$ values (Table 5.2). Vogel (1978) reported similar results with lower ^{13}C values for springbok living on Karoo bushes/shrubs from the Northern Cape compared to those consuming more grass from Namibia. In contrast, HK/CAL lamb had the highest $\delta^{15}\text{N}$ value ($12.9 \pm 0.56\text{‰}$).

Environmental parameters such as climate and soil condition are known to influence the ^{15}N content of feed, which in turn affects the $\delta^{15}\text{N}$ values of the products produced from it. Furthermore, organic fertilisers increase the ^{15}N level of nitrogen compounds of soil and plants, whereas the presence of legumes has the opposite effect on grassland ^{15}N -content, because it uses the nitrogen in the air as a nitrogen source (DeNiro

& Epstein, 1981; Piasentier *et al.*, 2003; Perini *et al.*, 2009). Perini *et al.* (2009) proposed that an increase in the $\delta^{15}\text{N}$ values of lamb meat could be due to the application of organic fertilisers or linked to arid conditions of the production site. Hence, the high $\delta^{15}\text{N}$ values for HK/CAL are likely due to the arid conditions of the farm. Another contributor towards ^{15}N -enrichment could also be the prevalence of CAM plants as Codron *et al.* (2005a) observed the highest $\delta^{15}\text{N}$ values for succulents. Conversely Devincenzi *et al.* (2014) found that $\delta^{15}\text{N}$ decreased linearly with the proportion of legume in the diet of lambs. This is likely the reason for the decrease in $\delta^{15}\text{N}$ values for KV ($9.5 \pm 0.26\text{‰}$) (Table 5.2). The plants collected from the KV farm included several of these leguminous plant species such as *Lebeckia leipoldtiana*, *Wiborgia monoptera* and *Wiborgia sericea* (Table 5.1).

Piasentier *et al.* (2003) found differences in the $\delta^{15}\text{N}$ values of lamb meat fed similar diets, but in different countries. Therefore, the different $\delta^{15}\text{N}$ values for the lamb meat of the various farms were expected (Table 5.2). This was specifically related to lamb from the Northern Cape, which is produced on typical Karoo plants which vary depending on the origin within the region. In fact, the lamb from the Western Cape and Free State farms (RU and FS) had the lowest ($P \leq 0.05$) $\delta^{15}\text{N}$ values, followed by the Northern Cape farms (CK, KV, HK/LO, NK and HK/CAL), HK/CAL having the highest $\delta^{15}\text{N}$ value. Overall $\delta^{15}\text{N}$ values showed the clearest discrimination between farms in the stable isotope ratios examined as a result of low within-group (animal to animal) variation and the greatest between farm variability (Table 5.2). According to Piasentier *et al.* (2003), the reported $\delta^{15}\text{N}$ values were not indicative of a specific region as distant regions might be similar. This was also evident in the current study as farms largely varying in diet and location had similar values such as CK (8.9 ± 0.41) and FS (8.2 ± 0.45) (Table 5.2).

5.3.2 Discriminating geographical origin from meat stable isotope ratios

Discriminant analysis (DA) was performed to test if the stable isotope ratios in lamb meat allow to discriminate the different farms of origin. The results are reported in Table 5.3 as the number and percentage of correctly classified observations. The stable isotope ratios allowed 97.62% correct classification of the meat samples for the estimation model and 96.43% for the validation model (Table 5.3). With the exception of CK, all the meat samples were 100% correctly classified (Table 5.3). The 25% misclassification of the CK is as a result of the variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between animals sourced from the CK area. The results obtained for the classification of lamb meat according to farm of origin confirm the potential of using stable isotope ratio analysis as a tool for the characterisation of the origin of meat, i.e. the authentication of farm of origin lamb meat and its traceability linked to a diet typical of the defined region.

Ward's hierarchical clustering technique (Ward, 1963) was applied for grouping farms based on the dissimilarities in the stable isotope ratio values of the meat samples. During the clustering process samples, which minimizes the agglomeration criterion, are clustered together. A binary clustering tree (dendrogram), whose root is the class that contains all the samples, is produced by all the succeeding clustering processes.

The dendrogram of the clustering is provided as Supplementary data (Fig. S5.1). Six classes were identified and the resulting classification sample codes used as observation labels in the DA plot.

Table 5.3 Classification of individual lamb meat samples, on the basis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percentage of observations correctly classified

Model	Actual origin	Classified origin ^a							Total
		CK	NK	HK/LO	KV	HK/CAL	RU	FS	
Estimation	CK	5	0	0	0	0	0	1	6
	NK	0	6	0	0	0	0	0	6
	HK/LO	0	0	6	0	0	0	0	6
	KV	0	0	0	6	0	0	0	6
	HK/CAL	0	0	0	0	6	0	0	6
	RU	0	0	0	0	0	6	0	6
	FS	0	0	0	0	0	0	6	6
% Correctly classified		83.33	100	100	100	100	100	100	97.62 ^b
Validation	CK	3	1	0	0	0	0	0	4
	NK	0	4	0	0	0	0	0	4
	HK/LO	0	0	4	0	0	0	0	4
	KV	0	0	0	4	0	0	0	4
	HK/CAL	0	0	0	0	4	0	0	4
	RU	0	0	0	0	0	4	0	4
	FS	0	0	0	0	0	0	4	4
% Correctly classified		75.00	100	100	100	100	100	100	96.43 ^c

^a The number of correctly classified observations are tabulated diagonally; ^b 97.6% of the observations for the estimation model correctly classified; ^c 96.4% of the observations for the validation correctly classified; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State.

The DA plots of the observations (labelled according to Ward's clusters) and loadings on the linear discriminant function scores are illustrated in Figure 5.2. The DA plots were used to visualise the grouping of observations within each farm of origin and the separation between farms of origin. The DA plots confirms that the farms of origin are very well discriminated on the axes extracted from the stable isotope ratios. In fact, Figure 5.2b shows that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values influence the location of samples along the axes. NK, HK/CAL and KV associate with higher $\delta^{15}\text{N}$ values, while RU, FS, CK and HK/LO associate with less negative $\delta^{13}\text{C}$ values. Figure 5.2a also indicates that the seven farms of origin could be classified in six clusters. Each farm was assigned to its own cluster, except for CK and HK/LO which were clustered together (cluster 3) (Fig. 5.2a).

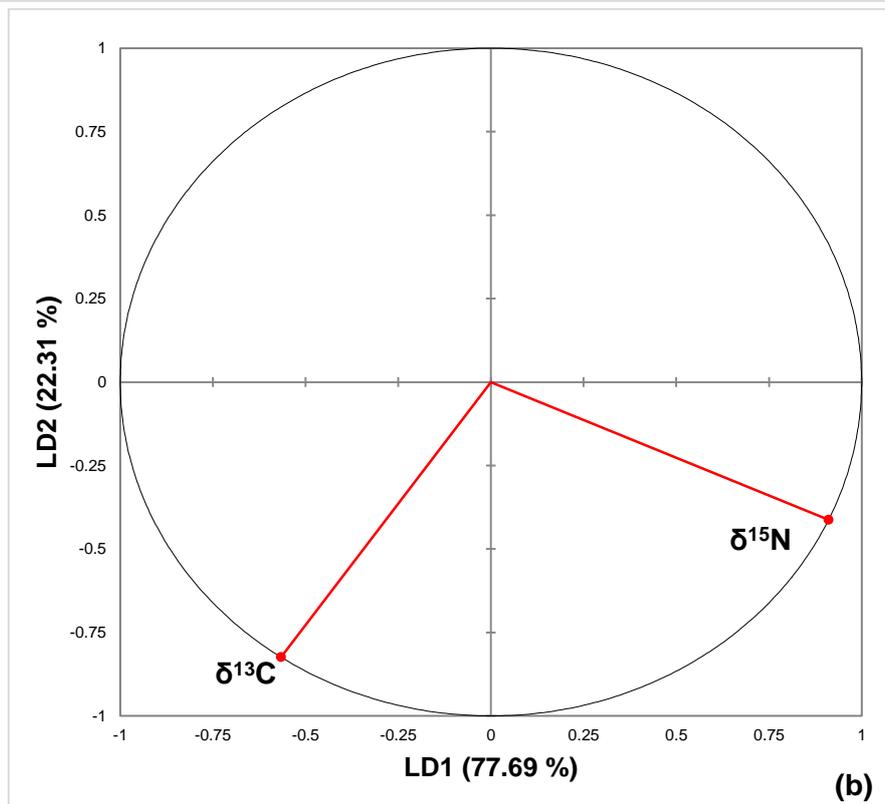
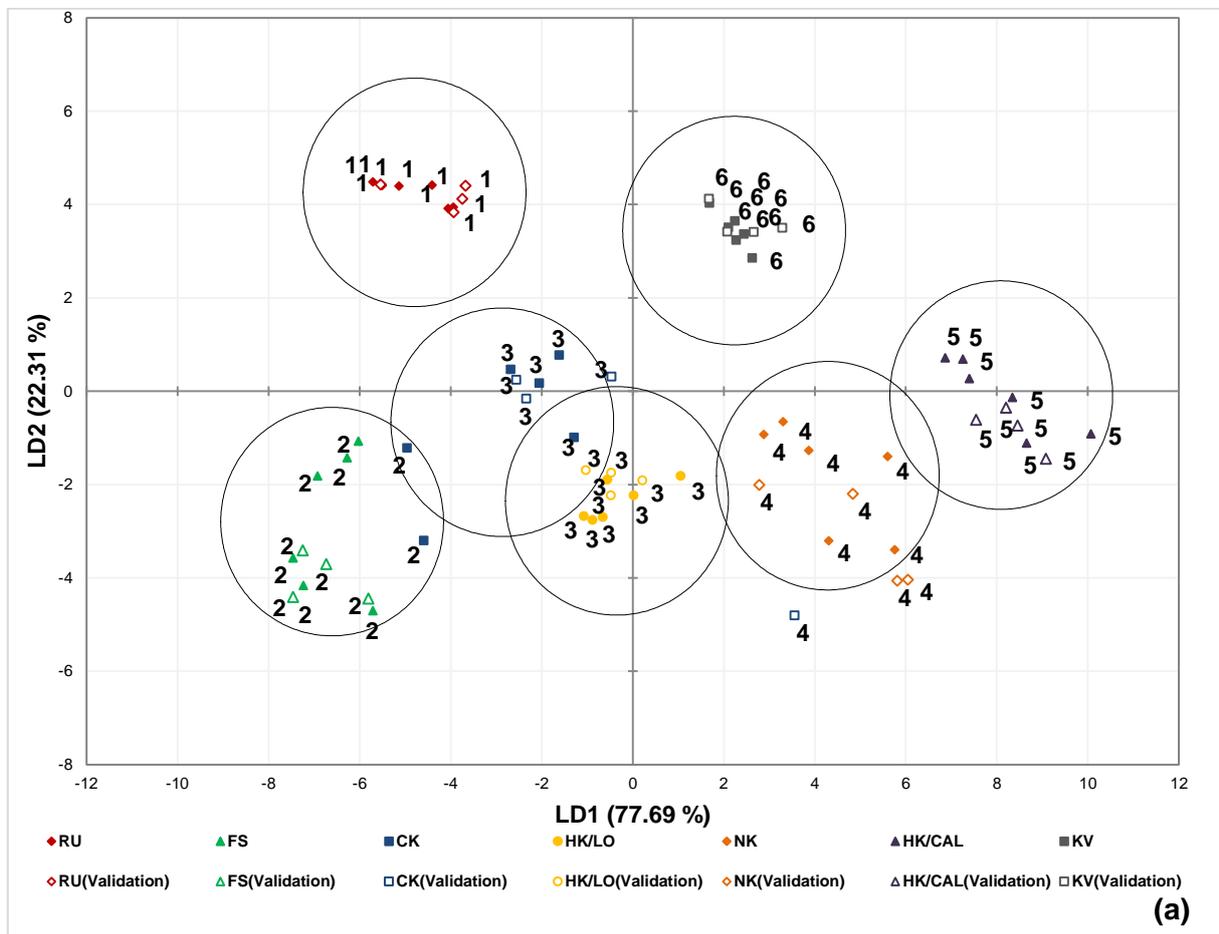


Figure 5.2 Discriminant analysis (DA) plot (a) and DA variable loadings plot (b) of the linear discriminant function scores on stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in lamb meat for the grouping of observations from different farms, using Ward cluster numbers (1 to 6) as observation labels. (LD) Linear Discriminant Score; (RU) Rûens; (FS) Free State; (CK) Central Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (NK) Northern Karoo; (HK/CAL) Hantam Karoo/Calvinia; (KV) Knersvlakte.

CK and FS had the most variable $\delta^{13}\text{C}$ values, where the isotope ratios of some of the individual animals were far from the mean of the group (Fig. 5.2). Such variation is likely as the amount and type of plant species consumed by individual animals is a complex behavioural act influenced by various factors (Holechek *et al.*, 1982). Three of the ten CK samples were also assigned to different clusters, two to cluster 2 (FS) and one to cluster 4 (NK) (Fig. 5.2a). The other Northern Cape farms (CK, NK and HK/LO) grouped more to the middle of the graph (Fig. 5.2); hence, having lower $\delta^{15}\text{N}$ values combined with less negative $\delta^{13}\text{C}$ values (Fig. 5.2b). Some of the plants collected from the farms, apart from that of HK/LO, included C_4 grasses, likely the cause of increase in $\delta^{13}\text{C}$ values (Table 5.1). C_4 grasses have carbon isotope ratios ranging from -9 to -18% (Gibson, 2009). Although the HK/LO production area did not feature any of such grasses, it did have various succulent CAM plant species (Table 5.1). As CAM plants result in intermediate $\delta^{13}\text{C}$ values a high inclusion in the diet could increase the $\delta^{13}\text{C}$ values (Capuano *et al.*, 2013).

Given the assumed similarity in diets between HK/CAL and HK/LO one expected the $\delta^{13}\text{C}$ values to group closely. However, the results showed a different grouping with HK/LO closer to the combined carbon and nitrogen isotope ratios of CK and NK (Fig. 5.2a) suggesting that the HK/CAL lambs consumed more Karoo bushes/shrubs compared to that of HK/LO and in effect also to that of the other areas. A more accurate approach to confirm the composition of the diet would be to identify the exact plant species consumed by using techniques such as micro-histological analysis of the faeces (Holechek *et al.*, 1982). Unfortunately, such an approach was out of the scope of the current study, but for future work it would provide valuable information regarding the effect of diet on the isotope ratios of the meat. Although carbon and nitrogen isotopes allowed a good discrimination between farms, the use of other isotopes (i.e. ^2H , ^{18}O and ^{34}S) has the potential to provide an additional level of geographical resolution (Kelly *et al.*, 2005; Crawford *et al.*, 2008). For instance, Perini *et al.* (2009) explored the use of several isotopes (^2H , ^{13}C , ^{15}N , ^{18}O and ^{34}S) as an effective tool to trace the geographical origin and diet of lambs.

5.4 Conclusions

The study proved that stable isotope ratio analysis of meat is a promising tool to evaluate extensive meat production associated with type of diet of which the ^{13}C isotope are particularly useful for indicating dietary differences. The results were reasonably representative of the observed vegetation of the different farms. Hence, one can conclude that the collection of plants from the animal production site provides an indication of the likely diet of the animals. Stable isotope ratio analysis is also useful when limited number of sample replicates is available. It provided evidence for the authenticity of extensively produced South African Dorper lamb based on origin, as well as diet. By applying discriminant analysis 97.62% and 96.43% of the observations for the estimation and validation were correctly classified, respectively. The results demonstrate the usefulness of multi-element fingerprints as indicators for authenticating the geographical origin of lamb in South Africa. Through the analysis of additional isotopes, one can even further improve the discriminative power of the technique. Further research would entail determining baseline data of stable isotope ratios for

regionally unique South African lamb so as to development robust classification models using a larger sample set which is representative of the different regions. The latter would provide the means of moving from the current farm of origin to region of origin authentication of sheep meat.

5.5 References

- Acocks, J. P. H. (1988). Veld types of South Africa. In: *Memoirs of the Botanical Survey of South Africa*. 3rd ed. Vol. 57.
- Almela, E., Jordan, M. J., Martinez, C., Sotomayor, J. A., Bedia, M. & Banon, S. (2010). Ewe's diet (pasture vs. grain-based feed) affects volatile profile of cooked meat from light lamb. *Journal of Agricultural and Food Chemistry*, **58**, 9641-9646.
- Bramley, C., Bienabe, E. & Kirsten, J. (2009). The economics of geographical indications towards a conceptual framework for geographical indication research in developing countries. In: *The Economics of Intellectual Property*. Pp. 109-141. New York, USA: World Intellectual Property Organization (WIPO).
- Brand, T. S. (2000). Grazing behaviour and diet selection by Dorper sheep. *Small Ruminant Research*, **36**, 147-158.
- Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S. D., Schlicht, C., Thomas, F., Monahan, F. J., Hoogewerff, J. & Rossmann, A. (2007). Multi-element (H, C, N, S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry*, **389**, 309-320.
- Capuano, E., Boerrigter-Eenling, R., Van der Veer, G. & Van Ruth, S. M. (2013). Analytical authentication of organic products: an overview of markers. *Journal of the Science of Food and Agriculture*, **93**, 12-28.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Codron, J., Codron, D., Lee-Thorp, J. A., Sponheimer, M., Bond, W. J., De Ruiter, D. & Grant, R. (2005b). Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. *Journal of Archaeological Science*, **32**, 1757-1772.
- Codron, D., Codron, J., Lee-Thorp, J. A., Sponheimer, M. & De Ruiter, D. (2005a). Animal diets in the Waterberg based on stable isotopic composition of faeces. *South African Journal of Wildlife Research*, **35**(1), 43-52.
- Codron, D., Codron, J., Sponheimer, M., Lee-Thorp, J. A., Robinson, T., Grant, C. C. & De Ruiter, D. (2005c). Assessing diet in savanna herbivores using stable carbon isotope ratios of faeces. *Koedoe*, **48**(1), 115-124.
- Crawford, K., McDonald, R. A. & Bearhop, S. (2008). Applications of stable isotope techniques to the ecology of mammals. *Mammal Review*, **38**, 87-107.
- DeNiro, M. J. & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495-506.
- DeNiro, M. J. & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341-351.

- Department of Agriculture, Forestry and Fisheries (DAFF). (1990). *Product Standards Act* (Act No.119 of 1990, No. R. 342). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2000). *Meat Safety Act* (Act No.40 of 2000, No. 1072). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2006). *Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa*. Government Gazette of 1 September 2006, No. R. 863. Pretoria, South Africa: Government Printer.
- Devincenzi, T., Delfosse, O., Andueza, D., Nabinger, C. & Prache, S. (2014). Dose-dependent response of nitrogen stable isotope ratio to proportion of legumes in diet to authenticate lamb meat produced from legume-rich diets. *Food Chemistry*, **152**, 456-461.
- Du Plessis, H. J. & Du Rand, G. (2012). The significance of traceability in consumer decision making towards Karoo lamb. *Food Research International*, **47**, 210-217.
- Du Toit, P. C. V. (1998). Diets selected by Merino and Dorper sheep in Karoo veld. *Archivos de Zootecnia*, **47**, 21-32.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Franke, B. M., Gremaud, G., Hadorn, R. & Kreuzer, M. (2005). Geographic origin of meat – elements of an analytical approach to its authentication. *European Food Research Technology*, **221**, 493-503.
- Gibson, D. J. (2009). *Grasses and Grassland Ecology*. Oxford, USA: Oxford University Press.
- Holechek, J. L., Vavra, M. & Pieper, R. D. (1982). Botanical composition determination of range herbivore diets: a review. *Journal of Range Management*, **35**(3), 309-315.
- Kelly, S., Heaton, K. & Hoogewerff, J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends in Food Science & Technology*, **16**, 555-567.
- Lee, C. M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC International*, **79**(2), 487-492.
- Low, A. B. & Rebelo, A. G. (1996). *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs & Tourism, Pretoria.
- Mucina, L. & Rutherford, M. C. (2006). South African vegetation map. South African National Biodiversity Institute (SANBI). Available from: <http://bgis.sanbi.org/vegmap/map.asp>.
- Palmer, A. R. & Ainslie, A. M. (2005). Grasslands of South Africa. In: *Plant Production and Protection Series: Grasslands of the World* (edited by J. M. Suttie, S. G. Reynolds & C. Batello). No. 34. Pp. 77-120. Rome, Italy: Food and Agriculture Organization of the United Nations.
- Perini, M., Camin, F., Bontempo, L., Rossmann, A. & Piasentier, E. (2009). Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions. *Rapid Communications in Mass Spectrometry*, **23**, 2573-2585.
- Piasentier, E., Valusso, R., Camin, F. & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science*, **64**, 239-247.

- Radloff, F. G. T., Van der Waal, C. & Bond, A. L. (2013). Extensive browsing by a conventional grazer? Stable carbon isotope analysis reveals extraordinary dietary flexibility among Sanga cattle of North Central Namibia. *Isotopes in Environmental and Health Studies*, 1-7.
- Resconi, V. C., Campo, M. M., Montossi, F., Ferreira, V., Sañudo, C. & Escudero, A. (2010). Relationship between odour-active compounds and flavour perception in meat from lamb fed different diets. *Meat Science*, **85**, 700-706.
- Sandberg, P. A., Loudon, J. E. & Sponheimer, M. (2012). Stable isotope analysis in Primatology: a critical review. *American Journal of Primatology*, **74**(11), 969-989.
- Shapiro, S. S. & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Vogel, J. C. (1978). Isotopic assessment of the dietary habitats of ungulates. *South African Journal of Science*, **74**, 298-301.
- Vogel, J. C., Fuls, A. & Ellis, R. P. (1978). The geographical distribution of Kranz grasses in South Africa. *South African Journal of Science*, **74**, 209-215.
- Vorster, M. & Roux, P. W. (1983). Veld of the Karoo areas. *Proceedings of the Annual Congress of the Grassland Society of South Africa*, **18**, 18-24.
- Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, **58**(301), 236-244.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Young, O. A., Lane, G. A., Priolo, A. & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food Agriculture*, **83**, 93-104.

Supplementary data

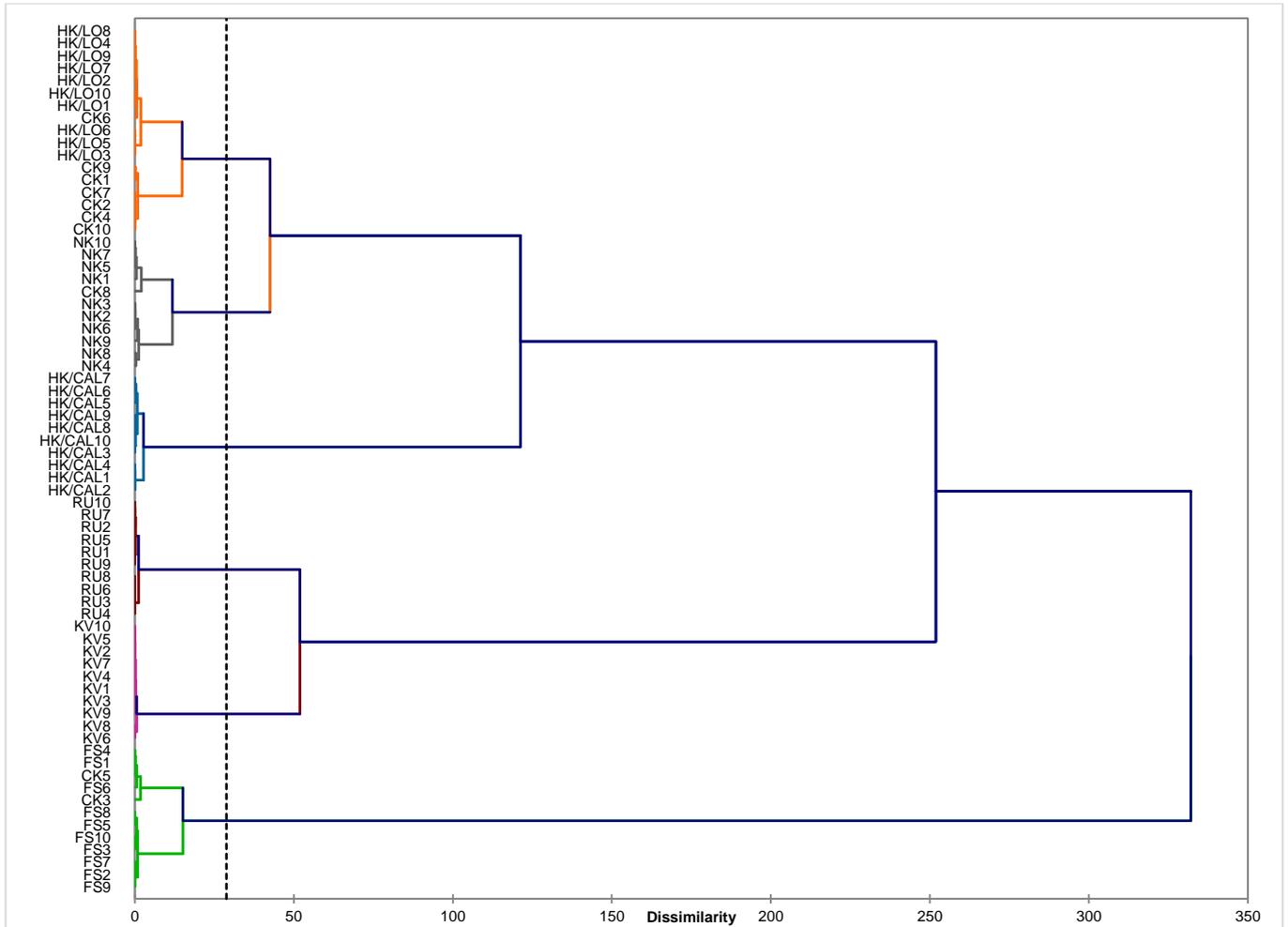


Figure S5.1 Dendrogram showing the clustering of the lamb meat samples using the two isotopic variables. (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State.

Supplementary data

Table S5.1 Details of plant species collected from the seven different farms

Area code	Common name	Family name	Species name	Description	Palatability	References ^a	Plant path	References ^b
CK	Ankerkaroo (Anchor karoo)	Asteraceae	<i>Pentzia incana</i>	Perennial dwarf shrub 200-250 mm tall	Palatability varies depending on region. Abundant and vital part of sheep's diet	DAFF, 2014; Le Roux et al., 1994	C ₃	
CK	Beslyn / Vanwyksbrak	Aizoaceae	<i>Galenia sarcophylla</i>	Perennial ephemeral prostrate herb 200-300 mm in diameter	Palatable and fattens sheep, has short life-span	DAFF, 2014; Le Roux et al., 1994	C ₃	
CK	Kortbeen boesmangras (small Bushman grass)	Poaceae	<i>Stipagrostis obtusa</i>	Perennial arid-climate grass up to 500 mm tall	Very palatable and grazed down short	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
CK	Kriedoring	Solanaceae	<i>Lycium cinereum</i>	Densely branched perennial shrub up to 1 m tall	Not particularly grazed, except for new growth and fruit	DAFF, 2014; Shearing, 1994	C ₃	
CK	Langbeen boesmangras (tall Bushman grass)	Poaceae	<i>Stipagrostis ciliata</i>	Perennial arid-climate grass up to 1 m tall	Very palatable and grazed down short	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
CK	Rivierganna	Chenopodiaceae	<i>Salsola glabrescens</i>	Shrub with woody stems, up to 1.5 m tall	Palatable, grazed less when it occurs in homogenous stands	Le Roux et al., 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002
CK	Silwerkaroo (Silver karoo)	Aizoaceae	<i>Plinthus karrooicus</i>	Perennial dwarf shrub 100-300 mm tall	Very palatable and drought-resistant. Presence is indication of potentially valuable bossieveld	DAFF, 2014; Le Roux et al., 1994	C ₃	Heaton, 1987
CK	Vyebossie / Doringvygie	Aizoaceae	<i>Ruschia intricata</i>	Perennial succulent leaved shrublet up to 400 mm tall	Moderate palatability	DAFF, 2014	CAM	Von Willert <i>et al.</i> , 1992
NK	Ankerkaroo (Anchor karoo)	Asteraceae	<i>Pentzia incana</i>	Dwarf shrub 200-250 mm tall	Palatability varies depending on region. Abundant and vital part of sheep's diet	DAFF, 2014; Le Roux et al., 1994	C ₃	
NK	Asbos	Aizoaceae	<i>Psilocaulon absimile</i>	Spreading shrub 250-800 mm tall	Unpalatable, but grazed under pressure	Le Roux et al., 1994	CAM	Von Willert <i>et al.</i> , 1992
NK	Blomkoolganna	Chenopodiaceae	<i>Salsola tuberculata</i>	Dome-shaped shrublet 200 mm tall	Palatable and highly resistant to grazing and drought	Le Roux et al., 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002
NK	Bloublommetjie	Asteraceae	<i>Felicia muricata</i>	Perennial soft shrublet 150-500 mm tall	Very palatable and grazed down short in most parts of the winter rainfall region. Fairly unpalatable in the Great Karoo. Not drought-resistant	DAFF, 2014; Le Roux et al., 1994; Van Breda & Barnard, 1991	C ₃	
NK	Boegoekaroo	Asteraceae	<i>Pteronia glauca</i>	Perennial woody dwarf shrublet 300-600 mm tall	Palatable and eaten by sheep	DAFF, 2014	C ₃	
NK	Brosdoring	Nyctaginaceae	<i>Phaeoptilum spinosum</i>	Woody shrub 1-1.8 m tall	Produce many palatable fruits for a limited period of the year	Le Roux et al., 1994	C ₃	

Supplementary data

Table S5.1 (Continued)

Area code	Common name	Family name	Species name	Description	Palatability	References ^a	Plant path	References ^b
NK	Driedoring bossie (Three-thorn)	Bignoniaceae	<i>Rhigozum trichotomum</i>	Perennial rigid, woody shrub 1-2 m tall	Pods and flowers grazed	DAFF, 2014; Le Roux et al., 1994	C ₃	
NK	Gannabos	Chenopodiaceae	<i>Salsola aphylla</i>	Perennial succulent leaved shrub up to 4 m tall	Palatable, very well grazed. Animals strip leaves from stems	DAFF, 2014; Shearing, 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002
NK	Kapokbossie (Wild Rosemary)	Asteraceae	<i>Eriosephalus ericoides</i>	Perennial woody shrublet up to 1 m tall	Palatability varies depending on region, habitat and season. When sheep graze the plants, the flower heads turn their lips black	DAFF, 2014; Le Roux et al., 1994	C ₃	
NK	Kortbeen boesmangras (small Bushman grass)	Poaceae	<i>Stipagrostis obtusa</i>	Perennial arid-climate grass up to 500 mm tall	Very palatable and grazed down short	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
NK	Kriedoring	Solanaceae	<i>Lycium cinereum</i>	Densely branched perennial shrub up to 1 m tall	Not particularly grazed, except for new growth and fruit	DAFF, 2014; Shearing, 1994	C ₃	
NK	Langbeen boesmangras (tall Bushman grass)	Poaceae	<i>Stipagrostis ciliata</i>	Perennial arid-climate grass up to 1 m tall	Very palatable and grazed down short	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
NK	Pienkloodganna	Chenopodiaceae	<i>Salsola calluna</i>	Shrublet 300 mm tall	Palatable and grazed by all types of animals	Shearing, 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002
NK	Rivierleegtebos / Perdekaroo / Perdebos	Asteraceae	<i>Rosenia humilis</i>	Perennial woody dwarf shrublet 400 mm tall	Not very palatable but provides feed when most other plants have been grazed, drought resistant. Young leaves have pleasant pine aroma	DAFF, 2014; Shearing, 1994	C ₃	
NK	Spekbos	Zygophyllaceae	<i>Zygophyllum gilfillanii</i>	Woody, much-branched shrublet 250-500 mm tall	Palatable and resistant to grazing and drought	Le Roux et al., 1994	C ₄	Ehleringer <i>et al.</i> , 1997
NK	Vingerhoedgras (Thimble grass)	Poaceae	<i>Fingerhuthia africana</i>	Perennial warm-climate grass up to 900 mm tall	Average grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
NK	Vyebossie / Doringvygie	Aizoaceae	<i>Ruschia intricata</i>	Perennial succulent leaved shrublet up to 400 mm tall	Moderate palatability	DAFF, 2014	CAM	Von Willert <i>et al.</i> , 1992
HK/LO	Ankerkaroo (Anchor karoo)	Asteraceae	<i>Pentzia incana</i>	Dwarf shrub 200-250 mm tall	Palatability varies depending on region. Abundant and vital part of sheep's diet	DAFF, 2014; Le Roux et al., 1994	C ₃	
HK/LO	Appelbos / Skilpadbos	Zygophyllaceae	<i>Zygophyllum lichtensteinianum</i>	Branched shrublet up to 750 mm tall	Palatable, very well grazed and highly resistant to drought	Shearing, 1994	C ₄	Ehleringer <i>et al.</i> , 1997
HK/LO	Bietoubos	Asteraceae	<i>Chrysanthemoides incana</i>	Shrub up to 2.5 m tall	Palatable and very well grazed	Shearing, 1994	C ₃	
HK/LO	Blomkoolganna	Chenopodiaceae	<i>Salsola tuberculata</i>	Dome-shaped shrublet 200 mm tall	Palatable and highly resistant to grazing and drought	Le Roux et al., 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002

Supplementary data

Table S5.1 (Continued)

Area code	Common name	Family name	Species name	Description	Palatability	References ^a	Plant path	References ^b
HK/LO	Krakerbossie (Kleinsaad klappiesbrak)	Aizoaceae	<i>Tetragonia fruticosa</i>	Perennial succulent leaved shrub up to 1.5 m tall	Very palatable and valuable grazing crop	Van Breda & Barnard, 1991	CAM	
HK/LO	Swartboegoe	Asteraceae	<i>Pteronia sordida</i>	Woody shrublet 150-300 mm tall	Unpalatable plant	Le Roux et al., 1994	C ₃	
HK/LO	Vygie	Aizoaceae	<i>Stoeberia beetzii</i>	Perennial succulent leaved shrublet up to 500 mm tall	Moderate palatability	Court, 2000	CAM	Von Willert <i>et al.</i> , 1992
KV	Ankerkaroo (Anchor karoo)	Asteraceae	<i>Pentzia incana</i>	Dwarf shrub 200-250 mm tall	Palatability varies depending on region. Abundant and vital part of sheep's diet	DAFF, 2014; Le Roux et al., 1994	C ₃	
KV	Bergganna	Fabaceae	<i>Lebeckia leipoldtiana</i>	Woody shrublet up to 1 m tall	Leaves, fruits and flowers may be grazed	Manning & Goldblatt, 1997	C ₃	
KV	Bleekkorrelertjie	Fabaceae	<i>Wiborgia monoptera</i>	Woody shrublet up to 1 m tall	Leaves, fruits and flowers may be grazed	Manning & Goldblatt, 1997	C ₃	
KV	Boegoekapokbossie	Asteraceae	<i>Eriocephalus punctulatus</i>	Shrublet up to 1 m tall	Palatability varies	Manning & Goldblatt, 1997	C ₃	
KV	Ghagras (Gha grass)	Poaceae	<i>Chaetobromus dregeanus</i>	Grass up to 500 mm tall	Palatable and very well grazed	Van Oudtshoorn, 2012	C ₄	
KV	Kleinwolfdoring	Fabaceae	<i>Wiborgia sericea</i>	Woody shrub up to 1.5 m tall	Leaves, fruits and flowers may be grazed. Highly drought resistant	Van Breda & Barnard, 1991	C ₃	
KV	Kraalbos	Aizoaceae	<i>Galenia africana</i>	Perennial aromatic dwarf shrublet up to 1 m tall	Plant is toxic and has been associated with liver damage and ascites in small stock	DAFF, 2014	C ₃	
KV	Medics	Fabaceae	<i>Medicago polymorpha</i>	Procumbent annual herb	Palatable and very well grazed	Retief & Herman, 1997	C ₃	
KV	Skilpadbessie (Tortoise berry)	Polygonaceae	<i>Nylandtia spinosa</i>	Much-branched rigid shrub up to 4 m tall	Edible berries with an astringent taste	Van Wyk, 2000	C ₃	
KV	Turknael (Musk heron's bill)	Geraniaceae	<i>Ereiodium moschatum</i>	Annual or biennial, erect to procumbent aromatic herb up to 300 mm tall	Palatable and readily ingested by sheep. Suspected of causing photosensitivity in sheep, especially when heavily grazed. Musk-like odour when crushed	Stroebe, 2002	C ₃	
HK/CAL	Ankerkaroo (Anchor karoo)	Asteraceae	<i>Pentzia incana</i>	Dwarf shrub 200-250 mm tall	Palatability varies depending on region. Abundant and vital part of sheep's diet	DAFF, 2014; Le Roux et al., 1994	C ₃	
HK/CAL	Appelbos/Skilpadbos	Zygophyllaceae	<i>Zygophyllum lichtensteinianum</i>	Branched shrublet up to 750 mm tall	Palatable, very well grazed and highly resistant to drought	Shearing, 1994	C ₄	Ehleringer <i>et al.</i> , 1997
HK/CAL	Berggansie	Asteraceae	<i>Pentzia sphaerocephala</i>	Woody dwarf shrub 300-600 mm tall	Very palatable and drought-resistant	Le Roux et al., 1994	C ₃	

Supplementary data

Table S5.1 (Continued)

Area code	Common name	Family name	Species name	Description	Palatability	References ^a	Plant path	References ^b
HK/CAL	Bitterbossie (Bitter bush)	Asteraceae	<i>Chrysocoma ciliata</i>	Perennial soft to woody shrublet 100-600 mm tall	Only the flowers are grazed. Presence is an indication of overgrazing, veld deterioration and Karoo encroachment	DAFF, 2014; Le Roux et al., 1994	C ₃	
HK/CAL	Doringbos	Solanaceae	<i>Lycium spp.</i>	Branched perennial shrub up to 1 m tall	Palatable and well grazed	DAFF, 2014	C ₃	
HK/CAL	Doringvygie	Aizoaceae	<i>Eberlanzia ferox</i>	Leaf-succulent shrub 400 mm tall	Young growth is readily razed in spite of spines	Le Roux et al., 1994	CAM	
HK/CAL	Kapokbossie (Wild Rosemary)	Asteraceae	<i>Eriocephalus ericoides</i>	Perennial woody shrublet up to 1 m tall	Palatability varies depending on region, habitat and season. When sheep graze the plants, the flower heads turn their lips black	DAFF, 2014; Le Roux et al., 1994	C ₃	
HK/CAL	Pienkloodganna	Chenopodiaceae	<i>Salsola calluna</i>	Shrublet 300 mm tall	Palatable and grazed by all types of animals	Shearing, 1994	C ₄	Ehleringer <i>et al.</i> , 1997; Voznesenskaya <i>et al.</i> , 2002
HK/CAL	Ribbokbos	Acanthaceae	<i>Justica orchioides</i>	Shrub up to 1.75 m tall	Palatable and grazed by animals when found	Shearing, 1994	C ₃	
HK/CAL	Skaapvygie (Royal carpet)	Aizoaceae	<i>Drosanthemum hispidum</i>	Perennial leaf-succulent shrub 600 mm tall	Palatable, very well grazed	DAFF, 2014	CAM	
HK/CAL	Springbokslaai	Aizoaceae	<i>Mesembryanthemum vaginatum</i> (Brownanthus or Ruschia vaginatum)	Leaf-succulent shrub	Palatable, very well grazed		CAM	Von Willert <i>et al.</i> , 1992
RU	Kweekgras (Bermuda grass)	Poaceae	<i>Cynodon dactylon</i>	Perennial grass up to 400 mm tall	Palatable and average to good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987; Vogel <i>et al.</i> , 1978
RU	Lucerne (Alfalfa)	Fabaceae	<i>Medicago sativa</i>	Perennial herb 300-400 mm tall	Palatable, very well grazed and used as a forage	DAFF, 2014; Retief & Herman, 1997	C ₃	
FS	Assegaaigras (Spear grass)	Poaceae	<i>Heteropogon contortus</i>	Perennial tropical grass up to 1 m tall	Palatable and good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	
FS	Kalahari-suurgas (Kalahari sour grass)	Poaceae	<i>Schmidtia kalahariensis</i>	Annual arid-climate grass up to 900 mm tall	Palatable and well grazed despite its sour taint	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Vogel <i>et al.</i> , 1978
FS	Knietjiesgras (Lehmann's love grass)	Poaceae	<i>Eragrostis lehmanniana</i>	Perennial arid-climate grass up to 600 mm tall	Palatable and good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
FS	Kweekgras (Bermuda grass)	Poaceae	<i>Cynodon dactylon</i>	Perennial grass up to 400 mm tall	Palatable and average to good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987; Vogel <i>et al.</i> , 1978
FS	Rooigras (Red grass)	Poaceae	<i>Themeda triandra</i>	Perennial tropical grass up to 1.5 m tall and prevalent in average to high rainfall areas	Very palatable and good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
FS	Steekgras (Tassel three-awn)	Poaceae	<i>Aristida congesta subsp. congesta</i>	Perennial warm-climate grass up to 900 mm tall	Palatable and average grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Sage, 2003

Supplementary data

Table S5.1 (Continued)

Area code	Common name	Family name	Species name	Description	Palatability	References ^a	Plant path	References ^b
FS	Vingerhoedgras (Thimble grass)	Poaceae	<i>Fingerhuthia africana</i>	Perennial warm-climate grass up to 900 mm tall	Average grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987
FS	Weeluisgras (Saw-tooth love grass)	Poaceae	<i>Eragrostis superba</i>	Perennial warm-climate grass up to 1 m tall	Very palatable and good grazing grass	DAFF, 2014; Van Oudtshoorn, 2012	C ₄	Heaton, 1987; Vogel <i>et al.</i> , 1978

^a References for plant description; ^b References for plant path identified; Descriptions: (herb) soft, usually annual; (shrub) woody, taller than 1 m; (subshrub) semi-woody, about 1 m tall; (dwarf shrub and shrublet) woody or semi-woody, shorter than 1 m; (CK) Central Karoo; (NK) Northern Karoo; (HK/LO) Hantam Karoo/Loeriesfontein; (KV) Knersvlakte; (HK/CAL) Hantam Karoo/Calvinia; (RU) Rûens; (FS) Free State.

References

Court, D. (2000). *Succulent flora of Southern Africa*. Rotterdam, the Netherlands: Random House Struik.

Department of Agriculture, Forestry and Fisheries (DAFF). (2014). Grootfontein Herbarium. Grootfontein Agricultural Development Institute [Internet document]. URL <http://gadi.agric.za//////////herbarium/her-select.php>. Accessed 23.09.2014.

Ehleringer, J. R., Cerling, T. E. & Helliker, B. R. (1997). C₄ photosynthesis, atmospheric CO₂, and climate. *Oecologia*, **112**, 285-299.

Heaton, T. H. E. (1987). The ¹⁴N/¹⁵N ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia*, **74**(2), 236-246.

Le Roux, P. M., Kotzé, C. D., Nel, G. P. & Glen, H. F. (1994). *Bossievelde: Grazing Plants of the Karoo and Karoo-like Areas*. Bulletin 428. Pretoria, South Africa: Department of Agriculture.

Manning, J. & Goldblatt, P. (1997). *Nieuwoudtville Bokkeveld Plateau and Hantam. South African Wild Flower Guide 9*. Cape Town, South Africa: The Botanical Society of South Africa.

Retief, E. & Herman, P. P. J. (1997). *Plants of the Northern Provinces of South Africa: Keys and Diagnostic Characters*. Strelitzia 6. p. 461. Pretoria, South Africa: National Botanical Institute.

Sage, R. F. (2003). The evolution of C₄ photosynthesis. *New Phytologist*, **161**, 341-370.

Shearing, D. (1994). *Karoo. South African Wild Flower Guide 6*. Cape Town, South Africa: The Botanical Society of South Africa.

Stroebel, J. C. (2002). Induction of photosensitivity in sheep with *Erodium moschatum* (L.) L'Hérit. *Journal of the South African Veterinary Association*, **73**(2), 57-61.

Van Breda, P. A. B. & Barnard, S. A. (1991). *100 Veld Plants of the Winter Rainfall Region: A Guide to the Use of Veld Plants for Grazing*. Bulletin No. 422. Cape Town, South Africa: Department of Agricultural Development.

Van Oudtshoorn, F. (2012). *Gids tot Grasse van Suider-Afrika*. Pretoria, South Africa: Briza Publications.

Van Wyk, B. (2000). *A Photographic Guide to Wild Flowers of South Africa*. Cape Town, South Africa: Struik Publishers.

Von Willert, D. J., Eller, B. M., Werger, M. J. A., Brinckmann, E. & Ihlenfeldt, H.-D. (1992). *Life Strategies of Succulents in Deserts*. Cambridge, UK: Cambridge University Press.

Voznesenskaya, E. V., Franceschi, V. R., Kirats, O., Artyusheva, E. G., Freitag, H. & Edwards, G. E. (2002). Proof of C₄ photosynthesis without Kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). *The Plant Journal*, **31**(5), 649-662.

Chapter 6

Near-infrared spectroscopy (NIRS) for the authentication of regionally unique South African lamb⁴

Abstract

The use of a portable MicroNIR spectrophotometer was explored to determine the potential of using Near-infrared spectroscopy (NIRS) as an analytical tool for the authentication of fresh South African lamb. Meat steaks of the *Longissimus thoracis et lumborum* muscle of lambs (n = 201) from nine different regions/origins were assessed. Spectral measurements were made 24 h post-mortem on the fresh meat samples, the analysis was repeated on the same samples after a 2-month freezing period. The subcutaneous fat of the carcasses were also scanned on-site directly after slaughter and again after 24 h post-mortem. Principal component analysis (PCA) of the spectral data (of the nine regions) showed that fresh meat of the Non-Karoo Rûens (RU) lamb grouped separately from that of the Karoo lamb, known as Central Karoo (CK), Northern Karoo (NK), Hantam Karoo (HK) and Bushmanland (BL). However, within the Karoo, CK was partially separated from the other Karoo regions. Through the use of partial least square discriminant analysis (PLS-DA), support vector machines (SVM) and linear discriminant analysis (LDA) sufficient classification of the samples was achieved. Using PLS-DA and the pro-processing techniques of standard normal variate (SNV) and multiplicative scattering correction (MSC) combined with Savitzky-Golay second derivative, a 95% and 91% correct classification for Karoo and Non-Karoo fresh lamb meat was respectively achieved. The device also allowed the successful authentication of fresh vs. frozen-then-thawed meat with 85% of the fresh and 81% of the frozen-then-thawed meat samples were correctly classified using LDA. Using the MicroNIR it is possible to obtain suitable spectral data for the classification of fresh lamb meat. However, a larger sample size might yield a more satisfactory classification in terms of the exact origin.

Keywords: Authentication; Extensive grazing, Lamb; NIRS; Origin

6.1 Introduction

The determination of the origin of food products is becoming increasingly important as consumers want to know the source of their products, as well as the verification of its authentic nature (Grunert, 2005). This is especially vital for food products sold at a premium price and where the quality characteristics of the products are associated with it through its method of production in a defined region of origin. In South Africa, most of the lamb meat produced in the Northern parts of the country is known as Karoo lamb (Weissnar & Du Rand, 2012). Karoo lamb is appreciated for its unique sensory quality (e.g. herbaceous aroma and flavour attributes)

⁴ Part of this work presented as a poster presentation: Erasmus, S. W., Manley, M., Muller, M. & Hoffman, L. C. (2016). MicroNIR spectroscopy for the authentication of South African lamb, presented at the *62nd International Congress of Meat Science and Technology (ICoMST)*, 14-19 August 2016, Bangkok, Thailand.

and this unique quality is attributable to the diet of the sheep, which mainly consist of the indigenous, herbaceous Karoo bushes and shrubs (Estler *et al.*, 2006; Chapter 3, Erasmus *et al.*, 2016a). As a result of the quality and value associated with the meat, there is a risk that the name may be misused by entities not even remotely linked to the region. Another concern is that the meat is sold as Karoo lamb when in actual fact it has been produced in a feedlot. Therefore, it is important that a method for the authentication of South African lamb, with special reference to Karoo lamb, be developed. Not only would such a method be able to distinguish Karoo lamb from lamb of a different origin (in this study termed Non-Karoo), but it would also provide scientific evidence to verify the unique nature of the product.

In general, the methods used to determine the authenticity of food products require sophisticated analytical equipment, highly trained staff, chemical reagents and sample preparation, which are usually time-consuming and expensive. These are some of the reasons why the need exists to explore the use of alternative, cost-effective and faster methods. Near infrared spectroscopy (NIRS) offers a possible solution and has been applied widely for the authentication of food products (Downey, 1996; Huck-Pezzei *et al.*, 2014) and for predicting meat and meat product quality (Prieto *et al.*, 2009). However, according to Prieto *et al.* (2009), NIRS have limited ability to estimate the sensory and technological attributes of meat due to the heterogeneity of the samples and their preparation. Conversely, prediction of the chemical composition of meat and classification thereof show promising results (Prieto *et al.*, 2009). Although NIRS is generally performed in the laboratory using benchtop instruments, recent technological advances have enabled the development of portable instrumentation. The small (45 mm in diameter and 42 mm in height) and light (60 g) hand-held MicroNIR could be regarded as a suitable device to use for the classification of origin of South African lamb meat samples. The hand-held instrument delivers fast, reliable and on-site measurement of samples and has been used in various studies for pharmaceutical analysis (Alcalà *et al.*, 2013), contamination of soil (O'Brien *et al.*, 2012), the authentication of fish species (O'Brien *et al.*, 2013), quality control of 'Tommy Atkins' mango (Marques *et al.*, 2016) and palm oil adulteration (Basri *et al.*, 2017). The published works verify the application of the device.

Previous research of descriptive sensory analysis (Chapter 3, Erasmus *et al.*, 2016a), fatty acid and volatile profiling (Chapter 4) and stable isotope ratio analysis (Chapter 5, Erasmus *et al.*, 2016b) revealed distinct sensory and chemical differences of lamb meat obtained from different farms within the mentioned regions. The differences found were related to diet linked to the origin. Given the time-consuming and costly nature of these analyses, the aim of the current work was to investigate NIRS as a potential tool for rapid and effective classification of lamb meat. The portable MicroNIR was used for this purpose as it enables easy, fast and non-destructive monitoring of the lamb meat. The work is also the first of its kind in South Africa, investigating the use of NIRS for the authentication of regionally unique lamb.

6.2 Materials and methods

6.2.1 Experimental layout and study regions

Lambs were sourced from different regions in South Africa, each unique in terms of its vegetation and the extensive grazing conditions (Table 6.1). Lamb meat was collected from four registered abattoirs in South Africa (1966/011382/07 Bredasdorp; 1968/005117/07 KLK Calvinia; ZA 105 GWK Groblershoop; ZA 155 GWK De Aar). Each abattoir was located in or close to a distinct region, where lambs are raised extensively on the natural vegetation. The regions selected for this study were Central Karoo (CK), Northern Karoo (NK), Bushmanland (BL), Hantam Karoo (HK), Eastern Karoo (EK) and Rûens (RU) (Chapter 1, Fig. 1.4). Other lamb types raised in the feedlot (FL), produced semi-extensively (SE) or originating from Namibia (NAM) (neighbouring country) were also collected to test against the extensively raised samples.

Table 6.1 Regions selected for this study on NIRS

Region of origin	Code	Description of typical extensive diet	No. of farms	No. of animals
Central Karoo	CK	Shrubs/bushes and grass	15	45
Hantam Karoo	HK	Shrubs/bushes and grass	12	36
Northern Karoo	NK	Shrubs/bushes and grass	7	21
Bushmanland	BL	Shrubs/bushes and grass	7	21
Eastern Karoo	EK	Shrubs/bushes and grass	3	9
Rûens	RU	Lucerne/alfalfa	12	36
Additional				
Namibia	NAM	Namibian savannah-type vegetation	2	6
Feedlot	FL	Concentrates	3	9
Semi-extensive	SE	Shrubs/bushes, grass and concentrates	6	18
Total			67	201

(NIRS) Near-infrared spectroscopy; (No.) Number.

Lambs were sourced from regions located within the Northern Cape (CK, NK, BL and HK), Eastern Cape (EK) and the Western Cape provinces (RU). The Northern Cape province mainly consists of the Karoo ecotype which is known for its unique range of vegetation types and particularly the presence of the indigenous Karoo bushes and shrubs (Acocks, 1988). Sheep farming is mainly practised in the Northern Cape province due to the dryness of the land (Cloete & Olivier, 2010). Sheep raised within this province is generally known as Karoo lamb (Fig. 6.1). The HK is mostly covered in unique Karoo bushes and fall within the succulent Karoo while, CK, NK and BL fall in the Nama-Karoo biome, comprising a combination of Karoo bush and savanna-type grasses (Estler *et al.*, 2006). EK lamb was obtained from the Eastern Cape and some of its vegetation can be considered as Karoo, based on the prevalence of the eastern mixed Nama-Karoo biome in the province. Common shrubs include Karoo bushes such as *Pentzia incana*, *Eriocephalus ericoides* and *Hermannia spp.*, while grasses, such as *Aristida spp.*, *Eragrostis spp.* and *Themeda triandra*, may dominate the landscape after

good summer rains (Bredenkamp *et al.*, 1996). Hence, for the purpose of the study EK lamb was tentatively named Karoo lamb. Lamb raised extensively in the EK are also produced on the natural vegetation and pastures, such as lucerne/alfalfa (*Medicago sativa*). An increase in the use of pastures is seen with the shift towards the coastline bringing with it an increase in rainfall. Major concerns are whether lamb meat can still be deemed Karoo lamb, given that the sheep receives markedly less herbaceous Karoo bushes and shrubs. The sheep selected from farms within the Western Cape province (RU) were extensively raised on lucerne situated within the fynbos biome and known as the Rûens shale renosterveld (Fig. 6.1). The Rûens are known for their lamb produced on these pastures however, depending on season, lamb may also be raised on stubble after the grain harvesting period (usually December-February) (Cloete & Cloete, 2010). Similar to Karoo lamb, the RU lamb is known for its unique sensory quality. It is often compared and thought superior to feedlot raised lamb.



Figure 6.1 Karoo lamb (left) and Rûens lamb (right).

6.2.2 Sampling and slaughtering

The animals were slaughtered according to standard South African procedures and regulations (DAFF, 2000). Three carcasses per farm, of any breed and gender, were randomly selected according to age (class A lambs have no permanent incisors, only milk teeth and would typically be around 10 months of age), fatness (class 2) and weight (approximately 18 kg). In South Africa carcasses are classified according to age, fat, conformation and damage classes (DAFF, 1990; DAFF, 2006). For the classification of carcasses based on the level of fatness, the fat depth is measured between the 3rd and 4th lumbar vertebrae, 25 mm from the midline. The fat depth for class 2 carcasses measures 1.0-4.0 mm (lean).

Twenty four hours after slaughter and refrigeration at 4°C, meat steaks (1.5-2.0 cm thick) were cut perpendicular to the grain of the left *Longissimus thoracis et lumborum* muscle of the carcass at the T₁₃ position (13th thoracic vertebrae) towards the 1st lumbar vertebrae. The steaks were laid down on one side, while the other side was exposed to the air and left to bloom for 30 min after which the NIR scans were acquired. The experimental set-up of the measurement procedure is represented in Figure 6.2. Samples were scanned (in triplicate) in diffuse reflectance mode between 908-1680 nm by placing the MicroNIR spectrometer (Viavi

Solutions, formerly JDSU, USA) on the exposed surface of the meat. Three scans were taken and averaged in order to reduce the sampling error and variation. The MicroNIR was placed flat on the surface of the meat, completely covering the connectable collar of the device upon scanning. The MicroNIR was fitted with a novel thin-film linear variable filter (LVF) and the two tungsten lamps allowed for illuminating a spot (3 mm in diameter) on the sample with the optimal focal distance being 3 mm from the sample. A 128-pixel indium gallium arsenide (InGaAs) detector was used to achieve a resolution of 30 x 250 $\mu\text{m}/50 \mu\text{m}$. The same samples were then vacuum packed and frozen at -20°C for 2 months, after which the same procedure was repeated to acquire the new set of scans for the frozen-then-thawed meat. The thawing procedure was performed as follow: samples were removed from the freezer and placed in a refrigerator to thaw at 4°C for 12 h before analysis. The meat steaks were then removed from the packaging and laid down on one side (same side as with the fresh samples), while the other side was slightly blotted dry, exposed to the air and left to bloom for 30 min (14°C room temperature) after which the NIR scans were acquired. Additional scans were also taken on the back surface (subcutaneous fat) of the carcasses at the 13th rib position after slaughter before refrigeration and after refrigeration at 4°C before the steaks were removed. The scans (three per carcass) were taken by placing the MicroNIR flat on the surface of the carcass, completely covering the connectable collar of the device upon scanning.



Figure 6.2 Practical measurement set-up for a lamb meat steak.

6.2.3 Statistical analysis

Mean spectra (per sample) were used for data analysis. The absorbance of reflectance spectra was measured in the NIR region (908-1680 nm) of the electromagnetic spectrum at 6.2 nm intervals, resulting in 125 wavelength bands. XLSTAT® statistical software (Version 2016.04.33113; Addinsoft, NY, USA) and Unscrambler X10.3 (Camo Software AS., Oslo, Norway) was used for pre-treatment and chemometric analysis of the NIR spectral data. Three pre-treatments: standard normal variate (SNV), multiplicative scattering correction (MSC) and a combination of SNV and MSC, respectively with Savitzky-Golay second derivative (15 points) (SG) were used. Pre-processing of NIR spectral data removes undesired scatter effects, such as baseline shift and non-linearities (Rinnan *et al.*, 2009). SNV and MSC removes undesired scattering, while SG calculates derivatives of the data to concentrate analysis on the shape of the spectra and eliminate offsets

and drifting baseline. It is important to limit the number of pre-processing steps in order to preserve valuable information and develop a model which is robust enough for future predictions (Rinnan *et al.*, 2009). Principal component analysis (PCA) was used to visualize sample grouping, while the partial least square discriminant analysis (PLS-DA) (± 0.5 cutoff criteria), support vector machine (SVM) and linear discriminant analysis (LDA) methods were applied for classification of origin.

6.3 Results and discussion

6.3.1 Fresh meat of five regions

The fresh meat samples were analysed first to determine trends in the data and variation between regions. Five of the nine regions (CK, HK, NK, BL and RU) were selected for this purpose as there were only limited number of animals collected from EK, SE, NAM and FL. Thus, it was decided not to include these regions. The sample grouping of four Karoo and one Non-Karoo regions were analysed using principal component analysis (PCA), where the spectral data was pre-processed using standard normal variate (SNV). PCA revealed grouping of samples based on origin (Fig. 6.3). The Karoo samples tended to group separately from the Non-Karoo (RU). However, within the Karoo group, CK were more partially separated from the other Karoo samples, while HK, BL and NK grouped closer.

Overall, the highest absorptions were observed at 930-960 nm (Ar-OH second overtones), 1070-1100 nm (Ar-CH second overtones), 1120-1190 nm (C-H second overtones), 1380 nm (Ar-OH first overtones) and 1470-1520 (N-H first overtones) (Fig. 6.4). Differences in chemical components of lamb meat (due to different diets) could be the reason for discrimination between samples. The Karoo samples associated with strong absorptions in the 1380 nm range, possibly as a result of the presence of phenolic or aromatic compounds which can be deposited in the meat through the consumption of herbaceous Karoo plants (Erasmus *et al.*, 2016a). However, to confirm this assumption the volatile profile of the meat should also be determined. If the identified regions were indeed related to the volatile profile of the meat, they could potentially be used for future analysis to class the samples according to origin.

The variation in the results can be due to the lack of homogeneity of the meat samples. In fact, the chemical composition of minced samples can be more accurately determined than that of intact meat samples (Cozzolino & Murray, 2002; Guy *et al.*, 2011). The muscle fibres of intact meat may also conduct light by internal reflections, absorb more energy and produce less reflectance compared to homogenised meat (Prieto *et al.*, 2009). Another source of possible variation taken into account in the study were the thickness of the meat steaks. In the work of Marques *et al.* (2016) the NIR radiation penetration into mango pericarp tissue was explored. They concluded that the radiation penetrates through fruit pericarp tissue up to 7.4 mm but not beyond 10.0 mm. Since the meat steaks were 15 mm to 20 mm thick, variation due to thickness were not considered as a factor.

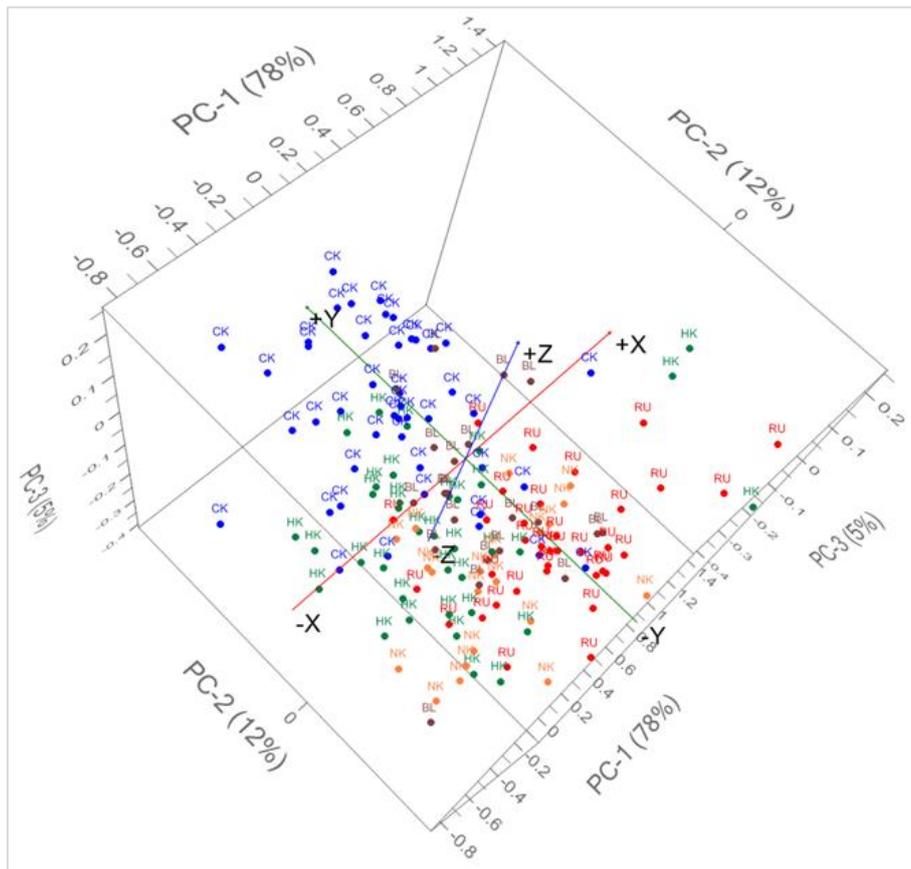


Figure 6.3 Principal component analysis (PCA) scores plot of the spectral data (standard normal variate pre-processed) for the different lamb types. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens.

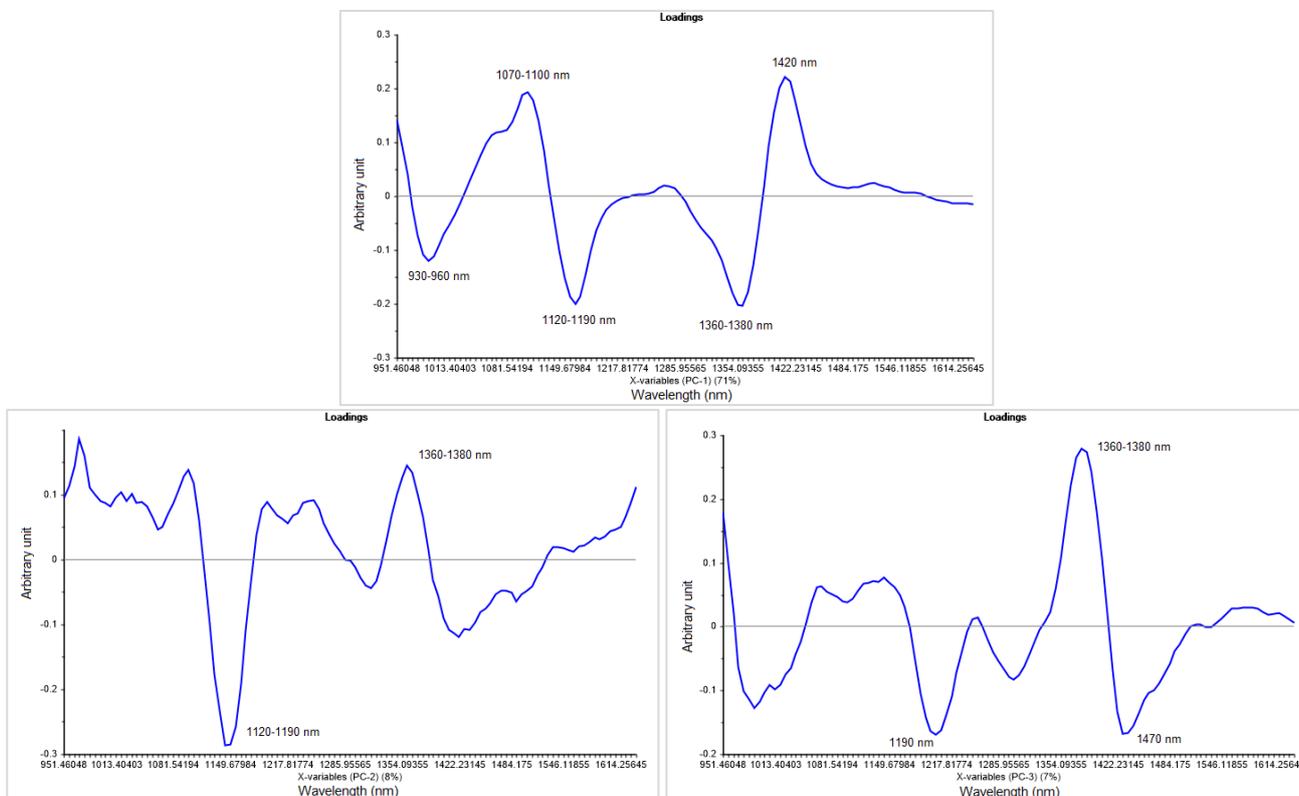


Figure 6.4 Loading line plots (for PC1, PC2 and PC3) of the spectral data showing the wavelengths with the highest absorptions (prominent positive and negative peaks).

The fresh meat samples of the five regions were classified according to origin (Karoo vs. Non-Karoo) using partial least-squares discriminant analysis (PLS-DA). A calibration (n = 106) and external validation (n = 53) sample set were used. For the calibration sample set, two of the three animals per farm were used, while the other animal was used for the validation set.

The PLS-DA results are shown in Table 6.2. In order to prevent over-fitting of the calibration model, the number of partial least squares (PLS) factors were reduced from 7 to 6 for multiplicative scattering correction (MSC) and SNV, and from 6 to 4 for MSC+Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points) (SG) and SNV+SG. Although the Pearson R² value decreased with the reduction in the number of PLS factors, a satisfactory classification of Karoo vs. Non-Karoo origin was still achieved with 95% and 98% correct classification for Karoo and 67% and 83% for Non-Karoo. As seen in Table 6.2, MSC+SG and SNV+SG pre-processing produced the best results.

Table 6.2 Correct classification rates of external validation set by PLS-DA analysis

Pre-processing	Calibration model		% Correct	
	R ²	RMSEP (‰)	Karoo (41)	Non-Karoo (12)
MSC ¹	0.74	0.59	95	67
MSC+SG ²	0.76	0.56	98	83
SNV ¹	0.75	0.58	98	67
SNV+SG ²	0.76	0.57	98	83

(PLS-DA) Partial least-squares discriminant analysis; ¹Six PLS factors used; ²Four PLS factors used; (%) Percentage; (R²) Pearson determination coefficient; (RMSEP) Root-mean-squared error of prediction (test set validated); (MSC) Multiplicative scattering correction; (SNV) Standard normal variate; (SG) Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points).

An important question arising from the results is whether or not the classification rate would improve if the selection of validation samples occurred on farm-base level. Hence, the PLS-DA models were re-evaluated using a new external validation set, where instead of selecting one animal per farm, the entire farm was used. Accordingly, farms were randomly selected for validation as following: five for CK (33%), four for HK and RU, respectively (33%) and two for NK and BL, respectively (29%). The results are indicated in Table 6.3. The results show an improvement (15% for MSC and SNV; 8% for MSC+SG and SNV+SG) for the classification of the Non-Karoo samples and only a slight decrease (3%) for that of the Karoo samples. This was not expected as in the previous model one animal per farm was taken for validation thereby including variation from all the farms in the calibration model. Thus, by selecting entire farms it was expected that the percentage correctly classified samples would decrease. Although unexpected, the results show that lambs from farms not included in the calibration model can be classified according to their origin.

Table 6.3 Correct classification rates of external validation set (entire farm-based) by PLS-DA analysis

Pre-processing	Calibration model		% Correct	
	R ²	RMSEP (‰)	Karoo (39)	Non-Karoo (11)
MSC ¹	0.76	0.52	95	82
MSC+SG ²	0.75	0.57	95	91
SNV ¹	0.75	0.54	95	82
SNV+SG ²	0.75	0.56	95	91

(PLS-DA) Partial least-squares discriminant analysis; ¹Six PLS factors used; ²Four PLS factors used; (%) Percentage; (R²) Pearson determination coefficient; (RMSEP) Root-mean-squared error of prediction (test set validated); (MSC) Multiplicative scattering correction; (SNV) Standard normal variate; (SG) Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points).

Apart from distinguishing Karoo lamb from Non-Karoo lamb, it is also necessary to evaluate the division of regions within the Karoo. Support vector machine (SVM) was used for the classification of the samples according to origin (Karoo vs. Non-Karoo) and region of origin (RU, HK, CK, NK, BL). Overall the best SVM classification for origin was achieved with MSC and SNV pre-processing of the spectral data. MSC provided a 95% correct classification for Karoo (39 out of 41) and 25% for Non-Karoo (3 out of 12). SNV gave 88% correct for Karoo (36 out of 41) and 67% correct for Non-Karoo (8 out of 12). In terms of SVM classification of the region of origin, MSC (C = 10, Gamma = 10, 83 SVs) and SNV (C = 1, Gamma = 10, 81 SVs) pre-processing gave the overall best results with 88% and 89% accuracy, respectively (Table 6.4).

Table 6.4 Correct classification rates of external validation set by SVM (C = 1, Gamma = 10) for lamb meat region of origin

Pre-processing	% Accuracy ¹	% Correctly classified ²				
		CK	HK	BL	NK	RU
MSC*	88	87	75	71	29	67
MSC+SG	72	67	75	29	0	67
SNV	89	87	67	71	43	58
SNV+SG	75	80	67	86	14	67

(SVM) support vector machine; ¹Training model; ²Classification of external validation set; (%) Percentage; *C adjusted to 10; (MSC) Multiplicative scattering correction; (SNV) Standard normal variate; (SG) Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points); (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens.

Through inspection of the external validation results it was noted that the SVM classification of CK lamb samples, from two farms, were consistently misclassified as Non-Karoo. This could suggest that these lambs were not raised extensively or that they received a large amount of supplementary feed. In terms of meat variation, one would also expect these sample to have a different sensory profile compared to the other CK lamb. It was decided to exclude the two farms, re-build the SVM calibration models (for both class and region

of origin classification) and re-test the model with the external validation set (data not shown). Overall there was no noteworthy improvement seen for the classification of the validation set. In fact, the samples excluded were not available to be misclassified for CK (as before), while the classification results of the other samples were the same. However, for the MSC+SG and SNV+SG pre-processing for SVM of origin, there was a large decrease in the percentage accuracy of the calibration models. There was also an increase in misclassifications for the validation set. This could signify the importance of having outliers in a robust classification model.

6.3.2 Fresh meat of five regions compared to additional regions

Using the five regions examined in section 6.3.1, a PLS-DA calibration model was created with the full dataset of the five regions, pre-processed using SNV and SG. The calibration model was evaluated using full internal cross-validation. The additional samples (EK, NAM, FL, SE) were used as the external validation set and classified as either Karoo or Non-Karoo. The results are shown in Figure 6.5 and Table 6.5.

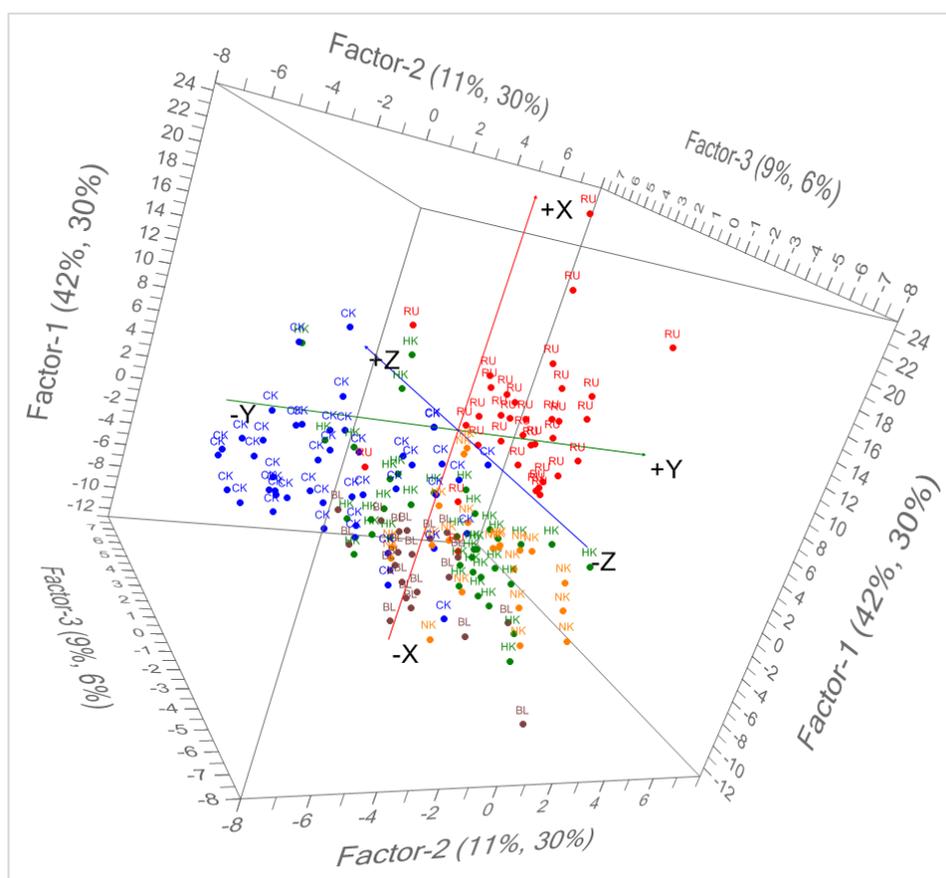


Figure 6.5 Partial least-squares discriminant analysis (PLS-DA) scores plot of the spectral data [Standard normal variate and Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points) pre-processed], acquired using a MicroNIR spectrophotometer, of the different fresh lamb steaks. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens.

Overall a satisfactory calibration model (four PLS factors) were generated with a R^2 value of 0.73. Almost all (99%) the Karoo samples were correctly classified, while 90% of Non-Karoo samples were correctly

assigned. The groupings are shown in Figure 6.5, where a clear separation between RU and the other Karoo regions can be seen.

Contrary to these results, none (except one SE sample) of the Non-Karoo samples (NAM, FL and SE) were correctly classified. Given the small sample size of these additional farms, these results are not unforeseen. The calibration model only includes variation of one Non-Karoo region (RU). RU lamb is raised extensively on lucerne pasture and can be distinguished from Karoo lamb raised extensively on indigenous Karoo plants. Hence, it is recommended to expand the calibration model with sufficient samples of NAM, FL and SE, so as to include their characteristic production parameters, for future prediction.

Table 6.5 Classification rates of additional validation set by PLS-DA analysis

Model (SNV+SG) ¹	Actual origin (No. of samples)	Classified origin	
		Karoo	Non-Karoo
Calibration $R^2 = 0.73$ RMSECV = 0.49‰	CK (45)	45 (100%)	-
	HK (36)	35 (97%)	1 (3%)
	NK (21)	21 (100%)	-
	BL (21)	21 (100%)	-
	RU (20) ²	2 (10%)	18 (90%)
Validation	EK (9)	9 (100%)	-
	NAM (6)	6 (100%)	-
	FL (9)	9 (100%)	-
	SE (18)	17 (94%)	1 (6%)

(PLS) Partial least-squares discriminant analysis; ¹Four PLS factors used; (%) Percentage; ² One outlier removed (sample RU5); (R^2) Pearson determination coefficient, (RMSECV) Root-mean-squared error of cross-validation, (SNV) Standard normal variate; (SG) Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points); (No.) Number; (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (EK) Eastern Karoo; (NAM) Namibia; (FL) Feedlot; (SE) Semi-extensive.

6.3.3 Fresh meat compared to frozen-then-thawed meat

Fresh meat samples were compared to that of frozen-then-thawed meat samples, irrespective of origin. The linear discriminant analysis (LDA) technique was used to evaluate the differences between the meat samples. The spectral data were pre-processed with MSC (Fig. 6.6) and the groupings visualised using PCA (Fig. 6.6) [spectral data pre-processed using SNV and SG (2nd derivative, 2nd order polynomial, 15 points)].

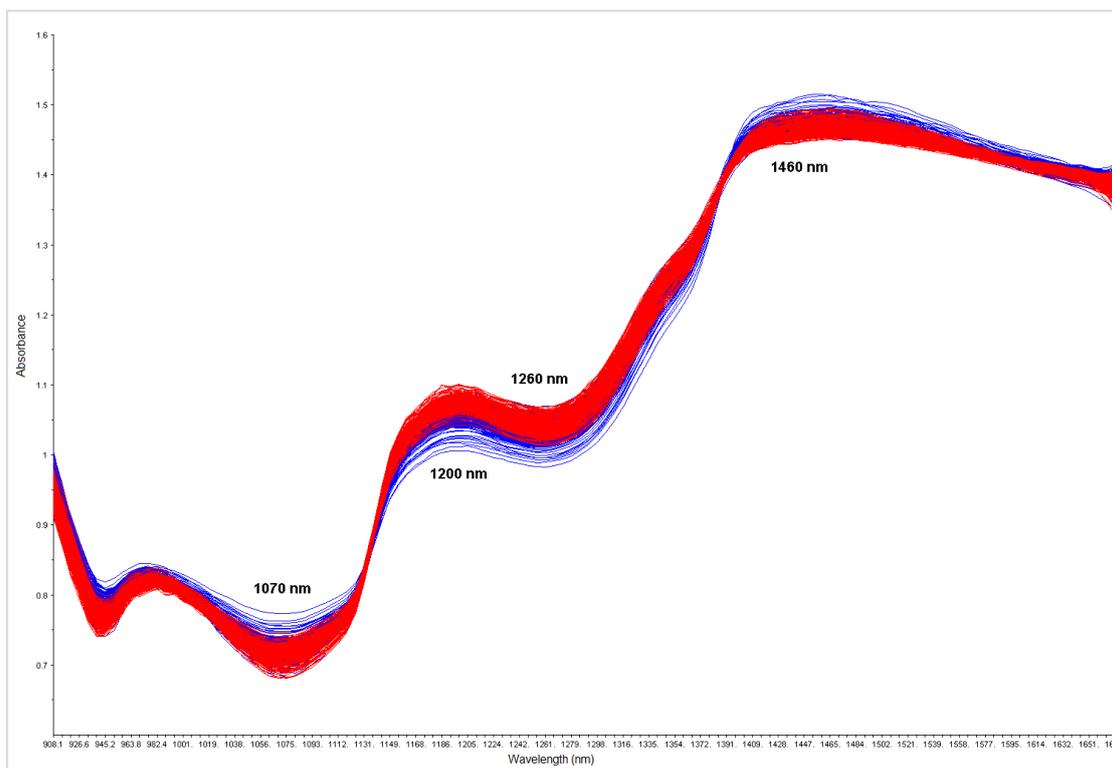


Figure 6.6 Multiplicative scattering correction (MSC) pre-processed NIR reflectance spectra, as acquired using a MicroNIR spectrophotometer, of the fresh (blue lines) and frozen-then-thawed (red lines) lamb steaks. Outliers removed: RU5 (fresh) and CK155 (frozen-then-thawed).

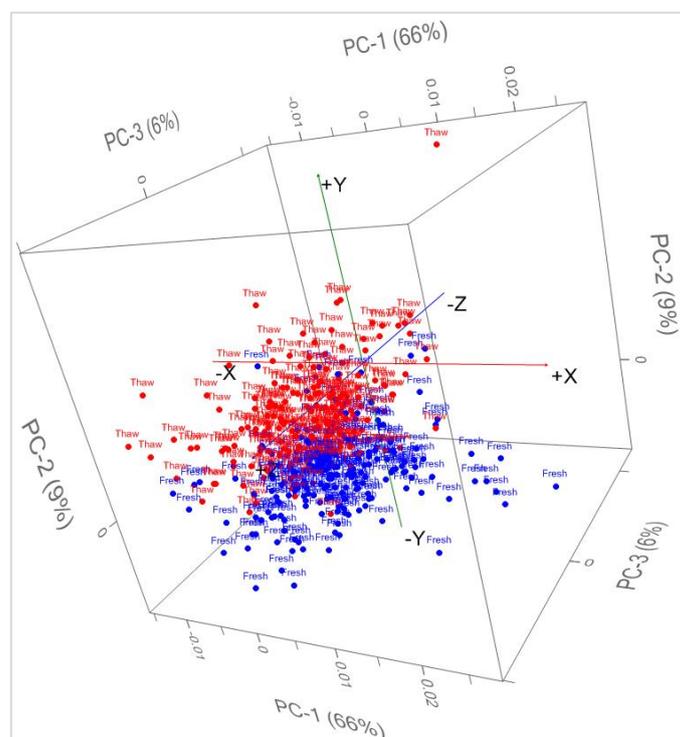


Figure 6.7 Principal component analysis (PCA) scores plot of the spectral data [Standard normal variate and Savitzky-Golay (2nd derivative, 2nd order polynomial, 15 points) pre-processed], acquired using a MicroNIR spectrophotometer, of the fresh (blue) and frozen-then-thawed (red) lamb steaks.

The difference between the fresh and frozen-then-thawed meat steaks can be seen at peaks 1070 nm, 1200 nm, 1260 nm and 1460 nm (Fig. 6.6). In fact, taking into account the prominent peaks of the loading line plots (Fig. 6.8), it is evident that the separate grouping in Figure 6.7 is driven by the higher absorption of fresh meat steaks in the regions 990-1000 nm, 1160-1200 nm and 1430-1460 nm; and frozen-then-thawed meat steaks in the regions 1100-1120 nm, 1250-1270 nm and 1410 nm.

It is known that water is a major component of fresh meat constituting about 75% with specific absorbance of O-H bonds at 980, 1450 and 1940 nm (Cozzolino & Murray, 2004; Prieto *et al.*, 2009). During the freezing process the meat structure is damaged (disruption of the muscle fibre structure and modification and/or denaturation of the proteins), reducing the water holding capacity (Leygonie *et al.*, 2012). Consequently, water is lost (thaw loss) when meat is defrosted. This is also reflected in the current findings as the fresh meat samples had higher absorptions at 1450 nm, indicative of the expected higher water content. Barbin *et al.* (2013) reported similar results for fresh and frozen-then-thawed pork meat with complex physical and chemical changes allowing samples to be separated using NIR technology.

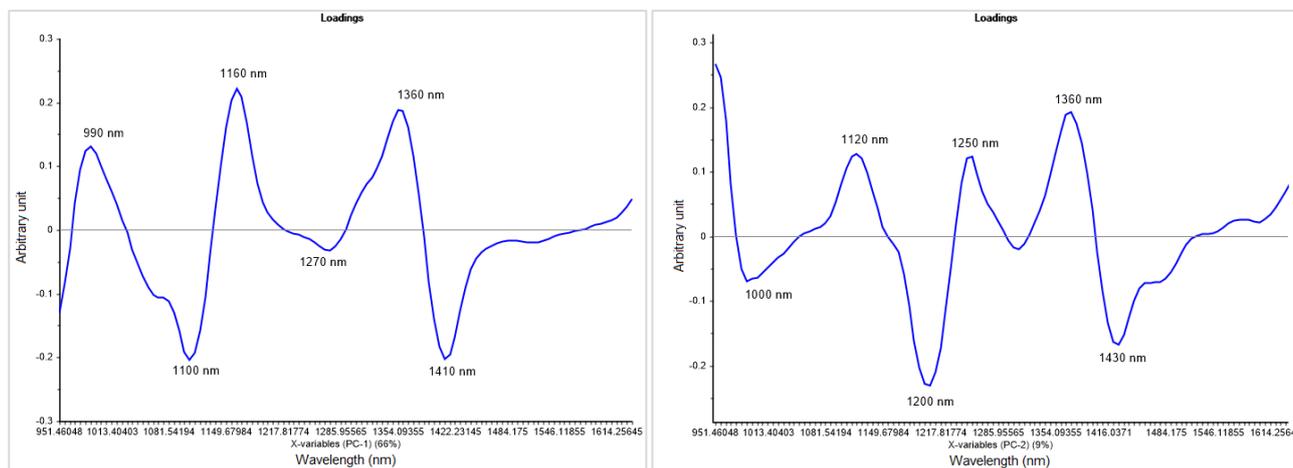


Figure 6.8 Loading line plots for: (left) PC1 showing prominent positive peaks at 990 nm, 1160 nm, 1360 nm and negative peaks at 1100 nm, 1270 nm, 1410 nm; (right) PC2 showing positive peaks at 1120 nm, 1250 nm, 1360 nm and negative peaks at 1000 nm, 1200 nm, 1430 nm.

The fresh and frozen-then-thawed meat samples were classified using LDA (Table 6.6). Three methods were employed. Overall the linear method gave slightly better results with only 15% of the fresh and 19% of the frozen-then-thawed meat samples incorrectly classified. The difference between the fresh and frozen-then-thawed meat is likely due to the change in physical structure (Prieto *et al.*, 2009). Damage to the muscle fibres is caused by the formation of ice crystals during the freezing process (Leygonie *et al.*, 2012). Large, extracellular ice crystals is formed and disrupts the physical structure. Together with the physical change in structure, other chemical changes may also occur. Lipid oxidation, protein oxidation and a decrease in colour stability are associated with frozen-then-thawed meat (Leygonie *et al.*, 2012). During frozen storage at -20°C unfrozen water is still available for biochemical reactions. Furthermore, mitochondrial and lysosomal enzymes, haem iron and other pro-oxidants are released from the damaged muscle cells and promote these reactions

(Leygonie *et al.*, 2012). Hence, both physical and chemical changes take place within the muscle during the freeze-storage-thaw process. The variation is reflected in the NIR spectra, making it possible to determine if the meat had been frozen or not.

Table 6.6 LDA classification rates of fresh and frozen-then-thawed lamb steaks

Method ¹	% Accuracy	Correctly classified (%)	
		Fresh meat (n = 200)	Frozen-then-thawed meat (n = 201)
Linear	83.04	170 (85%)	163 (81%)
Mahalanobis	81.80	164 (82%)	164 (82%)
Quadratic	81.30	167 (84%)	159 (79%)

(LDA) Linear discriminant analysis; (n) Number of samples; ¹ Two components used; (%) Percentage.

6.3.4 Subcutaneous fat assessment

Scans of the back surface of the carcasses were taken to determine whether or not it can be used to predict the origin. The spectral data were pre-processed with MSC and the absorbance curves are shown in Figure 6.9. The absorbance of the fresh meat samples were also included to show how it compares to that of the fat.

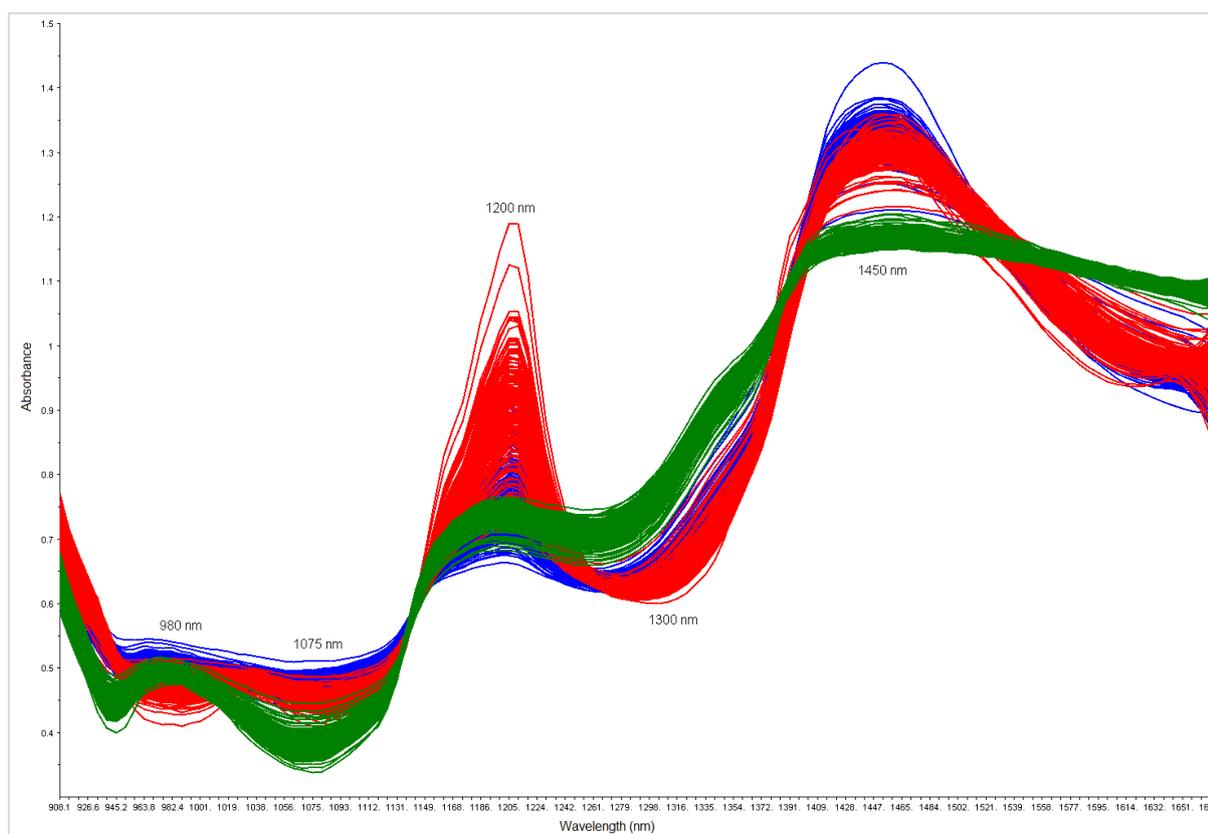


Figure 6.9 Multiplicative scattering correction (MSC) pre-processed NIR reflectance spectra, as acquired using a MicroNIR spectrophotometer, of the warm subcutaneous fat (blue lines), cold subcutaneous fat (red lines) and fresh lamb steaks (green lines).

Scanning of the warm subcutaneous fat on the slaughter line did not provide satisfactory results. Unlike the fresh meat samples, no groupings or trends were seen for the fat scans of the different regions using PCA and testing different pre-processing techniques (data not shown). This could be due to the rough environment within the abattoirs. Even scanning of the cold fat (after refrigeration at 4°C) did not improve the results. Fibre-optic probes could be a solution to the changing temperatures and humidity of the abattoirs (Prieto *et al.*, 2009). Fibre-optic probes have shown satisfactory results predicting meat quality online under industrial conditions.

As expected, the absorbance curve of the meat samples were completely different to that of the fat (Fig. 6.9). Variation in absorbance between the fat and fresh meat steaks can be seen at peaks 980 nm, 1075 nm, 1200 nm, 1300 nm and 1460 nm (Fig. 6.9). One of the major peaks (with absorption bands around 1200 nm) are related with C-H second stretching overtone (Cozzolino & Murray, 2004). This overtone could be characteristic of fat and fatty acid molecules (Morsy & Sun, 2013), likely the reason why the fat samples showed a higher absorbance at this wavelength (Fig. 6.9). Unfortunately, the MicroNIR does not measure absorbance in the following wavelength range, which are typical of fatty acids: 1742 nm, 1766 nm and 2200-2300 nm (Cozzolino & Murray, 2004). If measurement within these wavelengths were possible it could have increased the discrimination between the samples obtained from the different regions as the difference in diet is likely to cause variation in the fatty acid profiles (Chapter 4).

It was also noted that some of the carcasses were covered with a thin layer of meat over the fat. This thin layer is known as the *cutaneous trunci* muscle which helps the animal to twitch its skin (Seagren, 2009). This could affect the classification results as meat fibres have a different absorbance to that of fat tissue. In fact, to test this theory LDA was performed on the spectral data of the lamb meat and fat. The LDA method produced a model with 99.67% accuracy – which was expected given the clear difference between the absorbance curves of the meat and fat (Fig. 6.9). Of the 200 fresh meat samples, all of them were classified correctly. Similarly, 400 of the 402 fat samples were classified successfully (100%) with two samples (<0.5%) classified incorrectly. The two fat samples classified as meat, were HK56 and CK138. Although this is a small amount, taking into account the difference between the absorbance of the meat and fat, a misclassification was not expected. Investigation of the samples revealed that the carcasses were covered with the *cutaneous trunci* muscle, confirming that this muscle tissue is likely the cause of the misclassification. The variation of the subcutaneous fat is shown in Figure 6.10. Therefore, in order to prevent such variation for future analysis it is important that the sampling area is not covered by the mentioned layer of meat and the colour is consistent.



Figure 6.10 Variation of the subcutaneous fat of the lamb carcasses. The sampling area is indicated on the carcasses on the right.

6.4 Conclusions

NIRS showed considerable potential to be used for the classification of South African lamb in order to verify its authentic nature. Classification using PLS-DA and SVM was sufficient to distinguish between fresh Karoo and Non-Karoo lamb meat, as well as region of origin. Discrimination within the Karoo is weak, nevertheless it is strong between Karoo lamb vs. Non-Karoo lamb. The results also confirm that the portable MicroNIR is a suitable instrument to use for authenticating fresh meat. The device also enable the authentication of fresh vs. frozen-then-thawed meat using a straightforward statistical method. Scanning of the subcutaneous fat after slaughter did not give satisfactory results as temperature varies between abattoirs and the humidity cannot be regulated on the slaughter line. In addition, visual differences of the fat cover were also apparent where the fat of some carcasses were covered with the *cutaneous trunci* muscle. There is still a great need to extend the database by including more sheep samples from different regions in order to develop a robust classification model which can be used to detect the origin of the meat. By increasing the sample size, the variation is also increased. Ultimately, the wider the differences in sheep samples, the more accurate the model becomes. The results presented also serve as baseline data for future work. Considering the cost of the device, ease of measurement and flexibility of the measurement set-up, it is expected that retailers and producers would one day have a fast on-site measurement tool to distinguish lamb meat (based on its origin) in mislabelling attempts.

6.5 References

- Acocks, J. P. H. (1988). Veld types of South Africa. In: *Memoirs of the Botanical Survey of South Africa*. 3rd ed. Vol. 57.
- Alcalà, M., Blanco, M., Moyano, D., Broad, N. W., O'Brien, N., Friedrich, D., Pfeifer, F. & Siesler, H. W. (2013). Qualitative and quantitative pharmaceutical analysis with a novel hand-held miniature near infrared spectrometer. *Journal of Near Infrared Spectroscopy*, **21**, 445-457.
- Barbin, D. F., Sun, D.-W. & Su, C. (2013). NIR hyperspectral imaging as a non-destructive evaluation tool for the recognition of fresh and frozen-thawed porcine *longissimus dorsi* muscles. *Innovative Food Science and Emerging Technologies*, **18**, 226-236.
- Basri, K. N., Hussain, M. N., Bakar, J., Sharif, Z., Khir, M. F. A. & Zoolfakar, A. S. (2017). Classification and quantification of palm oil adulteration via portable NIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **173**, 335-342.
- Bredenkamp, G., Granger, J. E. & Van Rooyen, N. (1996). Eastern mixed Nama Karoo. In: *Vegetation of South Africa, Lesotho and Swaziland* (edited by A. B. Low & A. G. Robelo). Department of Environmental Affairs and Tourism, Pretoria.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Cozzolino, D. & Murray, I. (2002). Effect of sample presentation and animal muscle species on the analysis of meat by near infrared reflectance spectroscopy. *Journal of Near Infrared Spectroscopy*, **10**, 37-44.
- Cozzolino, D. & Murray, I. (2004). Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. *Lebensm.-Wiss.u.-Technol.*, **37**, 447-452.
- Department of Agriculture, Forestry and Fisheries (DAFF). (1990). *Product Standards Act* (Act No.119 of 1990, No. R. 342). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2000). *Meat Safety Act* (Act No.40 of 2000, No. 1072). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2006). *Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa*. Government Gazette of 1 September 2006, No. R. 863. Pretoria, South Africa: Government Printer.
- Downey, G. (1996). Authentication of food and food ingredients by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, **4**, 47-61.
- Erasmus, S. W., Hoffman, L. C., Muller, M., & Van der Rijst, M. (2016a). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems. *Small Ruminant Research*, **144**, 62-74. <http://dx.doi.org/10.1016/j.smallrumres.2016.07.020>.
- Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016b). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.

- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Grunert, K. G. (2005). Food quality and safety: consumer perception and demand. *European Review of Agricultural Economics*, **32**(3), 369-391.
- Guy, F., Prache, S., Thomas, A., Bauchart, D. & Andueza, D. (2011). Prediction of lamb meat fatty acid composition using near-infrared reflectance spectroscopy (NIRS). *Food Chemistry*, **127**, 1280-1286.
- Huck-Pezzei, V. A., Seitz, I., Karer, R., Schmutzler, M., De Benedictis, L., Wild, B. & Huck, C. W. (2014). Alps food authentication, typicality and intrinsic quality by near infrared spectroscopy. *Food Research International*, **62**, 984-990.
- Leygonie, C., Britz, T. J. & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, **91**(2), 93-98.
- Marques, E. J. N., De Freitas, S. T., Pimentel, M. F. & Pasquini, C. (2016). Rapid and non-destructive determination of quality parameters in the 'Tommy Atkins' mango using a novel handheld near infrared spectrometer. *Food Chemistry*, **197**, 1207-1214.
- Morsy, N. & Sun, D.-W. (2013). Robust linear and non-linear models of NIR spectroscopy for detection and quantification of adulterants in fresh and frozen-thawed minced beef. *Meat Science*, **93**, 292-302.
- O'Brien, N., Hulse, C. A., Friedrich, D. M., Van Milligen, F. J., Von Gunten, M. K., Pfeifer, F. & Siesler, H. W. (2012). Miniature near-Infrared (NIR) spectrometer engine for handheld applications. *SPIE Proceedings Next-Generation Spectroscopic Technologies V*, Vol. 8374.
- O'Brien, N., Hulse, C. A., Pfeifer, F. & Siesler, H. W. (2013). Near infrared spectroscopic authentication of seafood. *Journal of Near Infrared Spectroscopy*, **21**, 299-305.
- Prieto, N., Roehe, R., Lavín, P., Batten, G. & Andrés, S. (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science*, **83**, 175-186.
- Rinnan, Å, Van den Berg, F. & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *Trends in Analytical Chemistry*, **28**(10), 1201-1222.
- Seagren, M. A. (2009). The integumentary system. In: *Clinical Anatomy and Physiology Laboratory Manual for Veterinary Technicians*. Pp. 76. Canada: Mosby Elsevier.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.

Chapter 7

Stable isotope ratio analysis for the authentication of regionally unique South African lamb

Abstract

Stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of South African lambs from different regions were measured by isotope ratio mass spectrometry (IRMS) to evaluate it as a tool for the authentication of origin. Homogenised and defatted meat of the *Longissimus lumborum* (LL) muscle of lambs from different regions was assessed. The selected regions were from the Northern Cape, Western Cape and Eastern Cape provinces. The carbon isotope values were affected by the origin of the meat, mainly reflecting the diet. The Rûens (RU) and Hantam Karoo (HK) regions had the lowest ($P \leq 0.05$) $\delta^{13}\text{C}$ values, followed by Central Karoo (CK) and Eastern Karoo (EK), with Northern Karoo (NK), Namibia (NAM) and Bushmanland (BL) having the highest $\delta^{13}\text{C}$ values. The nitrogen isotopes values were highest for CK, Semi-extensive (SE), NAM and HK, while RU and Feedlot (FL) had the lowest $\delta^{15}\text{N}$ values ($P \leq 0.05$). The carbon isotopic ratios of NK, NAM and BL reveal a diet likely to have a high proportion of C_4 grass species. The low $\delta^{13}\text{C}$ values for HK are related to the presence of C_3 plants (i.e. Karoo bushes), while the higher values for $\delta^{15}\text{N}$ are likely caused by the presence of crassulacean acid metabolism (CAM) plants and the region's aridity. Support vector machine (SVM) classification achieved 58% accuracy with the highest percentage (%) correctly classified samples for RU (90%), CK (87%), HK (58%), NK (52%) and BL (52%). Classification of origin (Karoo vs. Non-Karoo) using discriminant analysis (DA) allowed 95% and 90% correct classification of the samples for the estimation model and validation models, respectively. The results confirm that IRMS provides sufficient discriminative power to classify lamb meat of varying origin using a larger sample size (more animals from different farms).

Keywords: Authentication; Extensive grazing; Lamb; Origin; Stable isotope ratios

7.1 Introduction

The multi-element approach, using ratios of stable isotopes to verify the origin and authenticity of food products, have been used in various studies ranging from coffee (Rodrigues *et al.*, 2009) to olive oil (Chiocchini *et al.*, 2016), wheat (Luo *et al.*, 2015), poultry (Rees *et al.*, 2016), beef (Heaton *et al.*, 2008; Horacek & Min, 2010) and lamb meat (Piasentier *et al.*, 2003; Camin *et al.*, 2007; Perini *et al.*, 2009; Chapter 5, Erasmus *et al.*, 2016). This is due to the fact that the isotopes can be linked to the origin through the diet (in the case of animal products), environmental conditions (i.e. altitude, precipitation, etc.) or the ingredients used for certain food products (e.g. water added to fruit juice) (Rossmann, 2001). Part of the need for verifying the origin of food products comes from the fact that consumers want to know where products come from, as well as the fact that no commercial fraud is involved. Products with higher commercial value is usually as a result of typical quality characteristics associated with region of origin, giving products an authentic nature and the

ability to stand out when compared to similar products without a geographical link. Some of these characteristic qualities may arise through its distinct or traditional production method.

In South Africa, most of the lamb produced in the Northern parts or Karoo region of the country is recognised as Karoo lamb (Weissnar & Du Rand, 2012). The meat is particularly known for its unique sensory quality (e.g. herbaceous aroma and flavour attributes) due to the sheep's diet, mainly consisting of indigenous, herbaceous Karoo bushes and shrubs (Estler *et al.*, 2006; Erasmus *et al.*, 2016). However, given the quality and value associated with Karoo lamb, there is a risk that the name may be misused by retailers selling lamb meat as Karoo lamb when in actual fact it has been produced in a feedlot or different region of origin. Similar to Karoo lamb, but less well-known, other characteristic South African lamb also exists, e.g. "Rûens lamb" of the Overberg region of the Western Cape. This lamb is raised on lucerne/alfalfa (*Medicago sativa*), typically cultivated in the region, while small grain stubble may also form part of the diet (Cloete & Olivier, 2010). It is believed that the characteristic diet of the sheep associated with a region and traditional farming practises provides the lamb meat with its unique sensory qualities. It is thus vital that an analytical method for the authentication of South African lamb is developed. Not only would such a method be able to distinguish lamb of Karoo from Non-Karoo origin, but it would also provide scientific evidence to verify the unique nature of the product.

Previous research (Chapter 5) using stable isotope ratio analysis revealed distinct isotopic differences of lamb meat obtained from different farms within the mentioned regions (Erasmus *et al.*, 2016). The differences found were related to diet linked to the origin. However, the classification was made at farm level. This raised questions in terms of whether a larger samples size, including more farms and regions with varying factors (i.e. breed, age and diet), would produce the same results. Also, the use of a larger sample set would be more representative of the different regions. Therefore, it was essential to perform stable isotope ratio analysis to extend and strengthen the baseline data and promote the development of robust classification models for region of origin authentication of South African sheep meat. Lambs from regions within different provinces of South Africa were sourced to investigate stable isotope ratio analysis as an analytical tool for the classification of lamb meat.

7.2 Materials and methods

7.2.1 Experimental layout and study regions

Nine regions, each unique in terms of vegetation and extensive grazing conditions, were selected for the purpose of the study (Table 7.1). Four regions were from the Northern Cape province, one from the Eastern Cape province, while the other was from the Western Cape province. Lambs obtained from Namibia (NAM) (neighbour country), feedlot (FL) and semi-extensive (SE) grazing conditions were also included (deemed types). Three slaughter ready lambs were sourced from each farm, where the number of farms per region ranged from 2 to 15 (Table 7.1). See General introduction (Chapter 1, Fig. 1.4) for the selected regions.

Table 7.1 Regions selected for this study on the carbon and nitrogen stable isotope ratio analysis

Region of origin	Code	Description of typical extensive diet	Nr. of farms	Nr. of animals
Central Karoo	CK	Shrubs/bushes and grass	15	45
Hantam Karoo	HK	Shrubs/bushes and grass	12	36
Northern Karoo	NK	Shrubs/bushes and grass	7	21
Bushmanland	BL	Shrubs/bushes and grass	7	21
Eastern Karoo	EK	Shrubs/bushes and grass	3	9
Rûens	RU	Lucerne/alfalfa	12	36
Additional				
Namibia	NAM	Namibian savannah-type vegetation	2	6
Feedlot	FL	Concentrates	3	9
Semi-extensive	SE	Shrubs/bushes, grass and concentrates	6	18
Total			67	201

(Nr.) Number.

7.2.2 Selected regions

The Northern Cape province is largely composed of the Karoo ecotype which features a variety of different biomes (Acocks, 1988; Estler *et al.*, 2006). The regions selected for this study are based on these biomes as they vary according to vegetation type. Due to this variation, a difference in diet is also expected as the sheep are mostly raised extensively. The Northern Cape/Karoo regions selected were the Central Karoo (CK), Hantam Karoo (HK), Northern Karoo (NK) and Bushmanland (BL). A “Karoo” region from the Eastern Cape was also included, the Eastern Karoo (EK). The borders of the Karoo are still widely disputed as some regions contain little and others none of the typical Karoo plants. For this reason, EK was also included as a Karoo region given that Karoo bushes are part of the vegetation. The HK is largely covered by the unique Karoo bushes and fall within the succulent Karoo biome, while CK, NK and BL fall in the Nama-Karoo biome, comprising a combination of Karoo bush and savanna-type or bushman grasses (Estler *et al.*, 2006). The “Non-Karoo” selected region is in the Western Cape and known as the Rûens (RU). Here lambs are typically raised on lucerne/alfalfa (*Medicago sativa*) pastures though, depending on season, they may also be raised on stubble after the grain harvesting period (usually from December to February) (Cloete & Olivier, 2010). For this study lambs were raised on lucerne.

Apart from the above-mentioned extensive enterprises, semi-extensive sheep farming is also practised and animals are provided with additional feed (i.e. concentrates) when they are not grazing in the veld. This system has less impact on the veld, allowing more time for the plants to recover from grazing, reducing the chances of over-grazing and erosion. In times of drought this system is largely used by Karoo farmers. For this reason, semi-extensive lambs were also included in the study.

7.2.3 Sample collection

Animals were slaughtered according to standard procedures and regulations in three registered abattoirs in South Africa (DAFF, 2000), as described in Chapter 6 (section 6.2.1 and 6.2.2). Extensively raised lambs (three per farm), classed A2 (DAFF, 1990; DAFF, 2006), of any breed and gender, and a carcass weight of approximately 18 kg were sourced. In total, 201 lambs sourced from 67 farms were used for the study (Table 7.1). Twenty-four hours after slaughter and refrigeration at 4°C, meat steaks (1.5-2 cm thick) were cut perpendicular to the grain of the left *Longissimus lumborum* muscle of the carcass at the L₄₋₅ position (4th to 5th lumbar vertebrae). The steaks were vacuum packed and stored at -20°C in absence of light until the analyses were conducted.

7.2.4 Sample preparation

The fat of a 5 g homogenised meat sample was extracted (Lee *et al.*, 1996). As a result of the effect of the biochemical isotopic fractionation, the fat was removed to correct for variation in isotopic ratios between proteins and lipids (Camin *et al.*, 2007). The resultant protein residue was freeze-dried and finely ground into a homogenous powder using a pestle and mortar. Powdered meat samples were then vacuum sealed and stored at -20°C until analysis.

7.2.5 Isotope ratio analysis

For the isotope analysis ~0.5 mg powdered meat samples were weighed into tin (for ¹³C/¹²C and ¹⁵N/¹⁴N) capsules and combusted individually in a Flash HT Plus elemental analyser (Thermo Fisher Scientific, Bremen, Germany). The resultant CO₂ and N₂ gas was introduced to an isotopic mass spectrometer (DELTA V Advantage) using a Continuous Flow Interface (ConFlo IV) (Thermo Fisher Scientific, Bremen, Germany). Isotope ratios are expressed in the conventional delta (δ) notation in parts per mil (‰), according to the following general formula:

$$\delta_{\text{‰}} = \frac{\mathbf{R} \text{ sample} - \mathbf{R} \text{ standard}}{\mathbf{R} \text{ standard}} \times 1000$$

where **R** represents the ratio between the abundant isotopes i.e. ¹³C/¹²C and ¹⁵N/¹⁴N. Standard refers to an international standard: V-PDB (Vienna PeeDee Belemnite) for δ¹³C and nitrogen air (N₂) for δ¹⁵N. The negative δ¹³C values of plant and animal tissues are attributed to the fact that V-PDB has relatively more ¹³C than most of the terrestrial biosphere (Sandberg *et al.*, 2012). Standard deviations of repeated measurements of in-house standards were less than 0.80‰. The in-house standard used was Merck Gel. Merck Gel is a proteinaceous gel produced by Merck and Seal. Seal is a seal bone crushed, demineralised and dissolved in acid, and then reconstituted in gel form. The in-house standard (Merck Gel) were calibrated against the International Atomic Energy Agency (IAEA) standards.

7.2.6 Statistical analysis

The data were statistically analysed using GLM (General Linear Models) procedure of SAS™ statistical software (Statistical Analysis System, Version, 9.4, 2006, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and XLSTAT® statistical software (Version 2016.04.33113; Addinsoft, NY, USA) and Unscrambler X10.3 (Camo Software AS., Oslo, Norway) for the multivariate statistical analysis. Pre-processing of the data involved using the Shapiro-Wilk test to test for deviation from normality (Shapiro & Wilk, 1965). Outliers in the data were identified and removed until the data was normally distributed in cases where the deviation from normality was significant ($P \leq 0.05$). After confirming that the data was symmetrically distributed, one-way ANOVA was carried out for each stable isotope ratio with region as factor. In order to compare region means, Fisher's Least Significant Differences (LSD) was calculated at a 5% significance level. A probability level of 5% was considered significant for all the significance tests. Significant patterns and associations in the collected data were identified with multivariate statistical techniques. To determine whether lamb meat from different regions could be classified based on their stable isotope ratios the vector support machine (SVM) classification method, linear discriminant analysis (LDA) and discriminant analysis (DA) were carried out. SVM and LDA were performed on the full sample set using Unscrambler X10.3, while XLSTAT® statistical software was used for DA. For DA, region separation was described and elucidated by means of linear functions of the variables (discriminant functions) that best separated regions, using farms (with their respective observations) from each region as a training set. The model was then validated using leave-N-out (LNO) cross-validation by using randomly selected farms (with their respective observations) from each region as a test set.

7.3 Results and discussion

The next sections will discuss the carbon and nitrogen isotope ratios of the different regions. Since the samples were defatted, the contribution of fat towards the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be ignored, especially given the variation in warm carcass weight and fat thickness (Table 7.2). Although, the carcass weights of CK, HK, NK, BL and SE did not vary significantly from each other, that of EK, RU and FL were the highest (significantly heavier than that of NK, BL and NAM) (Table 7.2).

NAM had the lowest warm carcass weight, which could result in the meat having a lower intra-muscular fat content as it is only usually deposited in later stages of growth (Warriss, 2010). Taking the fat thickness into account, only FL (7.4 ± 2.92 mm) and RU (3.6 ± 1.31 mm) differed significantly ($P \leq 0.05$) (Table 7.2). The higher degree of fat thickness was expected for FL as the animal were raised intensively in the feedlot and walked less than the extensively raised sheep. The variation due to fat content and the differences that exist in the isotopic ratios of protein compared to that of fat was corrected for by defatting the samples before analysis.

Table 7.2 Regions selected for this study and the means (\pm SD) for the carcass characteristics of the lamb

Origin	n	Breed	Gender	Age	Warm carcass weight (kg)	Fat thickness ¹ (mm)
CK	45	36 Dorper	33 rams	6-11 months	18.9 ^{ab} \pm 2.12	4.6 ^{bc} \pm 1.30
		9 Merino	12 ewes			
HK	36	27 Dorper	11 rams	4-7 months	18.7 ^{ab} \pm 2.09	5.4 ^{bc} \pm 1.43
		9 Merino	11 ewes			
			14 wethers			
NK	21	21 Dorper	13 rams	6-11 months	17.1 ^{bc} \pm 1.70	4.2 ^{bc} \pm 1.39
			7 ewes			
			1 wether			
BL	21	21 Dorper	15 rams	6-11 months	17.3 ^{bc} \pm 2.27	4.5 ^{bc} \pm 1.00
			6 ewes			
EK	9	3 Dorper	9 rams	6-11 months	20.4 ^a \pm 1.18	5.5 ^{ab} \pm 2.25
		6 Merino				
RU	36	3 Dorper	3 rams	6-10 months	21.0 ^a \pm 1.93	3.6 ^c \pm 1.31
		9 Merino	13 ewes			
		3 Merino cross	20 wethers			
		21 Dohne Merino				
Additional						
NAM	6	6 Dorper	2 rams	6-11 months	15.2 ^c \pm 1.15	4.8 ^{bc} \pm 0.60
			4 ewes			
FL	9	6 Merino	4 rams	6-11 months	21.1 ^a \pm 3.30	7.4 ^a \pm 2.92
		3 Dohne Merino x	2 ewes			
		Dorper	3 wethers			
SE	18	9 Dorper	14 rams	6-11 months	19.3 ^{ab} \pm 2.69	5.0 ^{bc} \pm 1.15
		9 Merino	4 ewes			

(SD) Standard Deviation; (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive; (EK) Eastern Karoo; (FL) Feedlot; (NAM) Namibia; (n) Number of lambs; ¹ Fat thickness measured between 3rd and 4th lumbar vertebrae; Least significant difference at the 5% level of significance equal to 2.73 (warm carcass weight) and 1.82 (fat thickness); ^{a-c} Values in the same column with different superscripts are significantly different ($P \leq 0.05$).

7.3.1 Variability of meat stable isotope ratios

Mean values of the ¹³C/¹²C and ¹⁵N/¹⁴N isotope ratios of meat protein are presented in Table 7.3 and the scatter plot illustrated in Figure 7.1. The $\delta^{13}\text{C}$ values are affected by the origin of the meat, mainly reflecting the diet. Overall, RU and HK had the lowest ($P \leq 0.05$) $\delta^{13}\text{C}$ values ($-23.6 \pm 2.00\text{‰}$ and $-21.6 \pm 1.72\text{‰}$, respectively), however, these two regions did not differ from each other (Table 7.3). The highest $\delta^{15}\text{N}$ values were obtained for CK, SE, NAM and HK, while RU and FL illustrated lower values, except for BL and NK (Table 7.3). Although the $\delta^{15}\text{N}$ values for FL and EK could be classified as low and intermediate, there was no significant difference ($P > 0.05$), possibly due to the large variation in nitrogen isotopic ratios between farms within the region.

Table 7.3 The means (\pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (in ‰, relative to the Vienna PeeDee Belemnite and atmospheric N_2) of lamb meat from varying origin

Code	Code	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Central Karoo	CK	15	$-19.9^{\text{bc}} \pm 1.41$	$8.8^{\text{a}} \pm 1.56$
Hantam Karoo	HK	12	$-21.6^{\text{cd}} \pm 1.72$	$7.8^{\text{ab}} \pm 1.07$
Northern Karoo	NK	7	$-15.9^{\text{a}} \pm 2.89$	$7.1^{\text{abcd}} \pm 2.13$
Bushmanland	BL	7	$-16.2^{\text{a}} \pm 2.12$	$6.5^{\text{bcd}} \pm 1.71$
Eastern Karoo	EK	3	$-19.4^{\text{bc}} \pm 1.71$	$7.5^{\text{abc}} \pm 3.18$
Rûens	RU	12	$-23.6^{\text{d}} \pm 2.00$	$5.4^{\text{d}} \pm 0.86$
Namibia	NAM	2	$-16.0^{\text{a}} \pm 2.13$	$8.1^{\text{ab}} \pm 1.28$
Feedlot	FL	3	$-18.1^{\text{ab}} \pm 1.20$	$5.5^{\text{cd}} \pm 2.52$
Semi-extensive	SE	6	$-18.7^{\text{b}} \pm 2.17$	$8.3^{\text{ab}} \pm 1.67$
LSD (P = 0.05)			2.49	2.05

(SD) Standard Deviation; (n) number of farms (three animals per farm); (LSD) Least significant difference; ^{a-d} Values in the same column for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with different superscripts are significantly different ($P \leq 0.05$).

Although a greater change in the proportion of carbon isotope to atmospheric carbon dioxide is caused by the C_3 -pathway of plants, more ^{13}C is included in the C_4 -pathway producing a smaller change, hence less negative $\delta^{13}\text{C}$ values (Piasentier *et al.*, 2003). This is the reason why C_4 plants, compared to C_3 plants, have higher $\delta^{13}\text{C}$ values in their tissue (Witt *et al.*, 1998; Gibson, 2009). In the same way, the carbon ratios are reflected in the meat of animals where C_3 and C_4 plants formed part of the diet (Codron *et al.*, 2005). Lambs raised on pasture (C_3 plants) have lower (more negative) $\delta^{13}\text{C}$ values than lambs supplemented with concentrates composed of grain and maize (C_4 plants) (Perini *et al.*, 2009). Although RU and HK had the lowest $\delta^{13}\text{C}$ values, this is not due to the supplementation of concentrates but the nature of the plants grazed. Comparable results were previously found for RU (-22.7‰) and HK (-22.9‰) (Erasmus *et al.*, 2016). Hence, the current results validate these values for the respective regions, where the diets of lucerne for RU and Karoo bushes/shrubs for HK are responsible for the low $\delta^{13}\text{C}$ values obtained.

The $\delta^{13}\text{C}$ values for NK, BL and NAM were the highest, similar to the values for Dorper lambs raised extensively on thornveld (Savanna biome) and grassland (Erasmus *et al.*, 2016). The C_4 grass species (i.e. *Cynodon dactylon*, *Eragrostis spp.*; *Stipagrostis spp.*, *Fingerhuthia africana*, *Schmidtia kalahariensis* etc.) were likely the main contributor towards the increase in $\delta^{13}\text{C}$ values (Erasmus *et al.*, 2016). Although the Karoo veld consist of both bush and grass species, depending on season, rainfall and geography, the availability and proportion of bushes and grasses may vary (Acocks, 1988; Estler *et al.*, 2006). It has also been shown that different breeds graze differently on the veld (Du Toit, 1998). For instance, the Dorper is a non-selective grazer and focuses on the woody plant types (i.e. Karoo bushes, shrubs and trees) (Du Toit, 1998). Yet, if there are

still some of the very palatable perennial grasses available in the veld the grasses would be preferred over the woody plants. The Merino is more selective towards grass and less adapted than the Dorper to graze the Karoo bushes (Du Toit, 1998). However, in winter and times of drought Karoo shrubs or bushes are mainly consumed due to the lack of soft forage (Acocks, 1988; Witt *et al.*, 1998). Characteristically, pure grazer animals (consuming C₄ plants) have the highest $\delta^{13}\text{C}$ values (-14.7 to -13.5‰), while browsers (consuming C₃ plants) tend to have the lowest values (-27.2 to -26.2‰) and mixed-feeders (consuming C₄ and C₃ plants) intermediate values (-23.0 to -19.0‰) (Codron *et al.*, 2005). Taking the grazing behaviour of the sheep and the results into account, one would expect the $\delta^{13}\text{C}$ values for NK and BL to be lower (and closer to CK) as only Dorper lambs were sourced from these regions (Table 7.2). Therefore, the assumption can be made that the vegetation of the NK and BL were most likely composed of more grass species than Karoo bushes/shrubs. This was expected as the vegetation changes, with a decrease in grass and increase in Karoo bush species, moving from the Central Karoo towards the Northern parts (Acocks, 1988). The Bushmanland is known for having a grass cover similar to that of the inner Namibian areas (Walter & Breckle, 1986), thereby also providing an explanation for the similar carbon isotopic ratios between BL and NAM (Table 7.3). The opposite was reported for springbok living on Karoo bushes or shrubs in the Northern Cape as they had lower (more negative) $\delta^{13}\text{C}$ values compared to the grass consuming springbok in Namibia (Vogel, 1978). Taking both isotopes into account, the isotopic signatures of NK, BL and NAM bear a resemblance to that of lambs from the Free State grasslands (Erasmus *et al.*, 2016) (Fig. 7.1).

When the $\delta^{15}\text{N}$ values are investigated, it is evident that RU and FL had the lowest ($P \leq 0.05$) ratios, although FL did not differ significantly from NK, BL and EK (Table 7.3). RU also had a reasonably low standard deviation suggesting that there was little variation of isotopic ratios between farms. The nitrogen values were in accordance with that obtained by Erasmus *et al.* (2016) for different RU lambs, that of Erasmus *et al.* (2016) was around 6.7‰ and that of the current study around 5.4‰. However, in the current study RU was overall the lowest when compared to the other treatments ($P \leq 0.05$). It is known that environmental and soil conditions influence the ¹⁵N content of plants and the resultant $\delta^{15}\text{N}$ values of the meat from animals raised on these plants. The use of organic fertilisers increase the $\delta^{15}\text{N}$ values of extensively reared lamb meat as it adds to the ¹⁵N level of nitrogen compounds of soil and plants (Piasentier *et al.*, 2003; Perini *et al.*, 2009). Conversely, leguminous plants use the atmospheric nitrogen as its nitrogen source resulting in a decrease of the $\delta^{15}\text{N}$ values of meat from legume-fed animals (Piasentier *et al.*, 2003; Perini *et al.*, 2009; Devincenzi *et al.*, 2014). Given that RU lambs were raised on lucerne pastures, the low $\delta^{15}\text{N}$ values were expected. The $\delta^{15}\text{N}$ values of meat from the feedlot lambs were also low, but with a high standard deviation, indicating substantial sample variation (Table 7.3). These variable results could be due to the feeding of sheep with concentrates containing legumes, such as dehydrated alfalfa, chickpea or soya bean. The high standard deviation is then likely due to some animals receiving more grains and maize than others. It can also be speculated that the FL lambs may have come from different extensive grazing regions and could have been in the feedlot for various time periods.

The tempo of change in isotopes values over time for different grazing conditions could offer an explanation, but it was not determined in the study. Thus, the change over time could be explored in future studies.

The highest $\delta^{15}\text{N}$ values were observed for CK, SE, NAM, HK and EK (Table 7.3). The increase in $\delta^{15}\text{N}$ values could be linked to the aridity of the origin or ^{15}N -enrichment through the presence of succulents [crassulacean acid metabolism (CAM) plants] (Perini *et al.*, 2009; Erasmus *et al.*, 2016). CAM plants have been reported as part of the vegetation of CK and HK (Erasmus *et al.*, 2016). In these earlier findings the $\delta^{15}\text{N}$ value were higher for HK (12.9‰) and NK (12.1‰), but similar for CK (8.9‰) (Erasmus *et al.*, 2016). The lower values for HK and NK could be as a result of less CAM plants or improved veld conditions of the selected farms as shown in Chapter 5 (Erasmus *et al.*, 2016). The variation in results between the two studies highlights the importance of building a large database for the isotopic values of regionally unique lamb. Although the potential of using stable isotope ratio analysis for the authentication of lamb meat could be shown from a farm-based level (Erasmus *et al.*, 2016), it was important to perform the validation from a region-based level in order for it to be representative of the region.

In view of the results in Fig. 7.1, four main groupings can be identified. The main groupings are illustrated in Figure 7.1 by plotting the mean $\delta^{13}\text{C}$ (y-axis) and $\delta^{15}\text{N}$ (x-axis) values. RU is grouped separately towards the lower left part of the plot, having low ratios for both isotopes and one outlier (RU1) (Fig. 7.1). NK, BL and NAM is grouped towards the top of the plot, having high $\delta^{13}\text{C}$ values and low $\delta^{15}\text{N}$ values. As mentioned, NK, BL and NAM have a similar isotopic signature to that of lambs raised on grass (Erasmus *et al.*, 2016). The other two groups are mainly composed of HK and CK lambs, respectively (Fig. 7.1). The vegetation of farms from the HK region are mainly covered in Karoo bushes/shrubs and CAM plants with limited number of grass species. As a result, HK have low ratios for carbon isotopes due to the presence of C_3 plants (i.e. Karoo bushes) and increased ratios for that of nitrogen, likely caused by the presence of CAM plants and the arid conditions on the farms (Fig. 7.1). CK grouped more towards the right side of the plot with higher values for $\delta^{15}\text{N}$ and intermediate values for $\delta^{13}\text{C}$ (Fig. 7.1). There is a great deal of overlap between HK and CK. Such overlapping was expected as they are both from the Karoo region. The lamb samples of FL1, FL3, SE4 and EK3 farms grouped rather separately with intermediate $\delta^{13}\text{C}$ values and low $\delta^{15}\text{N}$ values (Fig. 7.1). This could be indicative of their Non-Karoo origin yet, for future analysis a larger sample size is required to confirm whether or not these lamb types would group separately from Karoo lamb. In fact, lamb samples of SE1, SE2, SE3, SE6 and FL2 farms grouped with the Karoo samples (Fig. 7.1). Overall $\delta^{13}\text{C}$ values had the clearest discrimination between regions in the stable isotope ratios examined as a result of low within-group (farm to farm) variation and the greatest between region variability (Table 7.3). The $\delta^{15}\text{N}$ values had greater standard deviations with six of the nine regions (CK, HK, NK, EK, NAM and SE) not differing significantly from one another.

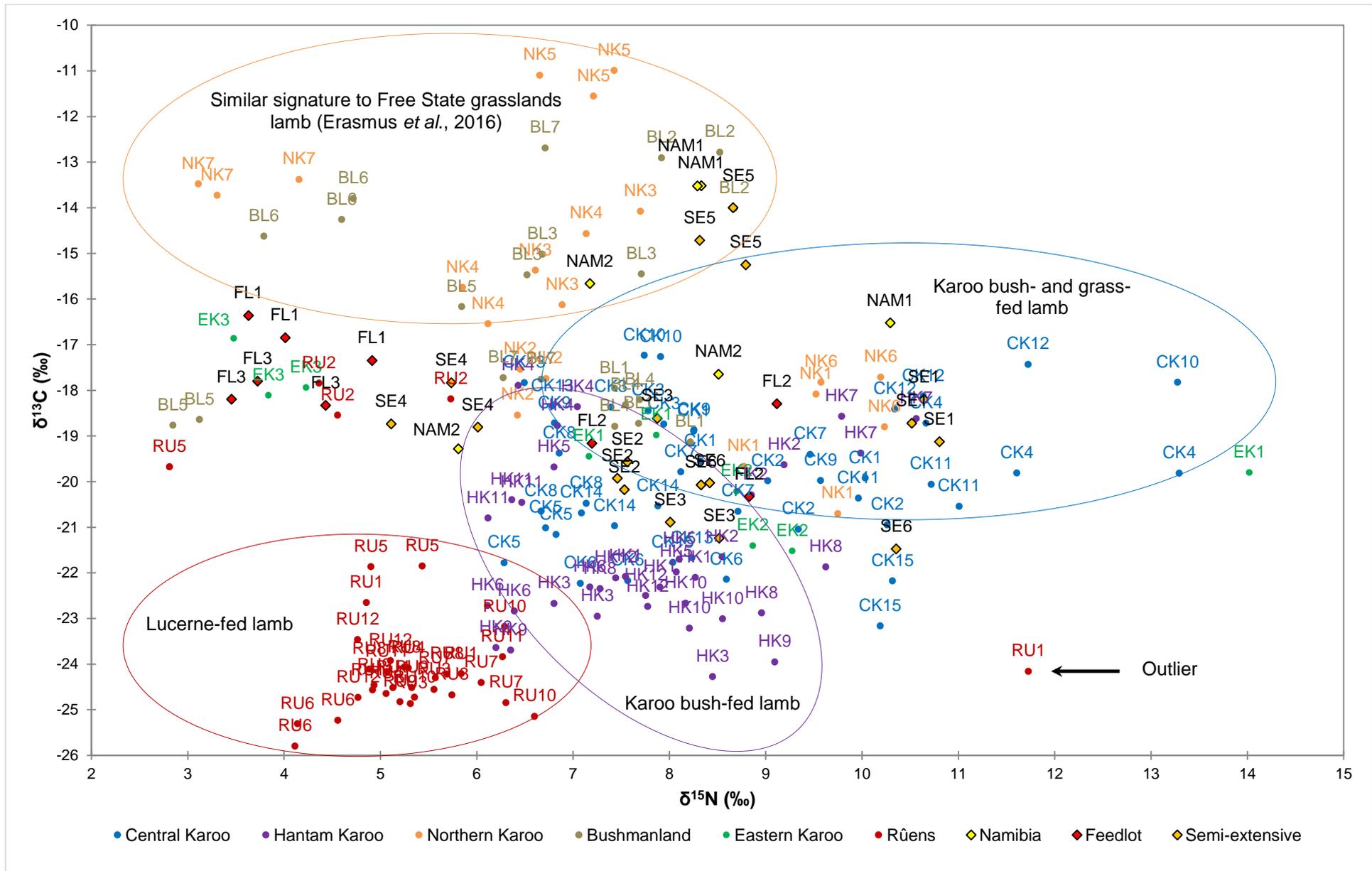


Figure 7.1 Scatter plot of the mean $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios of the individual meat protein samples from the different regions.

7.3.2 Discriminating geographical origin from meat stable isotope ratios

Support vector machine (SVM) was used for the classification of the samples according to region of origin (CK, HK, NK, BL, EK, RU, NAM, FL and SE). The classification plot is shown in Figure 7.2, while the values are presented in Table 7.4.

For the SVM classification model the following settings were used: nu-SVC, radial basis function (kernel type); gamma = 0.5; nu value = 0.5 and 10 cross validation segments. All the values were weighted. The model had a training accuracy of 58% and a validation accuracy of 49%. The accuracy of the model was low and is likely as a result of the high level of variation due to the nine regions included in the model. Visual inspection of Figure 7.2 reveals separate groupings of RU, HK, CK, BL and NK. According to the classification results the same regions obtained a higher classification rate than SE, EK, FL and NAM (Table 7.4). A large amount of misclassification occurred between CK and HK, with six CK samples being classified as HK and twelve HK samples as CK (Table 7.4). Only five samples of all the regions were misclassified as RU. However, the Non-Karoo types (SE, FL and NAM) were misclassified as Karoo regions. Although this is not the result which was expected, it could be due to the fact that these additional lamb types had a smaller sample size. Hence, a strong model was not created for them, while they also did not conform to the model created for the RU (Non-Karoo) lamb. On the other hand, SE lamb were raised on the veld and supplemented with concentrates. The reason for these lamb types being misclassified as Karoo, could be related to their diet and the amount of supplementation. Although the data were not available relating to the period and amount of supplementation, it could be that the supplementation were insufficient to have a significant effect on the isotopic signatures of the meat. If the period were indeed longer, different classification results might have been produced.

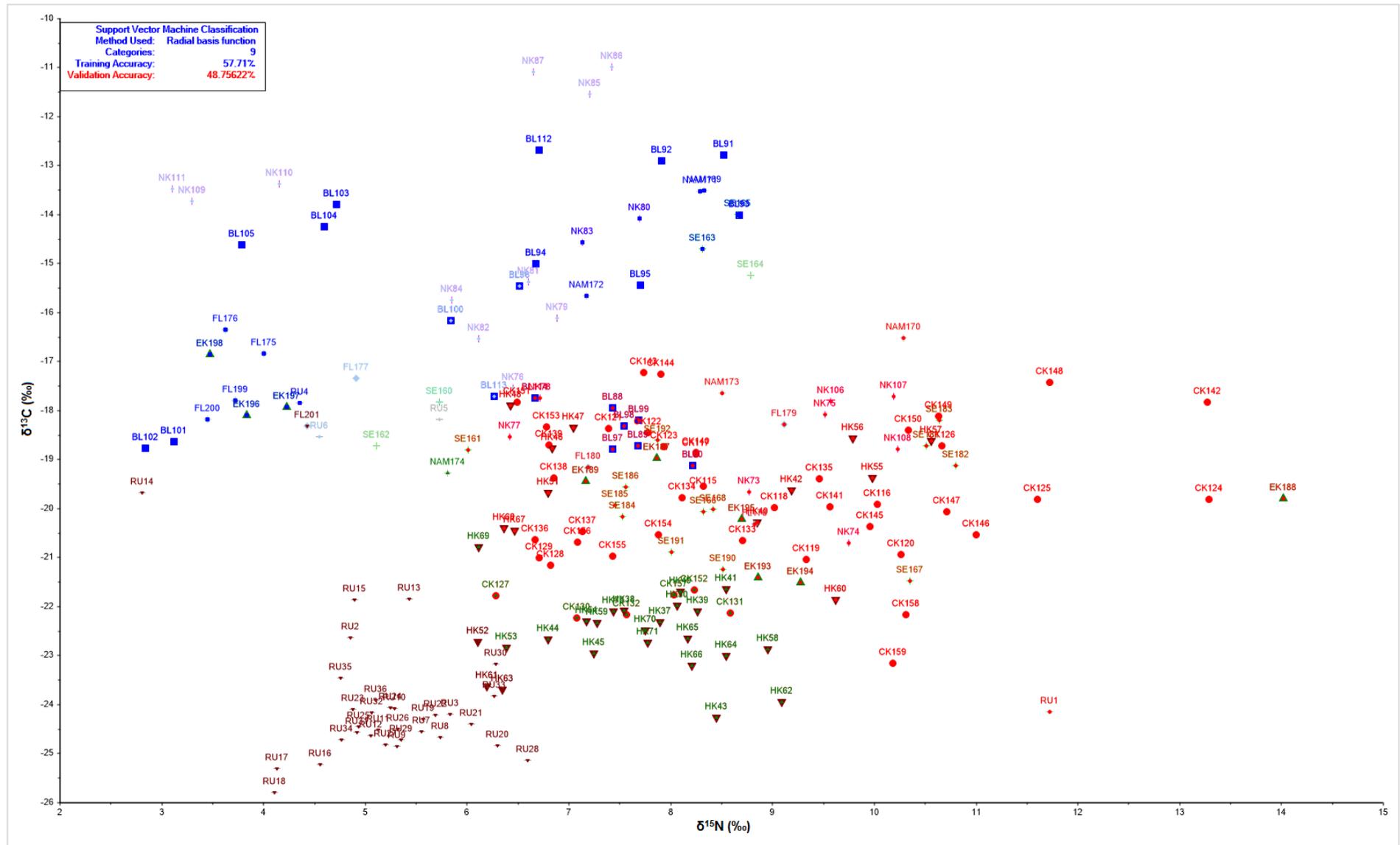


Figure 7.2 Support vector machine (SVM) classification plot of the mean $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios of the individual meat protein samples from the different regions. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive. (EK) Eastern Karoo, (NAM) Namibia and (FL) Feedlot not assigned to their own class

Table 7.4 Classification of individual lamb meat samples on the basis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percentage of observations correctly classified by SVM

Actual origin	Classified origin ^a									Total
	CK	HK	NK	BL	RU	SE	EK	FL	NAM	
CK	39	6	0	0	0	0	0	0	0	45
HK	12	21	0	0	3	0	0	0	0	36
NK	8	0	11	2	0	0	0	0	0	21
BL	7	0	3	11	0	0	0	0	0	21
RU	1	0	1	1	32	1	0	0	0	36
SE	13	0	1	2	0	2	0	0	0	18
EK	6	0	0	3	0	0	0	0	0	9
FL	3	0	1	4	1	0	0	0	0	9
NAM	2	1	0	3	0	0	0	0	0	6
% Correctly classified	86.67	58.33	52.38	52.38	89.89	11.11	0	0	0	57.71^b

(SVM) Support vector machine; (%) Percentage; ^a The number of correctly classified observations are tabulated diagonally; ^b 57.7% of the observations correctly classified; (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive; (EK) Eastern Karoo; (FL) Feedlot; (NAM) Namibia.

Following the SVM results, discriminant analysis (DA) was performed to test if the stable isotope ratios in lamb meat allow to discriminate the five different regions of origin with the highest successful SVM classification rates (RU, CK, HK, NK and BL). The DA models were evaluated using an external validation set, selecting the entire farm. Accordingly, farms (with 3 samples per farm) were randomly selected for validation as follows: five for CK (33%), four for HK and RU, respectively (33%) and two for NK and BL, respectively (29%). The results are reported in Table 7.5 as the number and percentage of correctly classified observations. The stable isotope ratios allowed 67% correct classification of the meat samples for the estimation model and 57% for the validation model (Table 7.5). Again, RU had the highest successful classification rate, followed by CK and HK. The high successful classification rate for RU could also be related to the diet of the animals. RU lamb mainly consumed lucerne, while lamb from the Karoo regions had access to a variety of species. Based on the variety of plant species in the Karoo, one could expect more variation in the isotope values of lamb meat from the Karoo region. CK and HK farms were also misclassified as described with SVM classification – hence confirming that their isotopic signatures are closely related. The latter most likely being as a result of their diet composed of Karoo veld vegetation. HK farms misclassified as CK could be due to an increase in C₄ grass species in their diets, while CK farms misclassified as HK could be related to the consumption of more C₃ Karoo bushes

Table 7.5 Classification of individual lamb meat samples on the basis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percentage of observations correctly classified by DA

Model	Actual origin	Classified origin ^a					Total
		CK	HK	NK	BL	RU	
Estimation	CK	22	8	0	0	0	30
	HK	6	16	0	0	2	24
	NK	6	0	4	5	0	15
	BL	3	0	5	7	0	15
	RU	0	1	0	0	23	24
% Correctly classified		73.33	66.67	26.67	46.67	95.83	66.67^b
Validation	CK	10	5	0	0	0	15
	HK	3	7	0	0	2	12
	NK	2	0	2	2	0	6
	BL	4	0	1	1	0	6
	RU	0	0	0	3	9	12
% Correctly classified		66.67	58.33	33.33	16.67	75.00	56.86^c

(DA) Discriminant analysis; (%) Percentage; ^a The number of correctly classified observations are tabulated diagonally; ^b 66.7% of the observations for the estimation model correctly classified; ^c 56.9% of the observations for the validation correctly classified; (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rùens.

The DA plots of the observations and loadings (in the top left corner) on the linear discriminant function scores are illustrated in Figure 7.3. The DA plots were used to visualise the grouping of observations within each region and the separation between regions. The DA plots confirms that the regions are reasonably well discriminated on the axes extracted from the stable isotope ratios. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values influence the location of samples along the axes (Fig. 7.3). CK and HK associate with higher $\delta^{15}\text{N}$ values, while RU and HK associate with lower (more negative) $\delta^{13}\text{C}$ values. These associations were also seen in Figure 7.1, with both Figures having similar groupings of the regions.

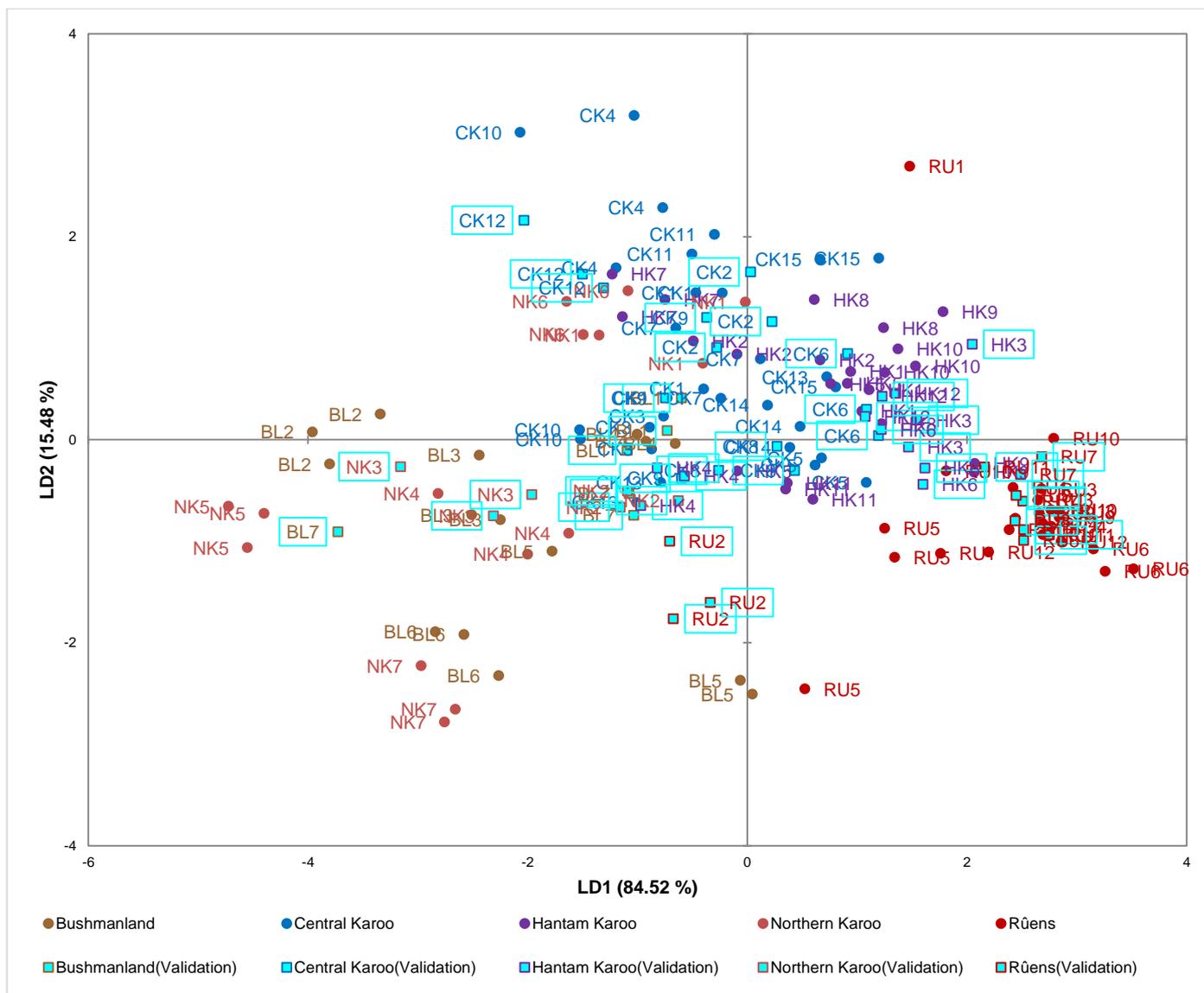


Figure 7.3 Discriminant analysis (DA) plot of the linear discriminant function scores on stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in lamb meat for the grouping of observations from different regions (variable loadings plot in the top left corner). (LD) Linear discriminant score.

The same dataset used for the classification of the five regions (Table 7.5) were used to classify the observations based on Karoo versus Non-Karoo origin. The results are shown in Table 7.6 as the number and percentage of correctly classified observations. The stable isotope ratios allowed 95% correct classification of the meat samples for the estimation model and 90% for the validation model (Table 7.6). For the estimation model the one Non-Karoo sample which was misclassified was an outlier (RU1) (Fig. 7.1). As the outlier was not removed before classification, the correct classification rates would be improved by removing it. The four misclassified Karoo samples were from HK9 and BL5 farms (two lambs per farm). This could indicate that the animals were given a high level of lucerne supplementation. Hence, a crucial question is whether or not these animals were raised extensively. Again for the validation model, two animals from one Karoo farm (HK6) were misclassified as Non-Karoo. An entire Non-Karoo farm (RU2) were misclassified as Karoo. This is a problem as the aim of the technique is to authenticate the meat and detect fraud. However, in Figure 7.1 RU2 farms

have intermediate $\delta^{13}\text{C}$ values and is grouped separately from the Karoo regions but closer to the FL type. Thus, the assumption can be made that the lambs from RU2 were not completely raised extensively on lucerne and likely received some form of grain or maize (C_4 plants) supplementation.

The successful classification rate was considerably improved by combining the regions within the Karoo and comparing it to a Non-Karoo region (RU). The lower success rate of the regional-based model could be related to the variation between regions within the Karoo. In order to improve their classification rate, it is important to extend the database even further by including more samples of the different regions for the development of the classification model in the future.

Table 7.6 Classification of origin (Karoo vs. Non-Karoo) of individual lamb meat samples (CK, HK, NK, BL and RU) on the basis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percentage of observations correctly classified by DA

Model	Actual origin	Classified origin ^a		
		Karoo	Non-Karoo	Total
Estimation	Karoo	80	4	84
	Non-Karoo	1	23	24
% Correctly classified		95.24	95.83	95.37^b
Validation	Karoo	37	2	39
	Non-Karoo	3	9	12
% Correctly classified		94.87	75.00	90.20^c

(DA) Discriminant analysis; (%) Percentage; ^a The number of correctly classified observations are tabulated diagonally; ^b 95.4% of the observations for the estimation model correctly classified; ^c 90.2% of the observations for the validation correctly classified.

Additionally, the linear discriminant analysis (LDA) technique was used to evaluate the differences between the regions based on their isotopic ratios. Unlike the results above, all the regions were included and a 68% correct classification was achieved. A large portion of the misclassifications for Karoo were due to HK ($n = 23$) and CK ($n = 9$) lambs being classified as Karoo. Then again, SE ($n = 15$), NAM ($n = 5$), FL ($n = 3$), and RU ($n = 1$) lamb(s) were misclassified as Karoo (Table 7.7). The findings indicate that more samples (at least 6 to 12 farms with 3 animals sourced per farm) are needed for the database to improve the classification rates as the accuracy of the linear model is also decreased with the inclusion of small samples sets (i.e. EK, NAM and FL).

The results obtained for the classification of lamb meat according to region of origin confirm the potential of using stable isotope ratio analysis as a tool for the characterisation of the origin of meat, i.e. the authentication of region of origin lamb meat and its traceability linked to a diet typical of the defined region. While carbon and nitrogen isotopes have shown to be effective for discriminating regions, an added level of geographical resolution can be achieved with the use of additional isotopes (i.e. ^2H , ^{18}O and ^{34}S) (Witt *et al.*, 1998; Kelly *et al.*, 2005; Crawford *et al.*, 2008). For example, Perini *et al.* (2009) used several isotopes (^2H ,

^{13}C , ^{15}N , ^{18}O and ^{34}S) to trace the geographical origin and diet of lambs, while Sun *et al.* (2011) used 21 elements (Be, Na, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Sb, Ba, Tl, Pb, Th and U) to determine the geographical origin of mutton from different regions of China.

Table 7.7 Classification of origin (Karoo vs. Non-Karoo) of lamb meat samples (of all the regions) on the basis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and percentage of observations correctly classified by LDA

Model ¹	Actual origin	Classified origin ^a		
		Karoo	Non-Karoo	Total
Linear (68.18% accuracy)	Karoo	92	40	132
	Non-Karoo	24	45	69
% Correctly classified		69.70	65.22	68.16^b

(LDA) Linear discriminant analysis; ¹ Two components used; (%) Percentage ^a The number of correctly classified observations are tabulated diagonally; ^b 68.2% of the observations for the correctly classified.

7.4 Conclusions

The findings reveal that the analysis of the stable isotopic ratios of carbon and nitrogen is a promising tool to use for the authentication of lamb meat. The carbon isotopes are particularly useful to evaluate the extensive diet in terms of C_3 , C_4 and CAM plant consumption. In effect by doing so, it allows discrimination between sheep production systems (in this study regions) based on their dietary differences. It is also effective in determining the nitrogen isotopic ratios, which is indicative of aridity and the presence of leguminous plants. The results verified that lamb meat from different farms of different regions can be discriminated using stable isotope ratios. Four main groups were identified: lucerne-fed lamb from the RU having low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values; grass-fed lamb from BL and NK having high $\delta^{13}\text{C}$ and low $\delta^{15}\text{N}$ values; Karoo bush- and grass-fed lamb from CK having intermediate $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values; and Karoo bush-fed lamb from HK having low $\delta^{13}\text{C}$ and intermediate $\delta^{15}\text{N}$ values. Hence, region of origin classification using support vector machines were more successful for observations from these regions. By applying discriminant analysis, 95% and 90% of the observations, for regions CK, HK, NK, BL and RU, for the estimation and validation (Karoo vs. Non-Karoo classification) were correctly classified, respectively. However, it is also evident that to increase the discriminative power a larger samples size per region is needed. Furthermore, to strengthen the geographical resolution future studies should include the analysis of additional isotopes such as, hydrogen, oxygen and sulphur. Overall the results confirm the importance and potential of isotopic fingerprints for the authentication of South African lamb.

7.5 References

Acocks, J. P. H. (1988). Veld types of South Africa. In: *Memoirs of the Botanical Survey of South Africa*. 3rd ed. Vol. 57.

- Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S. D., Schlicht, C., Thomas, F., Monahan, F. J., Hoogewerff, J. & Rossmann, A. (2007). Multi-element (H, C, N, S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry*, **389**, 309-320.
- Chiocchini, F., Portarena, S., Ciolfi, M., Brugnoli, E. & Lauteri, M. (2016). Isoscapes of carbon and oxygen stable isotope compositions in tracing authenticity and geographical origin of Italian extra-virgin olive oils. *Food Chemistry*, **202**, 291-301.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Codron, D., Codron, J., Sponheimer, M., Lee-Thorp, J. A., Robinson, T., Grant, C. C. & De Ruiter, D. (2005). Assessing diet in savanna herbivores using stable carbon isotope ratios of faeces. *Koedoe*, **48**(1), 115-124.
- Crawford, K., McDonald, R. A. & Bearhop, S. (2008). Applications of stable isotope techniques to the ecology of mammals. *Mammal Review*, **38**, 87-107.
- Department of Agriculture, Forestry and Fisheries (DAFF). (1990). *Product Standards Act* (Act No.119 of 1990, No. R. 342). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2000). *Meat Safety Act* (Act No.40 of 2000, No. 1072). Pretoria, South Africa: Government Printer.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2006). *Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa*. Government Gazette of 1 September 2006, No. R. 863. Pretoria, South Africa: Government Printer.
- Devincenzi, T., Delfosse, O., Andueza, D., Nabinger, C. & Prache, S. (2014). Dose-dependent response of nitrogen stable isotope ratio to proportion of legumes in diet to authenticate lamb meat produced from legume-rich diets. *Food Chemistry*, **152**, 456-461.
- Du Toit, P. C. V. (1998). Diets selected by Merino and Dorper sheep in Karoo veld. *Archivos de Zootecnia*, **47**, 21-32.
- Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. Cape Town, South Africa: Briza Publications.
- Gibson, D. J. (2009). *Grasses and Grassland Ecology*. Oxford, USA: Oxford University Press.
- Heaton, K., Kelly, S. D., Hoogewerff, J. & Woolfe, M. (2008). Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis. *Food Chemistry*, **107**, 506-515.
- Horacek, M. & Min, J.-S. (2010). Discrimination of Korean beef from beef of other origin by stable isotope measurements. *Food Chemistry*, **121**, 517-520.

- Kelly, S., Heaton, K. & Hoogewerff, J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends in Food Science & Technology*, **16**, 555-567.
- Lee, C. M., Trevino, B., & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC International*, **79**(2), 487-492.
- Luo, D., Dong, H., Luo, H., Xian, Y., Wan, J., Guo, X. & Wu, Y. (2015). The application of stable isotope ratio analysis to determine the geographical origin of wheat. *Food Chemistry*, **174**, 197-201.
- Perini, M., Camin, F., Bontempo, L., Rossmann, A. & Piasentier, E. (2009). Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions. *Rapid Communications in Mass Spectrometry*, **23**, 2573-2585.
- Piasentier, E., Valusso, R., Camin, F. & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science*, **64**, 239-247.
- Rees, G., Kelly, S. D., Cairns, P., Ueckermann, H., Hoelzl, S., Rossmann, A. & Scotter, M. J. (2016). Verifying the geographical origin of poultry: The application of stable isotope and trace element (SITE) analysis. *Food Control*, **67**, 144-154.
- Rodrigues, C. I., Maia, R., Miranda, M., Riberinho, M., Nogueira, J. M. F. & Máguas, C. (2009). Stable isotope analysis for green coffee bean: a possible method for geographic origin discrimination. *Journal of Food Composition and Analysis*, **22**, 463-471.
- Rossmann, A. (2001). Determination of stable isotope ratios in food analysis. *Food Reviews International*, **17**(3), 347-381.
- Sandberg, P. A., Loudon, J. E. & Sponheimer, M. (2012). Stable isotope analysis in Primatology: a critical review. *American Journal of Primatology*, **74**(11), 969-989.
- Shapiro, S. S. & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Sun, S., Guo, B., Wei, Y. & Fan, M. (2011). Multi-element analysis for determining the geographical origin of mutton from different regions of China. *Food Chemistry*, **12**, 1151-1156.
- Vogel, J. C. (1978). Isotopic assessment of the dietary habitats of ungulates. *South African Journal of Science*, **74**, 298-301.
- Walter, H. & Breckl, S.-W. (1986). The Karoo in South Africa. In: *Ecological Systems of the Geobiosphere 2: Tropical and Subtropical Zonobiomes*. p. 322. Berlin, Germany: Springer-Verlag.
- Warriss, P. D. (2010). The growth and body composition of animals. In: *Meat Science: An Introductory Text*. 2nd ed. p. 11. Wallingford, UK: CABI Publishing.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Witt, G. B., Moll, E. J., Beeton, R. J. S. & Murray, P. J. (1998). Isotopes, wool, and rangeland monitoring: let the sheep do the sampling. *Environmental Management*, **22**(1), 145-152.

Chapter 8

Proton transfer reaction-mass spectrometry (PTR-MS) for the authentication of regionally unique South African lamb⁵

Abstract

The volatile fingerprints of South African lamb meat and fat were measured by proton transfer reaction-mass spectrometry (PTR-MS) to evaluate it as a tool for the authentication of origin and feeding regime. Meat and fat of the *Longissimus lumborum* (LL) of lambs from six different regions were assessed. Analysis showed that the volatile fingerprints were affected by the origin of the meat. The classification of the origin of the lamb was achieved by examining the calculated and recorded fingerprints in combination with chemometrics. Four different partial least square discriminant analysis (PLS-DA) models were fitted to the data to classify lamb meat and fat samples into “region of origin” (six different regions) and “origin” (Karoo vs. Non-Karoo). Performance of the models was assessed by external validation. The estimation models classified samples 100% correctly. However, validation of the first two models gave only 42% (fat) and 58% (meat) correct classification of region. Whereas, the validation results of the second two models were better with 92% (fat) and 83% (meat) correct classification of origin.

Keywords: Extensive grazing; Geographical origin; Lamb fat; Lamb meat; PTR-MS

8.1 Introduction

Verifying the authentic nature and origin of food products is becoming increasingly important as consumers want to know the source and production method of their products (Grunert, 2005). This is particularly important for food products sold at a premium price as a result of quality characteristics associated with it. Some of these qualities may arise through distinct or traditional production methods and/or region of origin. Internationally, the small ruminant sector is currently giving attention to the labelling and branding of specific sheep meat (Rubino *et al.*, 1999). For example, *Patagonian lamb* (Argentina), *Northumbrian lamb* (UK), *Texelse Lambsham* (the Netherlands), *Cheviot lamb* (Wales), *Ronaldsday lamb* (Scotland), *Agneau Fermier du Quercy* (France), *Ternasco de Aragon* (Spain) and *Agnello della Maremma* (Italy) (Rubino *et al.*, 1999). In South Africa, most of the lamb produced in the Northern parts (or specifically the Karoo region) of the country is known as *Karoo lamb* (Weissnar & Du Rand, 2012). Consumers appreciate the meat for its unique sensory quality (e.g. herbaceous aroma and flavour attributes), attributable to the diet of the sheep, which mainly consist of the indigenous, herbaceous Karoo bushes and shrubs (Estler *et al.*, 2006). These plants are

⁵ Part of this work presented as a poster presentation: Erasmus, S. W., Muller, M., Alewijn, M., Koot, A., Van Ruth, S. M. & Hoffman, L. C. (2016). The sensory and chemical profiling of South African lamb: Evidence for its authenticity, presented at the 1st *Postgraduate Symposium on Food Fraud* hosted by Wageningen UR (University and Research), 23-24 June 2016, Wageningen, the Netherlands. Awarded 2nd best overall.

believed to function as a natural herb or spice, enhancing the overall palatability of the meat. As a result of the quality and value associated with Karoo lamb, there is a risk that the name may be misused by entities not even remotely linked to the region. Another concern is lamb sold as Karoo lamb when in actual fact it had been produced in a feedlot or a Non-Karoo region. Similar to Karoo lamb, other characteristic sheep production sites also exist. In the Overberg (southern seaboard) region of the Western Cape, lucerne/alfalfa (*Medicago sativa*) is typically cultivated and used as feed for sheep, while small grain stubble may also form part of the diet (Cloete & Olivier, 2010). The lamb produced within this region is known as “Rûens lamb”, where the typical diet of the sheep associated with the region and traditional farming practises gives the lamb meat its unique sensory qualities. It is vital that an analytical method for the authentication of South African lamb is developed. Not only would such a method be able to distinguish Karoo from Non-Karoo lamb, but it would also provide scientific evidence to verify the unique nature of the product.

Previous research of descriptive sensory analysis (Chapter 3, Erasmus *et al.*, 2016a) and stable isotope ratio analysis (Chapter 5, Erasmus *et al.*, 2016b), using a completely different sample set, revealed distinct sensory and isotopic differences of lamb meat obtained from different farms within the mentioned regions. The differences were related to diet linked to the origin. However, a limitation is that these methods can be time-consuming and expensive. Therefore, the need exists to explore the use of other analytical techniques to verify the origin and authentic nature of the meat. The use of near-infrared spectroscopy (Chapter 6) has already shown the potential for authenticating lamb meat using a rapid and portable technique.

Recent technological advances have brought forth the development of efficient and fast food authentication methods. One such method, which is relatively new in the field, is the use of proton transfer reaction-mass spectrometry (PTR-MS). PTR-MS is an emerging technique that allows direct (no pre-treatment of the sample required), fast (less than 1 min for a complete mass spectrum) and sensitive (at ppt level) monitoring of volatile organic compounds (VOCs) (Lindinger *et al.*, 1998; Biasioli *et al.*, 2011). Where VOCs are at the origin of both the aroma and flavour of food, and in the case of Karoo lamb possibly linking the meat with its origin. PTR-MS measures the VOCs in the headspace of a sample and combines the information to produce a single VOC fingerprint of the sample. The similarity of fingerprints or profiles between products can then be used to classify them into different categories. Recently, this method of authenticating food products has been achieved with several studies where products ranged from Dutch cheese (Galle *et al.*, 2011) to different fats used in animal feeds (Van Ruth *et al.*, 2010), olive oils of varying origin (Araghipour *et al.*, 2008) and pork meat of conventional, free-range and organic origin (Oliveira *et al.*, 2015).

There are currently no published results regarding the use of PTR-MS for the purpose of authenticating South African lamb. It is important to determine whether lamb from different regions can be distinguished from one another based on its volatile profile as dietary differences linked to the variation in vegetation within the regions is expected. Another crucial aspect of the study is to verify the direct link between the herbaceous plants and the lamb meat or fat. The presence of characteristic volatiles (such as terpenes) which connects the plants with the meat or fat may serve as validation for the unique sensory quality of Karoo lamb. Lambs

from farms within the Northern Cape and Western Cape provinces of South Africa and lambs obtained from semi-extensive grazing conditions were included in the study. The aim of the current work was to investigate PTR-MS as a potential tool for rapid and effective classification of lamb meat and fat. The work is also the first of its kind, investigating the use of PTR-MS for the authentication of regionally unique lamb.

8.2 Materials and methods

8.2.1 Experimental layout and study regions

Five regions, each unique in terms of its vegetation and the extensive grazing conditions, were selected for the purpose of the study (Table 8.1). See General introduction (Chapter 1, Fig. 1.4) for the selected regions. Four regions were from the Northern Cape province, while the other was from the Western Cape province. Lambs obtained from farms with semi-extensive grazing conditions were also included. Three slaughter ready lambs were sourced from each farm, where the number of farms per region ranged from 6 to 15 (Table 8.1).

Table 8.1 Regions selected for this study on PTR-MS

Region of origin	Code	Description of typical extensive diet	Number of farms	Number of animals
Central Karoo	CK	Shrubs/bushes and grass	15	45
Hantam Karoo	HK	Shrubs/bushes and grass	12	36
Northern Karoo	NK	Shrubs/bushes and grass	7	21
Bushmanland	BL	Shrubs/bushes and grass	7	21
Rûens	RU	Lucerne/alfalfa	12	36
Semi-extensive	SE	Shrubs/bushes, grass and concentrates	6	18
Total			59	177

(PTR-MS) Proton transfer reaction-mass spectrometry.

8.2.2 Selected regions

The arid Karoo ecotype is predominantly located within the Northern Cape province. It is described as having limited cropping potential due to the low annual rainfall (Palmer & Ainslie, 2005; Cloete & Olivier, 2010). Hence, the land is utilised for livestock production where sheep farming is practised in 82% of the province (Cloete & Olivier, 2010). The Karoo features a variety of different vegetation types and is made up of different biomes which form the sub-regions for this study (Acocks, 1988; Estler *et al.*, 2006). The four selected for this study are known as the Central Karoo (CK), Hantam Karoo (HK), Northern Karoo (NK) and Bushmanland (BL). It is widely argued whether some of these should be included as part of the Karoo and whether the lamb produced from these regions can be classified as Karoo lamb. HK lamb mainly comprise the unique Karoo bushes of the Karoo region and fall within the succulent Karoo biome, while CK, NK and BL fall in the Nama-Karoo biome,

comprising a combination of Karoo bush and savanna-type or bushman grasses (Estler *et al.*, 2006). The other selected region, known as the Rûens (RU), lies in the southern part of the country. Here lambs are typically raised on lucerne (*Medicago sativa*) pastures, however, depending on season, lamb may also be raised on stubble after the grain harvesting period (usually from December to February) (Cloete & Olivier, 2010). For this study lambs were raised on lucerne.

In addition to the above-mentioned extensive enterprises, semi-extensive sheep farming is also practised. Lambs are provided with additional feed (i.e. concentrates) when they are not grazing in the veld. This system has less impact on the veld, allowing more time for the plants to recover from grazing and reduces the chances of over-grazing and erosion. In times of drought this system is largely used by Karoo farmers.

8.2.3 Plants

Sixteen typical plant species consumed by sheep were collected from the Karoo region. These ranged from the fragrant Karoo bushes/shrubs to the dry, savanna-type grasses. The plant samples were dried at 60°C to a constant weight. After which they were ground to a fine powder with a pestle and mortar and a benchtop mill (1095 Knifetec™, Höganäs, Sweden). The plant samples collected are listed and described in Table 8.2.

8.2.4 Sample collection

Lambs were slaughtered according to standard South African procedures and the meat samples collected from the carcasses as described in Chapter 6. In total, 177 lambs from 59 farms were used for the study. Twenty-four hours after slaughter and refrigeration at 4°C, meat steaks (1.5-2 cm thick) were cut perpendicular to the grain of the left *Longissimus lumborum* muscle of the carcass at the L₂₋₃ position (2nd to 3rd lumbar vertebrae). The steaks were vacuum packed and stored at -20°C in absence of light until the analyses were conducted.

8.2.5 Sample preparation

Before the day of analysis, the meat samples were placed in a refrigerator and left to defrost for 24 h at 4°C. The subcutaneous fat was removed and ground to a fine powder with the use of liquid nitrogen and a Grindomix (GM 200, Retsch, Düsseldorf, DE, Germany) whilst the meat samples were cut finely and analysed using PTR-MS. The fat samples of the three animals per farm were pooled as one sample, representative of the single farm. It was decided to pool the fat samples as the PTR-MS analysis on the meat samples showed little variation between animals obtained from the same farm (data not shown). The powdered fat samples were used directly from the -20°C freezer on the day of analysis.

Table 8.2 Details of plant species analysed to determine their volatile fingerprints using PTR-MS

Species name	Code	Common name	Description and palatability ^a
<i>Pentzia incana</i>	Pentzia1	Ankerkaroo (Anchor karoo)	Perennial dwarf shrub 200-250 mm tall. Palatability varies between regions. Abundant and vital part of sheep's diet.
	Pentzia2		
	Pentzia3		
<i>Plinthus karrooicus</i>	Plinthus1	Silwerkaroo (Silver karoo)	Perennial dwarf shrub 100-300 mm tall. Very palatable, drought-resistant and presence indicative of valuable veld.
<i>Salsola glabrescens</i>	Salsola1	Rivierganna	Shrub with woody stems, up to 1.5 m tall. Palatable and grazed less when it occurs in homogenous stands.
<i>Salsola calluna</i>	Salsola2	Pienkloodganna	Shrublet 300 mm tall. Palatable and grazed by all types of animals.
<i>Rosenia humilis</i>	Rosenia1	Rivierleegtebos or Perdekaroo	Perennial woody dwarf shrublet 400 mm tall. Not very palatable but provides feed when most other plants have been grazed, drought resistant. Young leaves have pleasant pine aroma.
<i>Pteronia glauca</i>	Pteronia1	Boegoekaroo	Perennial woody dwarf shrublet 300-600 mm tall. Palatable and eaten by sheep.
<i>Eriocephalus ericoides</i>	Eriocephalus1	Kapokbossie (Wild Rosemary)	Perennial woody shrublet up to 1 m tall. Palatability varies between regions. Flower heads turns sheep lips black.
<i>Eriocephalus punctulatus</i>	Eriocephalus2	Boegoekapokbossie	Shrublet up to 1 m tall. Palatability varies between regions.
<i>Stipagrostis ciliata</i>	Stipagrostis1	Langbeen boesmangras (tall Bushman grass)	Perennial arid-climate grass up to 1 m tall. Very palatable and grazed down short.
<i>Stipagrostis obtusa</i>	Stipagrostis2	Kortbeen boesmangras (small Bushman grass)	Perennial arid-climate grass up to 500 mm tall. Very palatable and grazed down short.
<i>Themeda triandra</i>	Themeda1	Rooigras (Red grass)	Perennial tropical grass up to 1.5 m tall and prevalent in average to high rainfall areas. Very palatable and good grazing grass.
<i>Cynodon dactylon</i>	Cynodon1	Kweekgras (Bermuda grass)	Perennial grass up to 400 mm tall. Palatable and average to good grazing grass.
<i>Fingerhuthia africana</i>	Fingerhuthia1	Vingerhoedgras (Thimble grass)	Perennial warm-climate grass up to 900 mm tall. Palatable and average grazing grass.
<i>Aristida congesta subsp. congesta</i>	Aristida1	Steekgras (Tassel three-awn)	Perennial warm-climate grass up to 900 mm tall. Palatable and average grazing grass

(PTR-MS) Proton transfer reaction-mass spectrometry; ^a DAFF, 2014; Le Roux, Kotzé, Nel, & Glen, 1994; Manning & Goldblatt, 1997; Shearing, 1994; Van Oudtshoorn, 2012.

8.2.6 PTR-MS analysis

This technique allows the analysis of volatile compounds through protonation with H_3O^+ ions (generated in a hollow cathode ion source), analysing the mass in a mass spectrometer and detection as ion counts/s (cps) by a secondary electron multiplier (Lindinger *et al.*, 1998). The mass resolved fingerprint of the volatile profile can then be used for classifying samples according to their origin.

Preliminary studies were performed to determine the amount of sample, equilibration time and temperature to use for the analysis. Five grams of finely cut meat or powdered fat were placed in a 250 mL glass bottle (Schott Duran, Germany) which were equilibrated by being immersed in a water bath (water constantly agitated at 60 rpm) at 30°C for 30 min. Samples were randomly analysed in duplicate using a high sensitivity PTR-MS instrument (Ionicon Analytik, Innsbruck, Austria). The sample bottles were connected to the PTR-MS inlet flow, which was heated to 60°C, and the headspace air drawn at a flow rate of 55 mL/min, 32 mL/min of which was led into the PTR-MS via Teflon (0.25 mm) tubing. A constant drift voltage of 600 V and a pressure of 2.19 ± 0.01 mbar were maintained in the reaction chamber. A quadrupole mass spectrometer was used to analyse the masses, which were detected as ion counts per second (cps) using a secondary electron multiplier (SEM). The mass ion intensities were converted to volume mixing ratio (ppbv) values according to Lindinger *et al.* (1998). Mass spectral data were collected over the mass range m/z 20-160 using a dwell time of 200 ms and five cycles (1-5). The average of cycles 2-4 for each replicate sample was used for data analysis. Preliminary experiments showed stabilisation of the signal in these cycles. Hence, a reliable measurement of VOCs concentration is obtained. Blank sample (empty bottle) measurements of the background air for five cycles were made before the analysis of each sample with the average signal being subtracted from that of the sample spectra (Aprea *et al.*, 2007). The mass spectra of the duplicate measurements were averaged to obtain a mean mass spectrum for each sample. The plants samples were analysed according to the method of Nenadis *et al.* (2016). Plant samples were analysed in duplicate, 35 mg used per single analysis, over the mass range m/z 20-200 and allowed to equilibrate in a water bath at 25°C for 30 min. The following masses associated with the PTR-MS ion source were removed from the dataset: m/z 32 (O_2^+), m/z 37 and m/z 38 (major water cluster ions).

8.2.7 Statistical analysis

Statistical analysis of data was performed using the GLM (General Linear Models) procedure of SAS™ statistical software (Statistical Analysis System, Version, 9.4, 2006, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and XLSTAT® statistical software (Version 2016.04.33113; Addinsoft, NY, USA) and Unscrambler X10.3 (Camo Software AS., Oslo, Norway) for the multivariate statistical analysis. For the lamb meat data, mean values for the three sheep per farm were calculated before ANOVA. Pre-processing of the data involved using the Shapiro-Wilk test to test for deviation from normality (Shapiro & Wilk, 1965). When the deviation from normality was significant ($P \leq 0.05$) the outliers in the data were identified and removed until the data was normalised or symmetrically distributed. Following the confirmation of normality of

the data, one-way ANOVA was performed on the concentration data of the individual ions, with region as factor, in order to determine significant differences between the origin of the lamb meat and fat. Farms were taken as random repetitions for the regions and individual animals as subsamples. Fisher's Least Significant Differences (LSD) was calculated at a 5% significance level to compare region means. A probability level of 5% was considered significant for all the significance tests. Statistical techniques were used to find significant patterns and associations in the collected data. Principal component analysis (PCA) and discriminant analysis (DA) was used to visualise sample grouping and associations, while the partial least square discriminant analysis (PLS-DA) was applied for classification of origin. As PTR-MS involves more variables than samples, DA is not suitable to use and a PCA-like reduction of the variables is required before classification. PLS-DA is the solution as it combines both aspects. PLS-DA is a supervised clustering technique and performs a dimension reduction on the predictor variables, where the dimensions (components) extracted show maximum correlation with Y (class membership, defined using dummy variables) (Van Ruth *et al.*, 2010). Two farms per region were randomly selected to form part of the external validation set. These samples were not included in the estimation model and hence, the performance of the PLS-DA model was evaluated using the validation set. In addition, various data pre-treatment methods (none, log transformation, centre-reduce) were examined before the final classification model was selected. Correlations were determined by means of the Pearson's correlation coefficient (r) (Snedecor & Cochran, 1980).

8.3 Results and discussion

8.3.1 Mass spectra

Volatile profiles of the meat, fat and plant samples were analysed by PTR-MS and the mass spectral data used as "fingerprints". The masses and their corresponding signal intensities (ppbv) served as a pattern for comparison between samples. Mean fingerprint mass spectra for lamb meat and fat of the six different regions (CK, HK, NK, BL, RU and SE) are displayed in Figure 8.1a and 8.1b, respectively. Mean fingerprint mass spectra for lamb meat and fat of the Karoo and Non-Karoo regions are displayed in Figure 8.2a and 8.2b, respectively. Volatile fingerprints for the plant samples are shown in Figure 8.3. The headspace concentrations of the mentioned figures are indicated on a log scale.

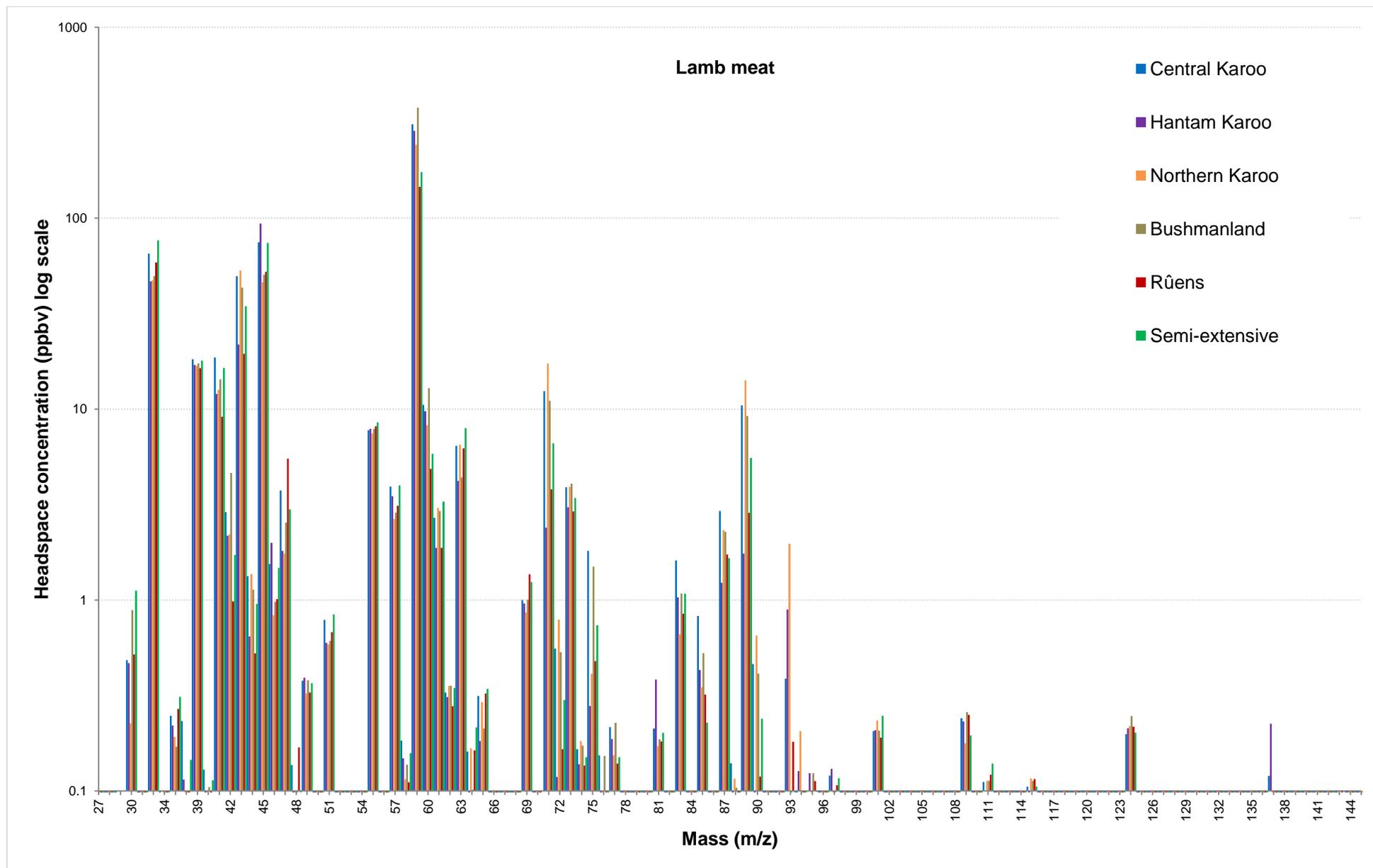


Figure 8.1a Mean fingerprint mass spectra for lamb meat of the different regions generated by proton transfer reaction-mass spectrometry (PTR-MS).

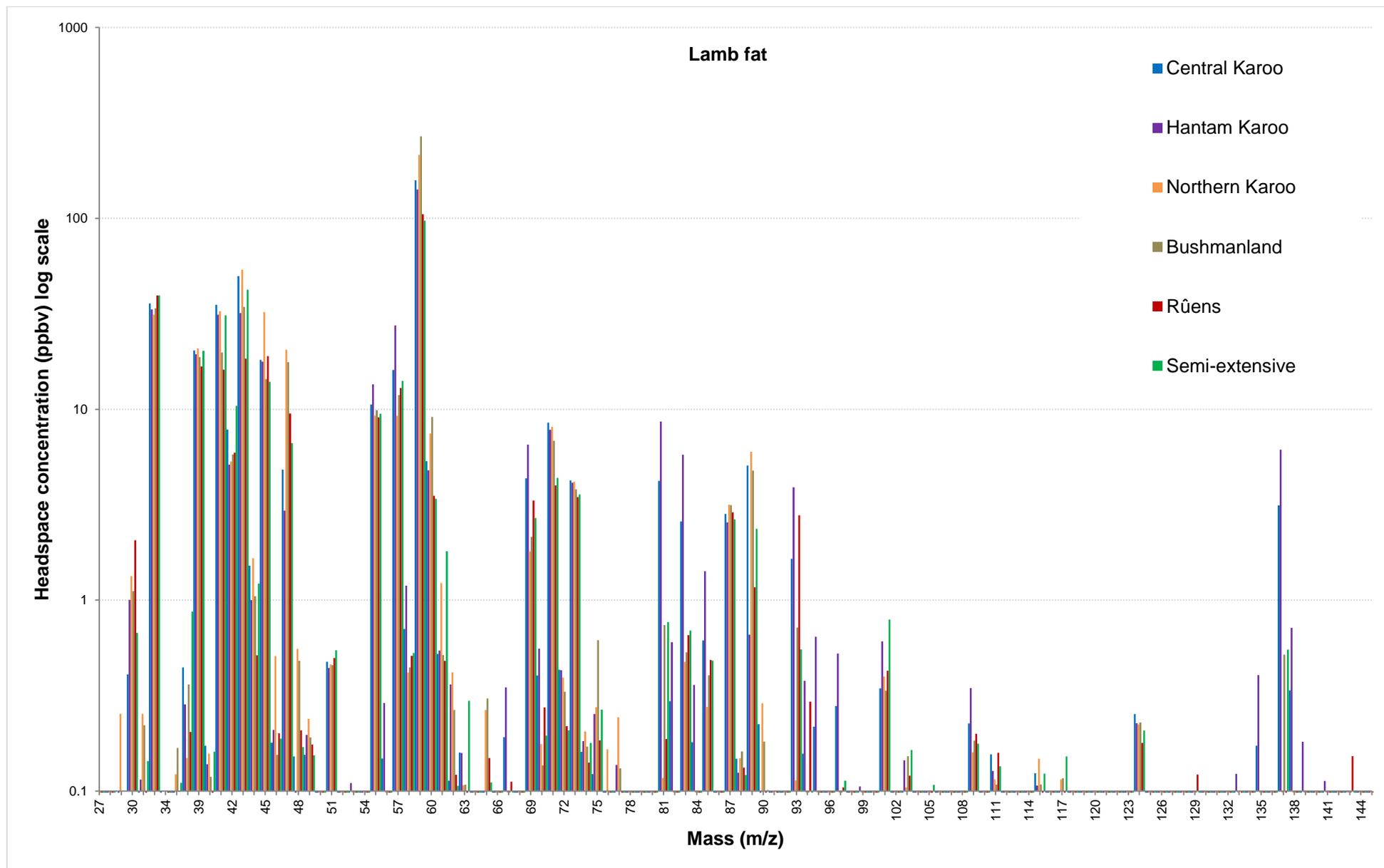


Figure 8.1b Mean fingerprint mass spectra for lamb fat of the different regions generated by proton transfer reaction-mass spectrometry (PTR-MS).

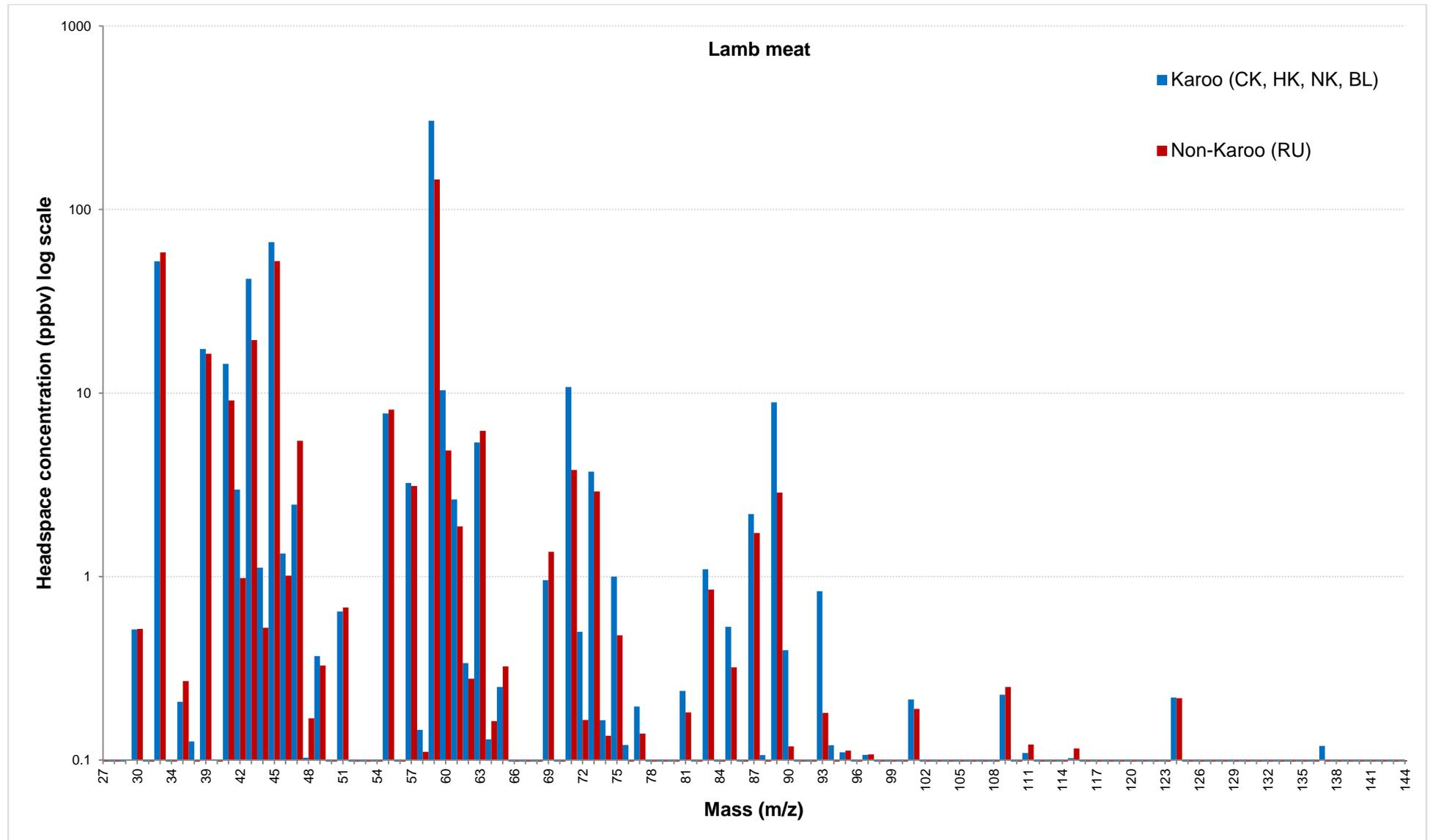


Figure 8.2a Mean fingerprint mass spectra for lamb meat of the Karoo and Non-Karoo regions generated by proton transfer reaction-mass spectrometry (PTR-MS). (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens.

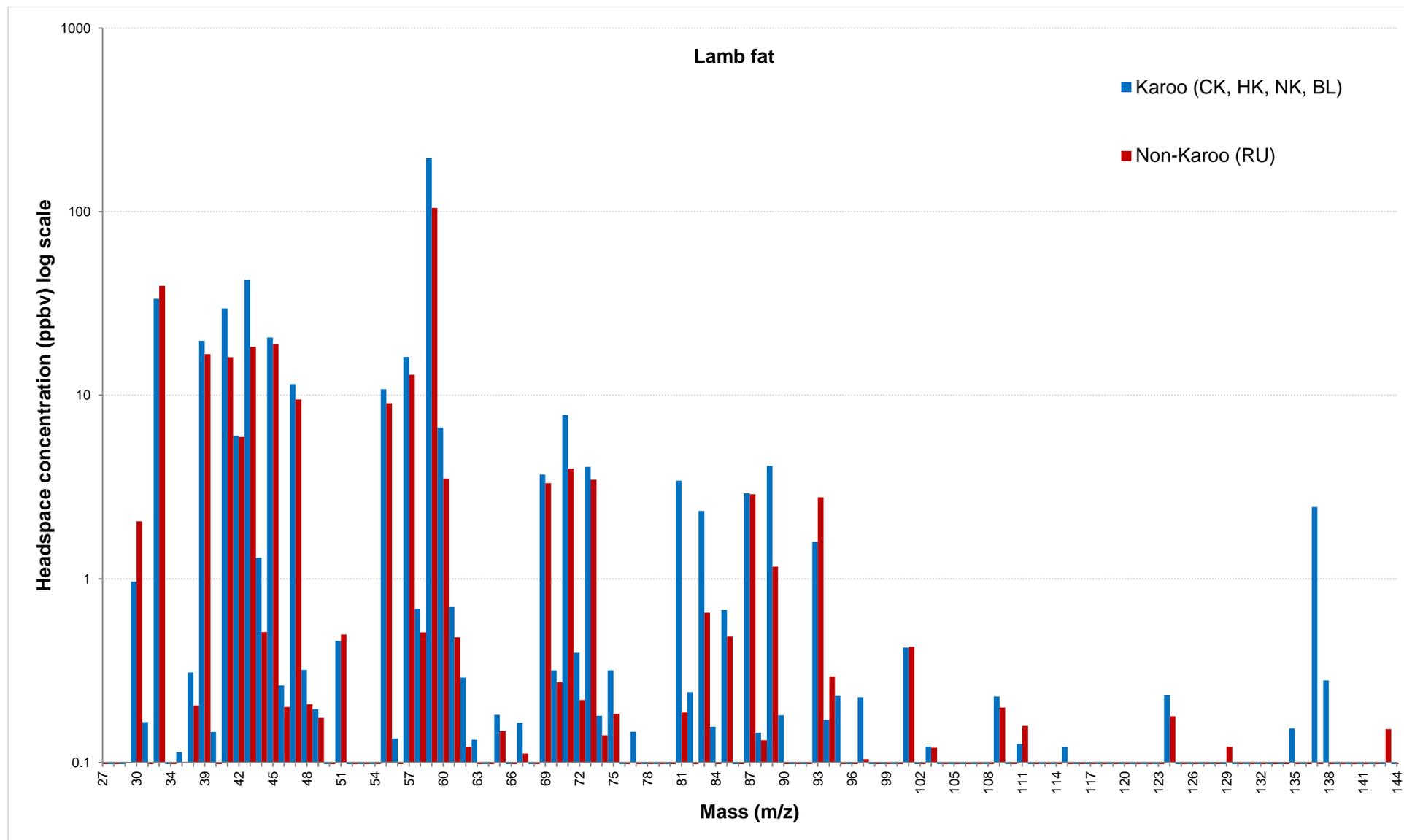
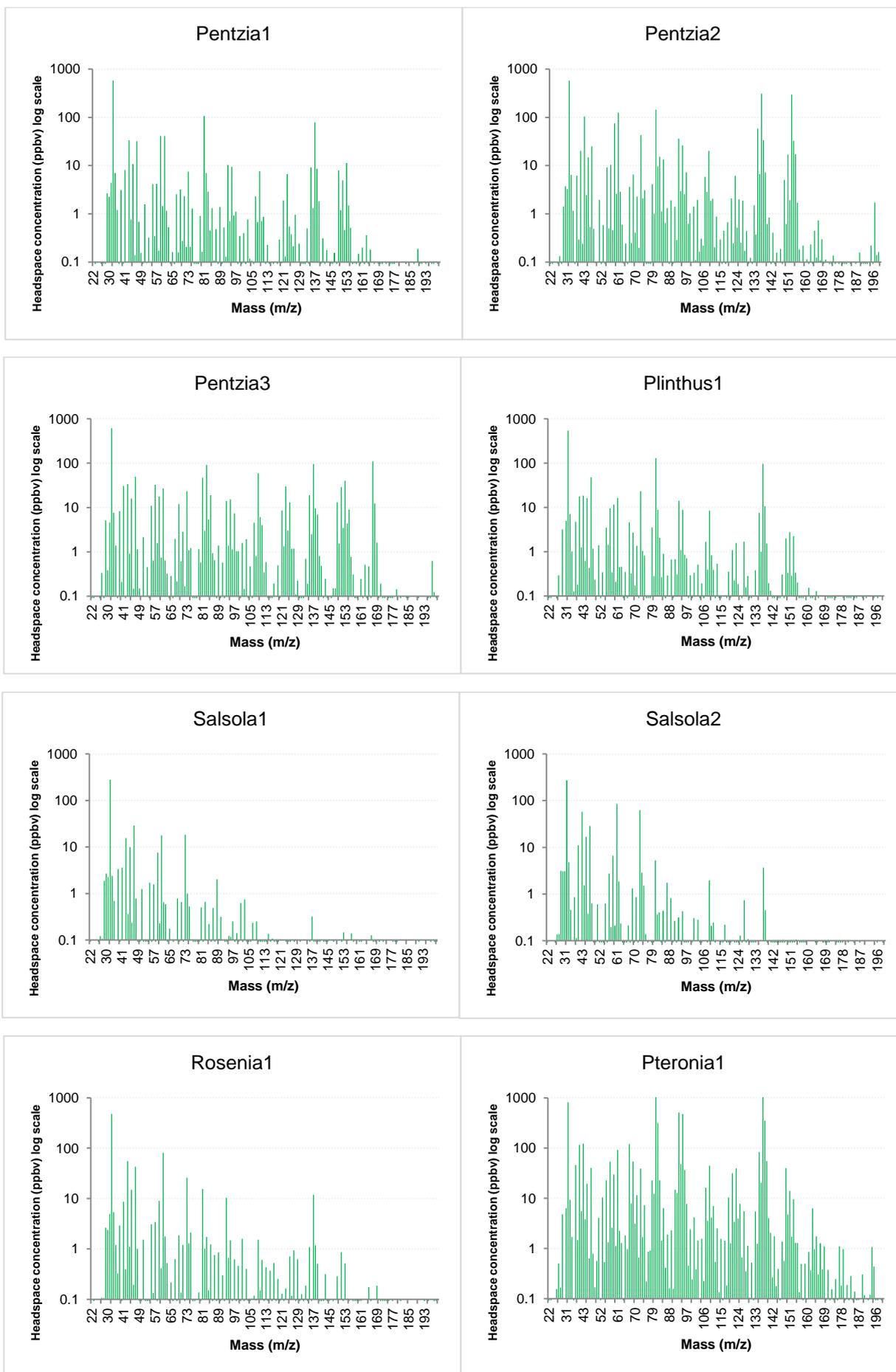


Figure 8.2b Mean fingerprint mass spectra for lamb fat of the Karoo and Non-Karoo regions generated by proton transfer reaction-mass spectrometry (PTR-MS). (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens.



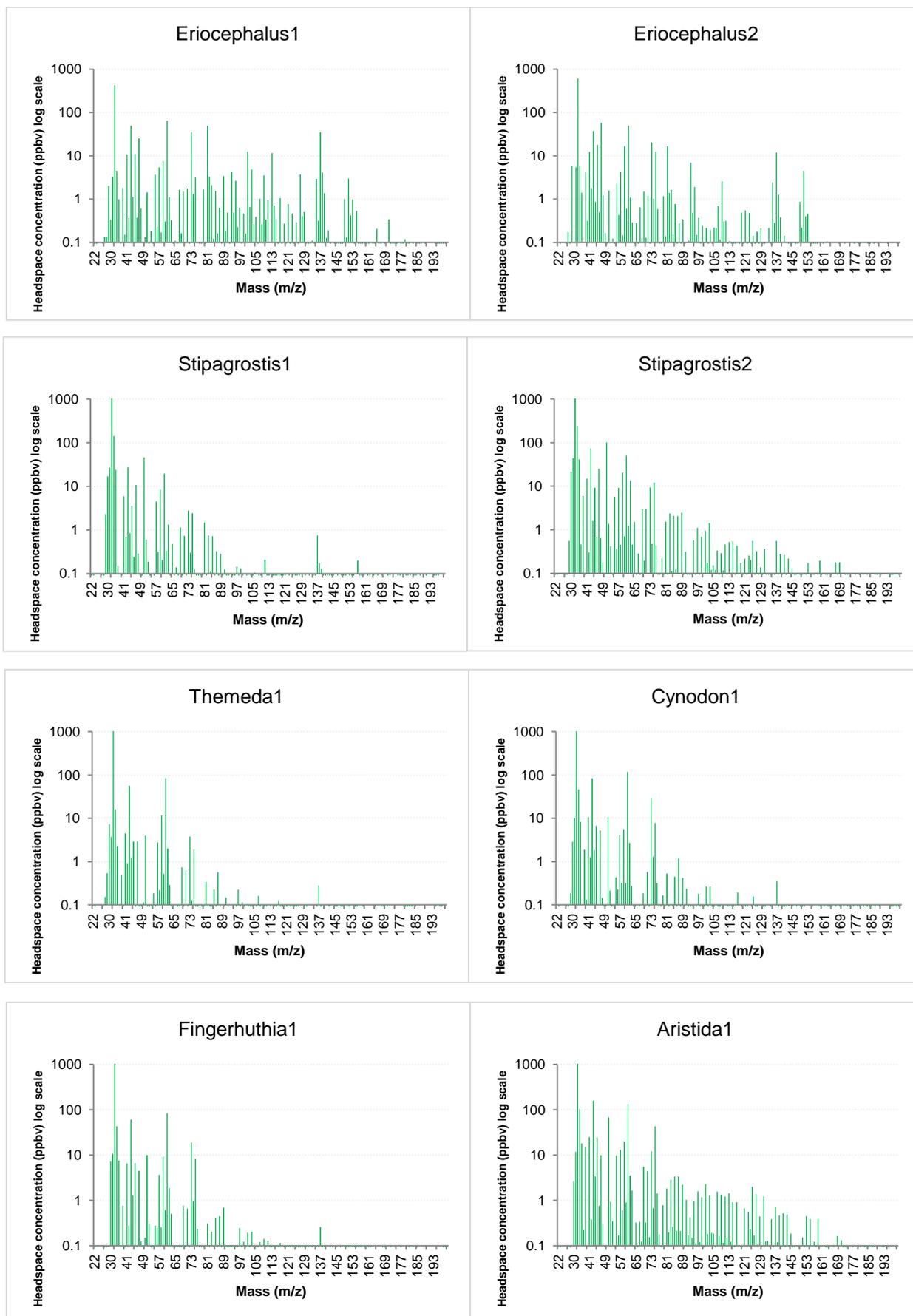


Figure 8.3 Volatile fingerprints of the plant samples generated by proton transfer reaction-mass spectrometry (PTR-MS).

The volatile compounds with higher volatility (lower mass) are abundant in the spectra, although lower masses also exist due to the fragmentation of larger compounds. The mass spectra of the plant samples showed many masses with high abundancies, demonstrating their complex VOC composition. Differences between spectra of the same plant species is also evident, suggesting some variation in their chemical composition due to geographical origin, seasonal effects, climatic conditions and the amount and part of the plant used. Through visual evaluation of the fingerprints, it is evident that the herbaceous plants (*Pentzia incana*, *Plinthus karrooicus*, *Rosenia humilis*, *Pteronia glauca* and *Eriocephalus spp.*) contains a greater amount of volatile compounds. This is particularly evident in the terpene regions (m/z 81, 93, 95, 135 and 137) (Tani *et al.*, 2003; Maleknia *et al.*, 2007). Terpenes are VOCs found in plants and provides a characteristic aroma. The herbaceous plants used in this study were previously analysed by a sensory panel as having the following aroma descriptors: eucalyptus, camphor, pine, thyme, camomile, sage-like, medicinal, lavender, rosemary, woody, floral and minty (Table 3.3, Chapter 3). Hence, the complex VOC composition was expected due to the herbaceous nature of the plants. However, an important question is whether or not these terpenes can be detected in the lamb meat and fat as it has been found that a diet of fragrant Karoo plants provides the meat and fat with an herbaceous sensory profile (Erasmus *et al.*, 2016a). Vasta *et al.* (2013) found that dietary supplementation with the essential oils of rosemary (*Rosmarinus officinalis*) and artemisia (*Artemisia herba alba*) did not affect lamb meat VOC profile, yet two sesquiterpenes (i.e. copaene and β -caryophyllene) were only detected in the supplemented samples.

In the fat samples, only the Karoo samples contained the ions m/z 77, 97, 135, 137 and 138, and had the highest concentration of the ions m/z 41, 43, 59, 71, 81, 83 and 89 (Fig. 8.2). However, for lamb meat the signals were much smaller between the two classes (Fig. 8.2). The ion m/z 137 was again only present for the Karoo samples with the ions m/z 43, 59, 71, 75, 89 and 93 having the highest concentrations (Fig. 8.2). Even though there are currently no lamb meat studies to compare the PTR-MS data, the probable identity of these ions are provided in Table 8.3. According to literature, compounds with mass ratios of m/z 81 and 137 are mostly identified as monoterpenes and largely derived from plants (Maleknia *et al.*, 2007). The typical aromas of these monoterpenes are described in literature as pine, camphor, fruity, citrus, lemon, orange, balsamic, spice, flower, lavender and turpentine (Calkins & Hodgen, 2007; Xiao *et al.*, 2016), relating to the descriptors of the herbaceous plants mentioned earlier. They are also the main chemical components of the essential oil of common herbs and spices, such as oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum*) (Viuda-Martos *et al.*, 2007). The fragrant Karoo bush, *Eriocephalus ericoides*, is generally known as “wild rosemary” due to its characteristic aroma (Table 8.2). Thus, one can make the assumption that the monoterpenes [i.e. α -pinene, camphor, 1,8-cineole (better known as Eucalyptol) and camphene] determined for rosemary (Viuda-Martos *et al.*, 2007), are likely to form part of its chemical composition. Other important mass ratios, as a result of monoterpenes, are m/z 93, 95, and 135 (Table 8.3). Hence, there is a likely link between the herbaceous Karoo bushes and the lamb samples obtained from these regions. Especially since

the Non-Karoo samples (RU) did not or hardly contained any of these volatiles (in view of the detected ions) and they also did not consume any of the herbaceous Karoo plants. However, one should carefully examine the results and take into account the fragmentation of monoterpene molecules.

The results show that m/z 135 was only detected for CK and HK in lamb fat (Fig. 8.1b). The mass is likely that of the protonated molecule of *p*-cymene according to Maleknia *et al.* (2007). Its most abundant fragment ion is observed at m/z 93 (Maleknia *et al.*, 2007), which is also seen in Figure 8.1b and 8.2b for lamb fat. Yet, RU also had a high concentration of m/z 93 without m/z 135 (being *p*-cymene) detected. This could mean that m/z 93 is a protonated molecule or fragment ion of another type of volatile, such as toluene (Maleknia *et al.*, 2007). For lamb meat, the same was seen although the Karoo samples had a higher concentration (NK>HK>CK>RU) of m/z 93 compared to the Non-Karoo samples (Fig. 8.1a and 8.2a). Therefore, the detection of a single ion should not be directly linked to the presence of a certain volatile compound as PTR-MS does not differentiate between the types of monoterpenes and their isomers as ions (Maleknia *et al.*, 2007). Although the RU lambs did not consume Karoo plants, they were extensively raised on lucerne pastures where the presence of terpenes could not be ruled out.

Figure 8.1 shows that the m/z 137 was present in the lamb meat of CK and HK, and in the lamb fat of CK, HK, BL and SE. This mass is likely the protonated molecules of camphene, 2-carene, limonene, β -myrcene, α -pinene, β -pinene, γ -terpinene, terpinolene, 1,8-cineole and/or linalool (Table 8.3). Accordingly, m/z 81 is also produced as fragment ions of the monoterpenes above (Fig. 8.1) (Maleknia *et al.*, 2007). A comparison of the mean intensities of the masses of Karoo and Non-Karoo lamb meat and fat is shown in Table S8.1 and S2 (Supplementary data). The results show that m/z 81 and 137 was significantly higher for HK meat (0.38 ± 0.35 ppbv and 0.23 ± 0.25 ppbv) and fat (8.63 ± 10.97 ppbv and 6.13 ± 7.82 ppbv) compared to the other samples (Table S8.1 and S2). Although m/z 81 and 137 was also higher for CK meat (0.21 ± 0.09 ppbv and 0.12 ± 0.06 ppbv) and fat (4.21 ± 3.85 ppbv and 3.13 ± 2.84 ppbv), the difference was not significant ($P > 0.05$) compared to the rest (Table S8.1 and S2). Similar results were seen for lamb fat of CK (2.59 ± 3.52 ppbv and 0.28 ± 0.24 ppbv) and HK (5.78 ± 6.82 ppbv and 0.53 ± 0.67 ppbv) for ratios m/z 83 and 97, respectively (Table S8.1 and S2). Out of the 136 masses, 66 differed significantly ($P \leq 0.05$) between regions for lamb meat (Table S8.1) and 90 for lamb fat (Table S8.2).

Table 8.3 Predominant ions (more than 1.0 ppbv) determined in PTR-MS analyses of the lamb meat and fat

Ions	Probable identity
30	aldehyde or imine ^g
33	methanol ^{c,d,e}
39	hexenyl acetate fragment ^f
41	alkyl fragment ^d ; hexanol fragment ^f
42	acetonitrile ^d
43	alkyl fragment ^d ; acetic acid ^{e,f} ; acetyl fragment ^e ; hexanol ^f ; 1-, 2- or 3-octanol fragment ^c
44	aldehyde or imine or amine ^g
45	acetaldehyde ^{d,e,f}
46 [#]	aldehyde or amine ^g
47	formic acid ^d ; ethanol ^{c,d,e,f}
55	butanal ^c
57	alkyl fragment ^d ; 2-propenal ^d ; butyl ^e ; hexanal ^f ; hexenal ^f ; hexanol ^f ; 1-, 2- or 3-octanol fragment ^c
58 [*]	imine or amine or azide ^g
59	2-propanone ^d ; acetone ^{e,f} ; propanal ^f ; hexenol fragment ^f
60	amine ^g
61	acetic acid ^{e,f} ; acetyl fragment ^e ; variety of esters (acetates) ^{c,f}
63 [#]	dimethyl sulfide ^f ; acetaldehyde hydrate ^f
69	1-octen-3-ol ^c ; isoprene ^{d,e} ; 3-hexen-2-ol ^d ; furan ^e ; pentanal ^{c,f} ; octanal ^c ; nonanal ^c
70 [*]	heterocyclic hydrocarbon (1H-1,2,3-triazole) or nitrile (propanenitrile) ^g
71	1-, 2- or 3-octanol fragment ^c
72	amine ^g
73	2-butanone ^{c,d,f} ; butanal ^{d,f}
75 [#]	methyl acetate ^{c,f} ; ethyl propionate ^c
81 ^a	terpene (fragment ion of: camphene; 2-carene; limonene; β -myrcene; α -pinene; β -pinene; γ -terpinene; terpinolene; 1,8-cineole; linalool) ^{d,e}
82 [*]	monoterpene fragment ^h
83	hexanal ^c ; 1,4-pentadien-3-one ^d
85	trans-2-methyl-butenal ⁱ ; trans-2-pentenal ⁱ
87	2,3-butanedione ^{c,d} ; hexanol ^{c,h} ; 2-pentanone ^{c,d} ; pentanal ^d
89	acetoin ^d ; butyric acid ^d ; butanoic acid ^f ; variety of esters (butyrates) ^{c,d,f}
90 [#]	N-hydroxy-N-methyl-acetamide ^g ; N-methoxy-acetamide ^g ; N,N-dimethyl-methanethioamide ^g
93	terpene (fragment ion of: <i>p</i> -cymene; α -pinene) ^e ; toluene ^{d,e}
94 [#]	2-, 3- or 4-methyl-pyridine ^g
95 [*]	terpene (fragment ion of: camphene; 2-carene; limonene; β -myrcene; α -pinene; β -pinene; γ -terpinene; terpinolene; 1,8-cineole; linalool) ^{d,e}
97 [*]	heptanal ^c
101 ^a	2-hexanone ^c
135	terpene (protonated molecule of: <i>p</i> -cymene) ^e
137 ^a	terpene (protonated molecule of: camphene; 2-carene; limonene; β -myrcene; α -pinene; β -pinene; γ -terpinene; terpinolene; 1,8-cineole; linalool) ^{d,e}
138 ^{b,*}	acid (3-aminobenzoic), ester or ketone ^g

(ppbv) parts per billion by volume; (PTR-MS) Proton transfer reaction-mass spectrometry; ^a More than 0.5, but less than 1.0 ppbv for lamb meat; ^b More than 0.5, but less than 1.0 ppbv for lamb meat and fat; [#] Predominant for lamb meat; ^{*} Predominant for lamb fat; ^c Buhr *et al.* (2002); ^d Galle *et al.* (2011); ^e Maleknia *et al.* (2007); ^f Van Ruth *et al.*, 2010; ^g Proton affinity list; ^h Asensio *et al.* (2007); ⁱ Spanel *et al.* (2002).

The fat samples showed an increase in the concentration of masses in the terpene regions and also showed distinct differences between regions, more so than the meat samples. In fact, when the mean concentration of m/z 81 and 137 is calculated to compare the total amount of terpenes in the lamb meat and fat (data not shown). The HK and CK fat contained 14.8 ± 1.77 ppbv and 7.3 ± 0.76 ppbv versus that of the meat being 0.6 ± 0.11 ppbv and 0.3 ± 0.07 ppbv, respectively. The higher content of volatiles in the fat compared to the meat is in keeping with previous sensory research on Karoo lamb where the fat was more discriminating in terms of its sensory quality (Erasmus *et al.*, 2016a). The reason for the increased discriminative power of the fat is likely due to the deposition of volatile compounds in the fat of animals (Berg & Walker, 2004; Vasta & Priolo, 2006). The volatile compounds can be deposited through ruminal fermentation or transferred directly from the forages eaten by the sheep (Vasta & Priolo, 2006). As a result, the accumulation of fat will have a more pronounced effect on the aroma and flavour of the meat. This is partly the reason why older sheep may have a different sensory profile compared to that of younger lambs (Berg & Walker, 2004). Through visual evaluation, it was noted that the meat steaks had a low level of marbling (i.e. intramuscular fat). This is due to the fact that intramuscular lipid deposition only occurs in later stages of growth and fat deposition (Warriss, 2010). This could also explain why the subcutaneous fat samples compared to that of the meat showed an increase in the concentration of masses in the terpene regions as these compounds are more prevalent in fat tissues. In addition, considering the important role of fat, one could expect that the meat of older sheep might have a better volatile fingerprint when analysed using PTR-MS. Overall the results already indicate the compounds which would likely be the strongest in discriminating the origin.

8.3.2 Groupings and associations

Principal component analysis (PCA) bi-plots which demonstrates the correlation between the 31 predominant ions for the lamb meat and 33 predominant ions for the lamb fat are provided as Supplementary data (Fig. S8.1). The discriminant analysis (DA) plots of the same data are provided in Figure 8.4. Principal component (PC) 1 and 2 explained 80.59% of the total variance for lamb meat and 68.28% for that of lamb fat (Fig. 8.4). Although not all of the variation is described, other intrinsic and extrinsic factors should also be kept in mind as they could have an influence on the volatile profile. The PCA bi-plots (Fig. S8.1) and DA plots (Fig. 8.4) shows that there are definite trends between the regions.

Along PC2 for lamb meat, the Karoo regions (HK, BL, NK and CK) grouped separately from the Non-Karoo, RU (Fig. 8.4). The same groupings were seen for lamb fat along PC1 (Fig. 8.4). However, for lamb meat there was a great deal of overlap between SE and CK which was not the case for lamb fat. Along PC2 for lamb fat, HK and CK grouped in the lower quadrants, while the NK, BL, RU and SE grouped in the upper quadrants (Fig. 8.4).

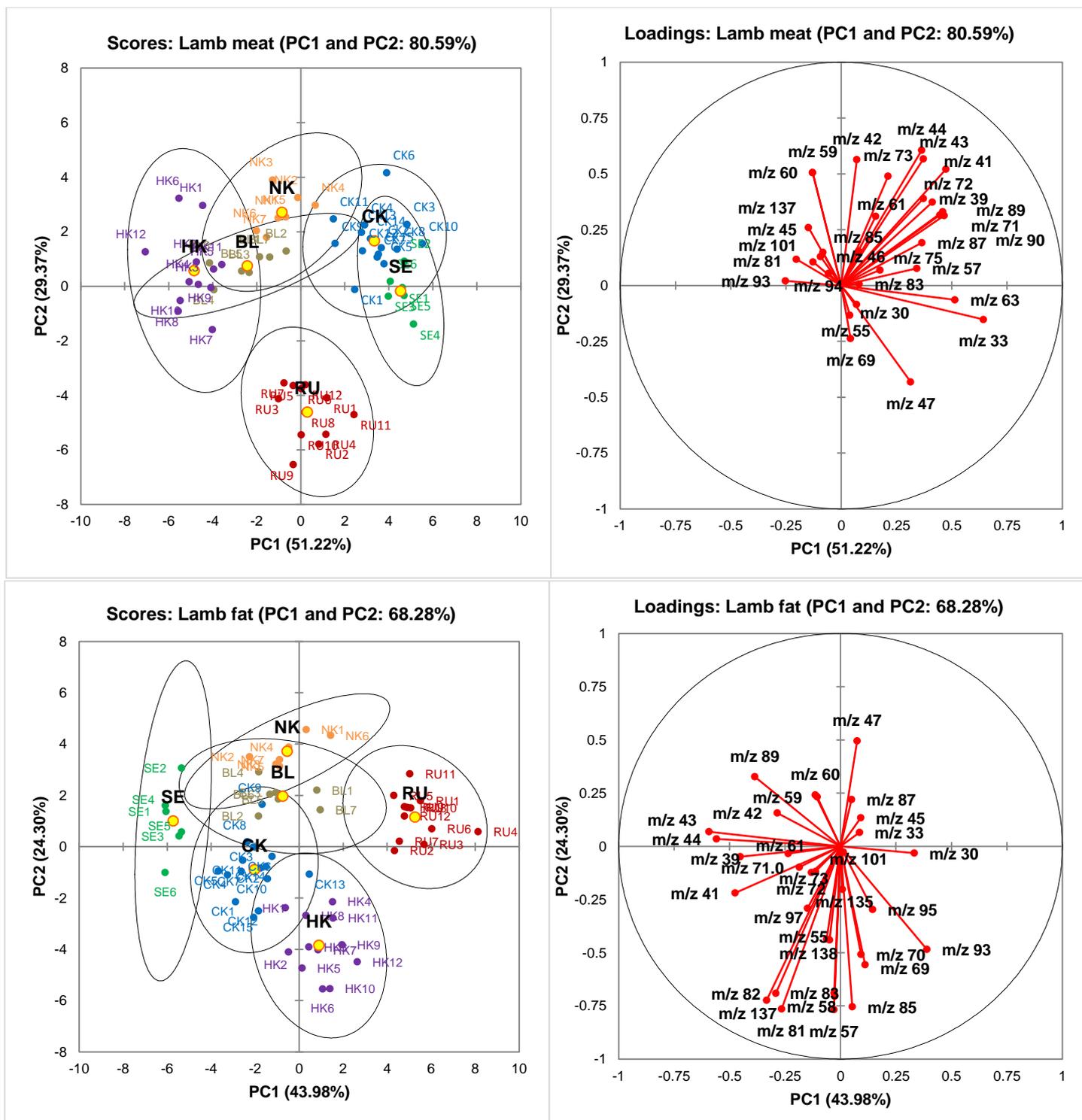


Figure 8.4 Discriminant analysis (DA) scores plots (left) and DA variable loadings plots (right) of the proton transfer reaction-mass spectrometry (PTR-MS) data (with log transformation) of the lamb meat (31 predominant ions) and fat (33 predominant ions) samples as affected by region of origin. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

Even though the samples appeared to group separately, overlap is seen between regions (i.e. NK and BL) which suggests that the groups are very similar in terms of their volatile profile. Unlike the fat, the lamb meat samples were more spread out and HK showed a great deal of overlap with RU (Fig. S8.1). The lamb meat of CK also grouped in the right quadrants along PC 1, while HK grouped in the left quadrants (Fig. 8.4 and Fig. S8.1). This indicated that although there is reasonable similarity between HK and CK, their separate groupings are also driven by associations with different ions. For example, CK associates with and had significantly higher concentrations for m/z 39 (18.24 ± 0.98 ppbv), 41 (18.64 ± 4.20 ppbv), 71 (12.42 ± 10.14 ppbv), 72 (0.56 ± 0.45 ppbv) and 73 (3.90 ± 0.67 ppbv) (Fig. 8.4 and Table S8.1). These ions could be fragments of hexenyl acetate, hexanol, 1-, 2- or 3-octanol, and 2-butanone or butanal (Table 8.3). The presence of these ions may also suggest a variation in the sensory profile of the meat as hexanol have been described as woody, cut grass, chemical-winey, fatty, fruity and weak metallic, while 1-octanol can have a penetrating aromatic odour and smell fatty, waxy, oily, burnt, citrus, walnut, moss, chemical and metal (Calkins & Hodgen, 2007).

8.3.2.1 Lamb fat

Along PC1 (Fig. S8.1) and PC2 (Fig. 8.4) for lamb fat, HK and CK associate with the ions m/z 41, 57, 58, 81, 82, 83, 85, 93, 95, 97 and 137, reflecting some of the ions which were previously pointed out in Figure 8.1 and 8.2. It is noteworthy that RU associate more with ions m/z 30, 33, 45, 47 and 87 (Fig. 8.4 and Fig. S8.1). Mass 33 could be methanol with m/z 47 as fragment ion due to the loss of water from its proton-bound dimer (Maleknia *et al.*, 2007). The Pearson correlation coefficients (r) showed significant and moderately negative correlations between m/z 47 and the following ions for lamb fat: m/z 57 ($r = -0.440$; $P = 0.0005$), 58 ($r = -0.476$; $P = 0.0001$), 82 ($r = -0.584$; $P = < 0.0001$), 85 ($r = -0.452$; $P = 0.003$) and m/z 138 ($r = -0.437$; $P = 0.001$). Similarly, m/z 87 showed moderately negative correlations with ions: m/z 81 ($r = -0.471$; $P = 0.0002$), 93 ($r = -0.423$; $P = 0.001$), 95 ($r = -0.425$; $P = 0.001$) and 137 ($r = -0.468$; $P = 0.0002$). In contrast, significant ($P = < 0.0001$) and strong positive correlations were observed between m/z 81 and the following ions: m/z 57 ($r = 0.693$), 58 ($r = 0.662$), 82 ($r = 0.800$), 83 ($r = 0.605$), 85 ($r = 0.618$) and 137 ($r = 0.970$). In addition, HK fat had significantly higher concentrations for m/z 57 (27.51 ± 17.37 ppbv), 58 (1.19 ± 0.79 ppbv), 82 (0.60 ± 0.73 ppbv), 83 (5.78 ± 6.82 ppbv), 85 (1.42 ± 1.22 ppbv) and 137 (6.13 ± 7.82 ppbv) (Table S8.2). These correlations may suggest that fat samples of regions with high concentrations for the ions m/z 57, 58, 81, 82, 83, 85 and 137 are likely to be from a Hantam Karoo region of origin.

8.3.2.2 Lamb meat

For lamb meat, the ions [m/z 39, 41, 43, 44, 61, 71, 72, 73, 87, 89, 90, significantly correlated with each other ($P = < 0.0001$)] which associate with CK where the same was seen for lamb fat, except for m/z 87 and 89 (Fig. 8.4). The ion m/z 87 could be indicative of the presence of 2,3-butanedione, hexanol, 2-pentanone or pentanal, while that of m/z 89 points to acetoin, butyric acid, butanoic acid or a variety of butyrates (Table 8.3). The concentration of m/z 89 was the highest for NK (14.17 ± 14.30 ppbv), CK (10.46 ± 8.61 ppbv) and BL ($9.22 \pm$

10.04 ppbv), significantly ($P \leq 0.05$) more than HK meat (1.75 ± 3.26 ppbv). The ions m/z 59 and 60 associates with NK and BK for both meat and fat (Fig. 8.4). In effect, the concentrations were the highest for BL meat and fat for m/z 59 (379.65 ± 64.87 ppbv and 269.11 ± 109.51 ppbv) and 60 (12.88 ± 2.15 ppbv and 9.11 ± 3.71 ppbv) (Table S8.1 and S8.2). Overall, the Karoo regions (CK, HK, NK and BL) had higher concentrations of m/z 59 and 60 for meat and fat, compared to that of the Non-Karoo regions (RU and SE) (Table S8.1 and S8.2). Mass 59 showed negative correlations with m/z 47 ($r = -0.318$, $P = 0.014$) and 55 ($r = -0.315$, $P = 0.015$), and a positive correlation with m/z 39 ($r = 0.419$, $P = 0.001$). Accordingly, RU grouped separately from NK and BL, and associates with m/z 47 and 55, but also with ions m/z 30 and 69 (Fig. 8.4). The ion m/z 55 correlates reasonably strongly positive with m/z 69 ($r = 0.726$, $P = < 0.0001$), 83 ($r = 0.652$, $P = < 0.0001$) and 101 ($r = 0.547$, $P = < 0.0001$). The probable identity of m/z 55 is butanal (Table 8.3), which has been described as smokey, fish, amylic, aldehyde-enal or dienal (Ba *et al.*, 2012). Similarly, m/z 69 and 83 are likely the aldehydes pentanal, octanal, nonanal or hexanal (Table 8.3), which are generated by lipid oxidation (Ortuño *et al.*, 2016). Although the concentrations of m/z 55, 69 and 83 did not differ significantly between regions for lamb meat (Table S8.1), they were the highest ($P \leq 0.05$) for HK fat (Table S8.2).

The ions m/z 33, 63 and 83 associates with SE and m/z 101 with HK. This could explain the overlap of the confidence circles of HK and SE along PC2 in the lower quadrants of Figure 8.4 for lamb meat. Mass 63 correlated negatively with m/z 137 ($r = -0.454$, $P = 0.0003$), also signified by the separate grouping of SE and HK along PC1 (Fig. 8.4) and the significant lower concentration of m/z 63 for HK (4.21 ± 1.08 ppbv) against SE (7.95 ± 3.10 ppbv) (Table S8.1). Conversely, m/z 63 correlated positively with m/z 61 ($r = 0.328$, $P = 0.011$), 89 ($r = 0.326$, $P = 0.012$) and 90 ($r = 0.348$, $P = 0.007$) which might explain the overlap of SE and CK (Fig. 8.4). When evaluating at the ions associating with RU fat, m/z 45 and 87 did not show the same association as seen with the meat. In fact, the concentration of m/z 45 and m/z 87 was highest ($P \leq 0.05$) for HK (93.83 ± 38.20 ppbv) and CK (2.34 ± 1.05 ppbv) meat, respectively (Table S8.1). For HK, m/z 45, 81, 93, 101 and 137 associate with the meat (Fig. 8.4). However, m/z 45 and 101 did not associate strongly with HK fat. The reason being that the concentration of acetaldehyde (m/z 45) was significantly highest in HK meat (and not for fat), whereas 2-hexanone (m/z 101) was higher in SE fat compared to HK fat (Table 8.3; Table S8.1 and S8.2). As expected m/z 137 showed a moderate positive correlation with m/z 81 ($r = 0.593$, $P = < 0.0001$) as it is the protonated terpene molecules, while m/z 81 is the fragment ions of these terpenes.

8.3.2.3 Variation between meat and fat

The reason for the variation in VOC profiles between the meat and fat could be explained based on their composition. Amino acids are the main component distinguishing them, being the building blocks of meat. Amino acids also play a vital role in the aroma and flavour development of meat as they are responsible for the Maillard reaction (i.e. non-enzymatic browning reaction between a reducing sugar and amino acid group), may undergo protein breakdown, protein oxidation and the development of heterocyclic compounds (Mottram, 1998; Calkins & Hodgen, 2007). Accordingly, the ions m/z 46, 63, 75, 90 and 94 were only predominant in the

meat samples (Table 8.3). Their origin is likely from the protein source as their probable identity could be an amine (m/z 46), dimethyl sulphide (m/z 63), thioamide (m/z 90) or methyl-pyridine (m/z 94) (Table 8.3). Sulphur- and nitrogen-containing volatile compounds are derived from the breakdown of amino acids and their concentration may be enhanced by the presence of aldehydes (Mottram, 1998; Vasta & Priolo, 2006). Given that aldehydes are largely derived from lipid oxidation, the high level of polyunsaturated fatty acids (PUFA) in grass-fed lamb meat may promote the production thereof (Mottram, 1998; Vasta & Priolo, 2006). Since the meat in the current study was not cooked (but only heated to 30°C in the glass bottles during the procedures), a small contribution of amino acid derived volatiles towards the VOC profile is expected. In addition, raw meat would generate less VOCs than cooked meat as a result of the extensive chemical reactions taking place during the cooking process (Mottram, 1998).

However, many VOCs are common in both raw and cooked meat and fat, such as the carbonyl compounds generated by the lipid oxidation. Hexanal (green grass, fatty and rancid), nonanal (sweet, fatty, plastic and soap), 1-octen-3-ol (mushroom) and octanal (geranium, herbal, citrus and floral) were the most abundant VOCs in the headspace of lamb meat (Ba *et al.*, 2012; Vasta *et al.*, 2013; Ortuño *et al.* 2016). Mass ratios which could be the mentioned compounds or other aldehydes are m/z 55, 57, 59, 69, 73, 83, 85, 87 and 97 (Table 8.3). For lamb fat, HK and CK associated with and had the highest concentrations for m/z 57 (27.51 ± 17.37 ppbv and 16.09 ± 2.60 ppbv), 83 (5.78 ± 6.82 ppbv and 2.59 ± 3.52 ppbv), 85 (1.42 ± 1.22 ppbv and 0.61 ± 0.26 ppbv) and 97 (0.53 ± 0.67 ppbv and 0.28 ± 0.24 ppbv) (section 8.3.2.1) (Table S8.2). Based on literature and if these compounds were lipid oxidation products, one would expect HK and CK to contain lower concentrations thereof. In studies where rosemary and thyme leaves were used as supplementary feed, a decrease in lipid oxidation and rancidity were seen due to the antioxidant compounds present in the plants (Nieto *et al.*, 2010a; Nieto *et al.*, 2010b). Given the high terpene concentrations of HK and CK, a similar trend was expected. Ortuño *et al.* (2016) confirmed that the formation of VOCs in meat kept under strong pro-oxidizing conditions can be delayed through bioavailable diterpenes from rosemary in the lamb diet. Since the fatty acid profiles were not determined one can only speculate about the degree of lipid oxidation. In fact, the concentration of PUFA (and resultant oxidation products) may also be affected by the dietary plants as Vasta *et al.* (2013) found that artemisia, but not rosemary, altered the fatty acid profile of the meat. They suggested that the terpenes present in rosemary could have been degraded by ruminal bacteria or been less effective in impairing ruminal biohydrogenation.

8.3.3 Classifications

Four partial least square discriminant analysis (PLS-DA) models were fitted on two different PTR-MS mass spectra datasets: one dataset for lamb meat and the other for lamb fat. Each dataset comprised 59 samples (farms) and models were fitted to classify the samples into their “region of origin” (six different regions) and “origin” (Karoo vs. Non-Karoo). The full mass spectra were used for the classification.

8.3.3.1 Classification into region of origin

The PLS-DA estimation models achieved a 100% correct classification rate (Table 8.4). However, validation of the PLS-DA models for the lamb meat and fat samples resulted in 58% and 42% of the samples being correctly classified into their region of origin (Table 8.4). High correct classification rates (100%) of the validation set were observed for RU, CK and HK. For lamb fat, only three samples of Karoo origin were misclassified as RU (Non-Karoo) and similarly one for lamb meat. For both lamb fat and meat validation, one particular SE sample (SE5) was classified as CK. This could suggest that the sheep from this farm received additional feed (i.e. concentrates) making the chemical profile similar to that of sheep from the Karoo fed shrubs and/or bushes and grass. Furthermore, the groups of NK, BL and SE were very small compared to the others and an improved classification success rate may be possible using a larger number of samples.

The PLS-DA scores plots are provided as Supplementary data (Fig. S8.2). Although the samples of each region are grouped together, they are spread out and a great deal of overlap in the clustering is seen where samples from two or more regions appear close together. Groupings of the different regions can be seen with that of RU separate from the other regions along axis 1 and 2 (Fig. S8.2). Along axis 2, NK, BL and CK are grouped separately from that of RU and HK for lamb fat. The fat samples of HK were not as closely grouped as the rest. However, the HK lamb fat associated and correlated positively with the majority of the predominant ions: m/z 55, 57, 58, 69, 70, 81, 82, 83, 85, 93, 95, 97, 135, 137 and 138. Suggesting again that the fat of HK contain a greater concentration of these volatiles. Positive correlations were also seen for the following ions of HK meat: m/z 81, 133 and 137, and negative correlations for m/z 64, 65 and 115. The CK fat samples had a weak positive correlation with the ions m/z 41, 43 and 44, while RU correlated negatively with the same ions. This is in accordance with the results reported in section 8.3.2. Lamb meat of CK also showed positive correlations with m/z 36, 39, 40, 41 and 58, while RU showed negative correlations with m/z 41, 43, 44, 47, 48 and 79. The fat of NK had quite strong correlations with m/z 29, 48, 76 and 77, and lamb meat with m/z 91, 92 and 93. It is important to note that quite a few ions mentioned above, were not present in concentrations above 1.0 ppbv. Although similar trends and groupings were seen as described in section 8.3.2. Classification using the full mass spectra is still important as some of the ions, present in low concentration, may have a low odour detection threshold but still contribute significantly towards the overall aroma and flavour of the meat and fat. They can also be key to classifying the samples into the different regions of origin.

Table 8.4 Classification of region of origin of lamb samples, on the basis of PTR-MS volatile fingerprints and percentage of observations correctly classified

Model	Actual origin	Classified origin ^a						Total
		CK	HK	NK	BL	RU	SE	
Estimation	CK	13	0	0	0	0	0	13
Lamb meat	HK	0	10	0	0	0	0	10
	NK	0	0	5	0	0	0	5
	BL	0	0	0	5	0	0	5
	RU	0	0	0	0	10	0	10
	SE	0	0	0	0	0	4	4
% Correctly classified		100	100	100	100	100	100	100^b
Validation	CK	2	0	0	0	0	0	2
Lamb meat	HK	0	2	0	0	0	0	2
	NK	0	0	0	1	1	0	2
	BL	1	0	0	1	0	0	2
	RU	0	0	0	0	2	0	2
	SE	2	0	0	0	0	0	2
% Correctly classified		100	100	0	50.00	100	0	58.33^c
Estimation	CK	13	0	0	0	0	0	13
Lamb fat	HK	0	10	0	0	0	0	10
	NK	0	0	5	0	0	0	5
	BL	0	0	0	5	0	0	5
	RU	0	0	0	0	10	0	10
	SE	0	0	0	0	0	4	4
% Correctly classified		100	100	100	100	100	100	100^d
Validation	CK	1	0	0	0	1	0	2
Lamb fat	HK	0	1	0	0	1	0	2
	NK	1	0	0	1	0	0	2
	BL	0	0	1	0	1	0	2
	RU	0	0	0	0	2	0	2
	SE	1	0	0	0	0	1	2
% Correctly classified		50.00	50.00	0	0	100	50.00	41.67^e

(PTR-MS) Proton transfer reaction-mass spectrometry; ^a The number of correctly classified observations are tabulated diagonally; ^b 100% of the observations for the estimation model correctly classified; ^c 58.33% of the observations for the validation correctly classified; ^d 100% of the observations for the estimation model correctly classified; ^e 41.67% of the observations for the validation correctly classified; (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

8.3.3.2 Classification into origin

Given the importance regarding the classification of Karoo lamb for the purpose of certification, the groups were separated into two “origins”. One being Karoo and the other Non-Karoo. Given that RU lamb were only fed lucerne/alfalfa (*Medicago sativa*) the samples were labelled as Non-Karoo. Although physically raised in the Karoo, SE lamb is provided with concentrates which makes them unsuitable to be certified as Karoo lamb. Hence, SE was also labelled as Non-Karoo for PLS-DA. The results are shown in Table 8.5 and the observations on the first two dimensions of the PLS-DA models for all the samples are presented in Figure 8.5.

Table 8.5 Classification of origin of lamb samples, on the basis of PTR-MS volatile fingerprints and percentage of observations correctly classified

Model	Actual origin	Classified origin ^a		
		Karoo	Non-Karoo	Total
Estimation	Karoo	33	0	33
Lamb meat	Non-Karoo	0	14	14
% Correctly classified		100	100	100^b
Validation	Karoo	8	0	8
Lamb meat	Non-Karoo	2	2	4
% Correctly classified		100	50.00	83.33^c
Estimation	Karoo	33	0	33
Lamb fat	Non-Karoo	0	14	14
% Correctly classified		100	100	100^d
Validation	Karoo	7	1	8
Lamb fat	Non-Karoo	0	4	4
% Correctly classified		87.50	100	91.67^e

(PTR-MS) Proton transfer reaction-mass spectrometry; ^a The number of correctly classified observations are tabulated diagonally; ^b 100% of the observations for the estimation model correctly classified; ^c 83.33% of the observations for the validation correctly classified; ^d 100% of the observations for the estimation model correctly classified; ^e 91.67% of the observations for the validation correctly classified.

Along axis 1, the Karoo and Non-Karoo regions are clearly separated with the Karoo samples on the left quadrants and the Non-Karoo samples on the right quadrants (Fig. 8.5). The separation between the two groups is greater for the lamb meat samples compared to the lamb fat samples. Compared to the region of origin classification (section 8.3.3.1), the classification into Karoo or Non-Karoo origin was more successful. This is reflected in the classification results, with a 100% success rate for the estimation models, while for the validation models 83.33% of the lamb meat samples were correctly classified and 91.67% of the lamb fat samples (Table 8.5). For the validation of lamb fat one CK sample (CK10) was incorrectly classified as Non-Karoo, while for lamb meat two SE samples (SE2 and SE5) were incorrectly classified as Karoo.

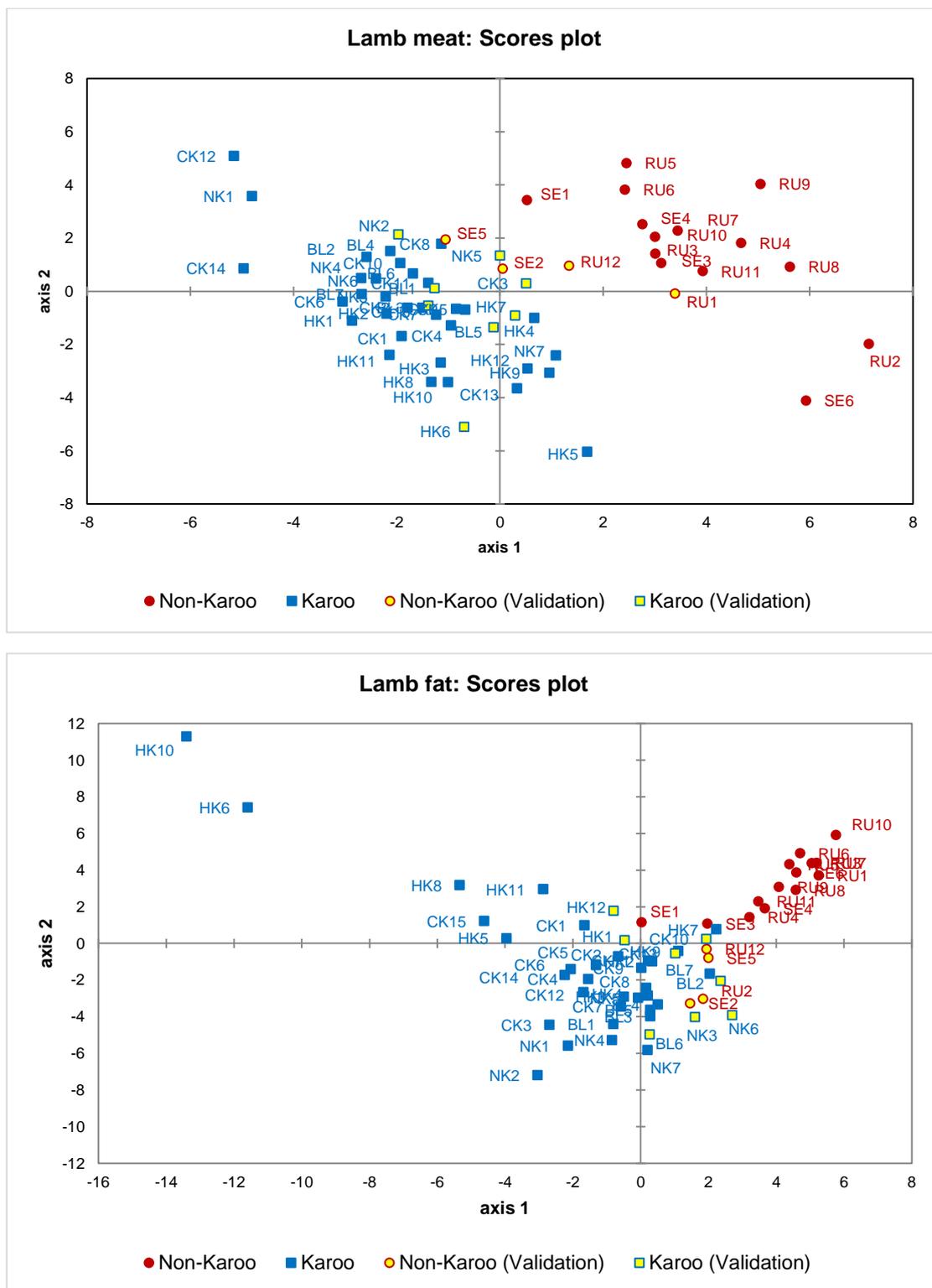


Figure 8.5 Partial least-squares discriminant analysis (PLS-DA) scores plot on axes 1 and 2 of the mass spectral data of lamb meat (upper) and fat (lower) determined by proton transfer reaction-mass spectrometry (PTR-MS) for origin (Karoo vs. Non-Karoo) classification. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

8.3.4 Factors influencing classification

A combination of factors are responsible for the composition of volatile compounds in lamb meat. These include *ante-mortem*, as well as *post-mortem* factors which affect the composition of meat and consequently, have an influence on the volatile profile of the meat. *Ante-mortem* factors include the age, gender, breed and diet of the animal, while *post-mortem* factors include the storage and handling of the meat (Spanier *et al.*, 2004). In terms of extensively produced sheep, the environment and vegetation plays an important role as it has been shown that it influences the chemical (Chapter 4 and 5) as well as the sensory profile (Chapter 3) of the meat. Therefore, the volatile composition of lamb meat and fat from various regions is dependent on an intricate variety of influences which could alter the classification of origin. However, the results of this study show that despite all the factors affecting the final product, a separation of lamb meat and fat according to geographic origin (Karoo vs Non-Karoo) can be successfully achieved. Although, within the Karoo, the regions showed separate groupings a larger sample size (including more of the regional variation) could possibly increase the success rate for the classification into region of origin.

8.4 Conclusions

The study proved that PTR-MS is a promising tool to evaluate extensive meat production associated with type of diet of which the monoterpene mass ratios are particularly useful for indicating dietary differences. Multivariate statistical analysis allowed the successful classification of samples into their region of origin using the masses as predictor variables. However, separation on a regional scale showed less strength, likely due to the small sample size per region. Classification of an origin-based scale proved to be more successful as samples were grouped according to their Karoo or Non-Karoo origin. For the time being such an approach might be more effective in determining the origin of the meat. The volatile composition of the fat also had a higher concentration of key monoterpenes, validating the direct link with the herbaceous plant samples, which could serve as markers for future classification purposes. In order to accurately identify the characteristic volatile compounds distinguishing Karoo lamb from other types of lamb, future research should include the detailed analysis of the volatile profile of samples using a technique with a higher resolution such as PTR-TOF-MS (coupling of PTR-MS with a time-of-flight spectrometer). Especially since PTR-MS data was insufficient to distinguish different types of terpenes. Overall the considerable differences between the Karoo and Non-Karoo samples indicated the typicality of Karoo lamb with the result being promising in the view of development of an authentication test. Hence, the PTR-MS method could be developed to incorporate auto-sampling procedures of subcutaneous fat to provide a maximum throughput of sample analyses and a future screening technique for origin determination of lamb that is both fast and accurate.

8.5 References

Acocks, J. P. H. (1988). Veld types of South Africa. In: *Memoirs of the Botanical Survey of South Africa*. 3rd ed. Vol. 57.

- Aprea, E., Biasioli, F., Märk, T. D. & Gasperi, F. (2007). PTR-MS study of esters in water and water/ethanol solutions: Fragmentation patterns and partition coefficients. *International Journal of Mass Spectrometry*, **262**, 114-121.
- Araghipour, N., Colineau, J., Koot, A., Akkermans, W., Rojas, J. M. M., Beauchamp, J., Wisthaler, A., Märk, T. D., Downey, G., Guillou, C., Mannina, L. & Van Ruth, S. (2008). Geographical origin classification of olive oils by PTR-MS. *Food Chemistry*, **108**, 374-383.
- Asensio, D., Peñuelas, J., Llusía, J., Ogaya, R. & Filella, I. (2007). Interannual and interseasonal soil CO₂ efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. *Soil Biology & Biochemistry*, **39**, 2471-2484.
- Ba, H. V., Hwang, I., Jeong, D. & Touseef, A. (2012). Principle of meat aroma flavors and future prospect. In: *Latest Research into Quality Control* (edited by M. S. F. Nezhad). ISBN: 978-953-51-0868-9. InTech, DOI: 10.5772/51110.
- Berg, E. P. & Walker, E. L. M. (2004). Sheep. In: *Encyclopedia of Meat Sciences* (edited by W. K. Jensen). Pp. 1291-1295. Oxford, UK: Elsevier.
- Biasioli, F., Yeretian, C., Gasperi, F. & Märk, T. D. (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. *Trends in Analytical Chemistry*, **30**(7), 968-977.
- Buhr, K., Van Ruth, S. & Delahunty, C. (2002). Analysis of volatile flavour compound by Proton Transfer Reaction-Mass Spectrometry: fragmentation patterns and discrimination between isobaric and isomeric compounds. *International Journal of Mass Spectrometry*, **221**, 1-7.
- Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, **77**, 63-80.
- Cloete, S. W. P. & Olivier, J. J. (2010). South African industry. In: *The International Sheep and Wool Handbook* (edited by D. J. Cottle). Pp. 95-112. Nottingham, UK: Nottingham University Press.
- Department of Agriculture, Forestry and Fisheries (DAFF). (2014). Grootfontein Herbarium. Grootfontein Agricultural Development Institute [Internet document].
URL <http://gadi.agric.za//////////herbarium/her-select.php>. Accessed 23.09.2014.
- Erasmus, S. W., Hoffman, L. C., Muller, M., & Van der Rijst, M. (2016a). Variation in the sensory profile of South African Dorper lamb from extensive grazing systems. *Small Ruminant Research*, **144**, 62-74.
<http://dx.doi.org/10.1016/j.smallrumres.2016.07.020>.
- Erasmus, S. W., Muller, M., Van der Rijst, M. & Hoffman, L. C. (2016b). Stable isotope ratio analysis: A potential analytical tool for the authentication of South African lamb meat, *Food Chemistry*, **192**, 997-1005. <http://dx.doi.org/10.1016/j.foodchem.2015.07.121>.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Galle, S. A., Koot, A., Soukoulis, C., Cappellin, L., Biasioli, F., Alewijn, M. & Van Ruth, S. M. (2011). Typicality and geographical origin markers of protected origin cheese from the Netherlands revealed by PTR-MS. *Journal of Agricultural and Food Chemistry*, **59**, 2554-2563.

- Grunert, K. G. (2005). Food quality and safety: consumer perception and demand. *European Review of Agricultural Economics*, **32**(3), 369-391.
- Le Roux, P. M., Kotzé, C. D., Nel, G. P. & Glen, H. F. (1994). *Bossievelde: Grazing Plants of the Karoo and Karoo-like areas*. Bulletin 428. Pretoria, South Africa: Department of Agriculture.
- Lindinger, W., Hansel, A. & Jordan, A. (1998). Proton-transfer-reaction mass spectrometry (PTR-MS): On-line monitoring of volatile organic compounds at pptv levels. *Chemical Society Reviews*, **27**, 347-354.
- Maleknia, S. D., Bell, T. L. & Adams, M. A. (2007). PTR-MS analysis of reference and plant-emitted volatile organic compounds. *International Journal of Mass Spectrometry*, **262**, 203-210.
- Manning, J. & Goldblatt, P. (1997). *Nieuwoudtville Bokkeveld Plateau and Hantam*. *South African Wild Flower Guide 9*. Cape Town, South Africa: The Botanical Society of South Africa.
- Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, **62**(4), 415-424.
- Nenadis, N., Heenan, S., Tsimidou, M. Z. & Van Ruth, S. (2016). Applicability of PTR-MS in the quality control of saffron. *Food Chemistry*, **196**, 961-967.
- Nieto, G., Díaz, P., Bañón, S. & Garrido, M. D. (2010a). Dietary administration of ewe diets with a distillate from rosemary leaves (*Rosmarinus officinalis* L.): Influence on lamb meat quality. *Meat Science*, **84**, 23-29.
- Nieto, G., Díaz, P., Bañón, S. & Garrido, M. D. (2010b). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet. *Meat Science*, **85**, 82-88.
- Oliveira, G. B., Alewijn, M., Boerrigter-Eenling, R. & Van Ruth, S. M. (2015). Compositional signatures of conventional, free range, and organic pork meat using fingerprint techniques. *Foods*, **4**, 359-375.
- Ortuño, J., Serrano, R. & Bañón, S. (2016). Use of dietary rosemary diterpenes to inhibit rancid volatiles in lamb meat packed under protective atmosphere. *Animal*, **10**(8), 1391-1401.
- Palmer, A. R. & Ainslie, A. M. (2005). Grasslands of South Africa. In: *Plant Production and Protection Series: Grasslands of the World* (edited by J. M. Suttie, S. G. Reynolds & C. Batello). No. 34. Pp. 77-120. Rome, Italy: Food and Agriculture Organization of the United Nations.
- Rubino, R., Morand-Fehr, P., Renieri, C., Peraza, C. & Sarti, F. M. (1999). Typical products of the small ruminant sector and the factors affecting their quality. *Small Ruminant Research*, **34**, 289-302.
- Shapiro, S. S. & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Shearing, D. (1994). *Karoo*. *South African Wild Flower Guide 6*. Cape Town, South Africa: The Botanical Society of South Africa.
- Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*. 7th ed. Ames, USA: The Iowa State University Press.

- Spaniel, P., Van Doren, J. M. & Smith, D. (2002). A selected ion flow tube study of the reactions of H_3O^+ , NO^+ , and O_2^+ with saturated and unsaturated aldehydes and subsequent hydration of the product ions. *International Journal of Mass Spectrometry*, **213**, 163-176.
- Spanier, A. M., Flores, M., Toldrá, F., Aristoy, M.-C., Bett, K. L., Bystricky, P. & Bland, J. M. (2004). Meat flavor: contribution of proteins and peptides to the flavor of beef. In: *Quality of Fresh and Processed Foods* (edited by F. Shahidi, A. M. Spanier, C.-T. Ho & T. Braggins). Pp. 33-49. Vol. 542. New York, USA: Kluwer Academic/Plenum Publishers.
- Tani, A., Hayward, S. & Hewitt, C. N. (2003). Measurement of monoterpenes and related compounds by proton transfer reaction-mass spectrometry (PTR-MS). *International Journal of Mass Spectrometry*, **223-224**, 561-578.
- Van Oudtshoorn, F. (2012). *Gids tot Grasse van Suider-Afrika*. Pretoria, South Africa: Briza Publications.
- Van Ruth, S. M., Rozijn, M., Koot, A., Garcia, R. P., Van der Kamp, H. & Codony, R. (2010). Authentication of feeding fats: Classification of animal fats, fish oils and recycled oils. *Animal Feed Science and Technology*, **155**, 65-73.
- Vasta, V., Aouadi, D., Brogna, D. M. R., Scerra, M., Luciano, G., Priolo, A. & Salem, H. B. (2013). Effect of the dietary supplementation of essential oils from rosemary and artemisia on muscle fatty acids and volatile compound profiles in Barbarine lambs. *Meat Science*, **95**, 235-241.
- Vasta, V. & Priolo, A. (2006). Ruminant fat volatiles as affected by diet. A review. *Meat Science*, **73**, 218-228.
- Viuda-Martos, M., Ruíz-Navajas, Y., Fernández-López, J. & Pérez-Álvarez, J. A. (2007). Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. *Acta Chimica Slovenica*, **54**, 921-926.
- Warriss, P. D. (2010). The growth and body composition of animals. In: *Meat Science: An Introductory Text*. 2nd ed. p. 11. Wallingford, UK: CABI Publishing.
- Weissnar, T. & Du Rand, G. (2012). Consumer perception of Karoo lamb as a product of origin and their consequent willingness to purchase. *Food Research International*, **47**, 272-278.
- Xiao, Z., Ma, S., Niu, Y., Chen, F. & Yu, D. (2016). Characterization of odour-active compounds of sweet orange essential oils of different regions by gas chromatography-mass spectrometry, gas chromatography-olfactometry and their correlation with sensory attributes. *Flavour and Fragrance Journal*, **31**, 41-50.

Supplementary data

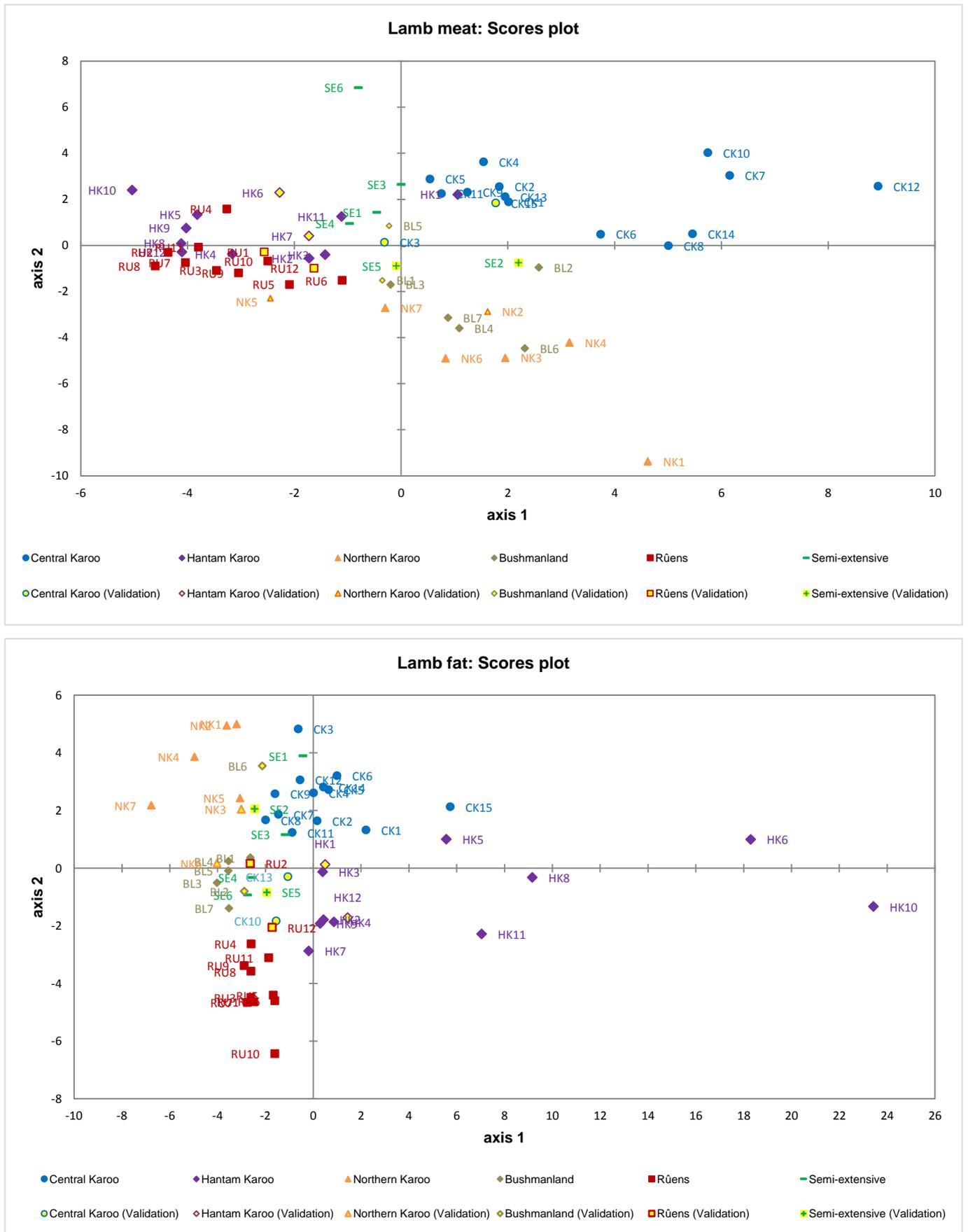


Figure S8.2 Partial least-squares discriminant analysis (PLS-DA) scores plot on axes 1 and 2 of the mass spectral data of lamb meat (upper) and fat (lower) determined by proton transfer reaction-mass spectrometry (PTR-MS) for region of origin classification. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

Supplementary data

Table S8.1 The means (\pm SD) of the ions determined in PTR-MS analyses of the lamb meat from the different regions

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
22	0.04 \pm 0.02	0.04 \pm 0.02	0.03 \pm 0.02	0.03 \pm 0.01	0.03 \pm 0.02	0.03 \pm 0.02	0.02
23	0.01 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01	0.01
24	<0.01	<0.01	<0.01	nd	<0.01	<0.01	<0.01
25	<0.01	<0.01	nd	<0.01	nd	nd	<0.01
26	0.01 ^{ab} \pm 0.01	0.00 ^b \pm 0.03	0.00 ^{ab} \pm 0.04	0.01 ^a \pm 0.00	0.01 ^{ab} \pm 0.00	<0.01 ^{ab}	<0.01
27	0.06 ^a \pm 0.03	0.03 ^b \pm 0.02	0.04 ^{ab} \pm 0.02	0.04 ^{ab} \pm 0.01	0.03 ^b \pm 0.02	0.03 ^b \pm 0.01	0.02
28	0.03 ^{ab} \pm 0.02	0.03 ^{ab} \pm 0.01	0.03 ^a \pm 0.02	0.02 ^b \pm 0.02	0.02 ^b \pm 0.01	0.03 ^{ab} \pm 0.01	0.02
29	<0.01	nd	nd	nd	nd	nd	<0.01
30	0.32 ^c \pm 0.40	0.47 ^{bc} \pm 0.60	0.23 ^c \pm 0.34	0.89 ^{ab} \pm 0.46	0.52 ^{bc} \pm 0.62	1.12 ^a \pm 0.94	0.54
31	0.09 \pm 0.12	0.04 \pm 0.04	0.03 \pm 0.05	0.06 \pm 0.07	0.08 \pm 0.06	0.10 \pm 0.15	0.09
33	59.46 ^b \pm 5.31	46.63 ^c \pm 5.58	47.30 ^c \pm 5.30	49.71 ^{bc} \pm 4.56	58.53 ^b \pm 6.64	76.52 ^a \pm 28.91	10.05
34	0.01 \pm 0.04	nd	0.01 \pm 0.02	<0.01	0.01 \pm 0.01	0.01 \pm 0.01	0.03
35	0.25 ^{abc} \pm 0.13	0.22 ^{abc} \pm 0.07	0.19 ^{bc} \pm 0.07	0.17 ^c \pm 0.06	0.27 ^{ab} \pm 0.13	0.31 ^a \pm 0.08	0.10
36	0.19 ^a \pm 0.13	0.11 ^{bc} \pm 0.04	0.07 ^c \pm 0.03	0.08 ^{bc} \pm 0.04	0.08 ^{bc} \pm 0.04	0.15 ^{ab} \pm 0.06	0.07
39	18.24 ^a \pm 0.98	17.01 ^{bc} \pm 1.41	16.85 ^c \pm 0.84	17.34 ^{abc} \pm 1.03	16.39 ^c \pm 0.68	17.96 ^{ab} \pm 1.02	0.99
40	0.13 ^a \pm 0.05	0.07 ^{cd} \pm 0.05	0.09 ^{bcd} \pm 0.02	0.10 ^{abc} \pm 0.04	0.06 ^d \pm 0.02	0.11 ^{ab} \pm 0.04	0.04
41	18.64 ^a \pm 4.20	12.02 ^{cd} \pm 6.93	12.64 ^{bcd} \pm 2.28	14.32 ^{bc} \pm 5.58	9.12 ^d \pm 1.20	16.45 ^{ab} \pm 1.97	4.23
42	2.89 ^a \pm 1.28	2.17 ^{ab} \pm 1.68	2.21 ^{ab} \pm 1.12	1.97 ^{abc} \pm 1.25	0.98 ^c \pm 0.30	1.73 ^{bc} \pm 0.39	1.14
43	49.65 ^a \pm 20.03	21.82 ^b \pm 11.27	41.84 ^a \pm 20.86	43.38 ^a \pm 19.39	19.47 ^b \pm 9.64	34.61 ^{ab} \pm 12.04	15.54
44	1.33 ^a \pm 0.44	0.64 ^{cd} \pm 0.34	1.10 ^{ab} \pm 0.45	1.13 ^{ab} \pm 0.37	0.53 ^d \pm 0.23	0.96 ^{bc} \pm 0.31	0.35
45	74.76 ^{ab} \pm 23.10	93.83 ^a \pm 38.20	46.17 ^c \pm 18.71	50.53 ^{bc} \pm 23.95	52.33 ^{bc} \pm 15.02	51.58 ^{bc} \pm 20.19	24.81
46	1.54 ^{ab} \pm 0.58	1.99 ^a \pm 0.91	0.84 ^c \pm 0.43	0.98 ^{bc} \pm 0.62	1.01 ^{bc} \pm 0.35	0.95 ^{bc} \pm 0.52	0.60
47	3.16 ^b \pm 2.31	1.81 ^b \pm 1.12	1.75 ^b \pm 1.18	2.55 ^b \pm 2.57	5.50 ^a \pm 2.34	2.98 ^b \pm 1.68	1.91
48	0.14 ^{ab} \pm 0.09	0.09 ^b \pm 0.04	0.10 ^b \pm 0.03	0.10 ^b \pm 0.05	0.17 ^a \pm 0.06	0.10 ^b \pm 0.04	0.06
49	0.38 \pm 0.14	0.39 \pm 0.09	0.32 \pm 0.10	0.38 \pm 0.12	0.33 \pm 0.04	0.37 \pm 0.05	0.10
50	0.03 \pm 0.02	0.03 \pm 0.03	0.04 \pm 0.02	0.02 \pm 0.03	0.03 \pm 0.03	0.03 \pm 0.03	0.02
51	0.73 ^b \pm 0.08	0.60 ^{cd} \pm 0.04	0.58 ^d \pm 0.07	0.61 ^{cd} \pm 0.08	0.68 ^{bc} \pm 0.05	0.84 ^a \pm 0.22	0.09
52	0.03 \pm 0.02	0.02 \pm 0.01	0.03 \pm 0.02	0.02 \pm 0.02	0.03 \pm 0.01	0.02 \pm 0.01	0.01
53	0.07 \pm 0.05	0.04 \pm 0.03	0.06 \pm 0.05	0.07 \pm 0.04	0.04 \pm 0.03	0.06 \pm 0.05	0.04
54	0.02 ^{ab} \pm 0.01	0.01 ^{ab} \pm 0.01	0.02 ^{ab} \pm 0.01	0.02 ^a \pm 0.02	0.02 ^{ab} \pm 0.01	0.01 ^b \pm 0.01	0.01
55	7.76 \pm 1.13	7.88 \pm 1.43	7.46 \pm 1.13	7.87 \pm 1.05	8.12 \pm 1.52	8.52 \pm 2.95	1.46
56	0.03 \pm 0.03	0.03 \pm 0.04	0.02 \pm 0.03	0.03 \pm 0.03	0.04 \pm 0.06	0.02 \pm 0.03	0.04
57	3.72 ^a \pm 0.35	3.50 ^{ab} \pm 0.93	2.66 ^c \pm 0.45	2.87 ^c \pm 0.40	3.11 ^{bc} \pm 0.39	3.98 ^a \pm 0.90	0.58
58	0.18 ^a \pm 0.07	0.15 ^{ab} \pm 0.05	0.12 ^b \pm 0.05	0.14 ^{ab} \pm 0.04	0.11 ^b \pm 0.03	0.16 ^{ab} \pm 0.02	0.05
59	255.55 ^{bc} \pm 70.00	286.70 ^{ab} \pm 202.97	242.35 ^{bcd} \pm 55.42	379.65 ^a \pm 64.87	145.70 ^d \pm 35.66	174.29 ^{cd} \pm 76.43	103.73
60	8.66 ^{bc} \pm 2.39	9.74 ^{ab} \pm 7.04	8.22 ^{bcd} \pm 1.92	12.88 ^a \pm 2.15	4.87 ^d \pm 1.13	5.83 ^{cd} \pm 2.60	3.58
61	2.70 \pm 1.43	1.88 \pm 1.36	3.03 \pm 1.20	2.93 \pm 1.30	1.88 \pm 1.36	3.27 \pm 2.63	1.46
62	0.33 ^{ab} \pm 0.08	0.31 ^{ab} \pm 0.07	0.35 ^{ab} \pm 0.10	0.36 ^a \pm 0.07	0.28 ^b \pm 0.09	0.35 ^{ab} \pm 0.08	0.08

Supplementary data

Table S8.1 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
63	6.43 ^a ± 2.44	4.21 ^c ± 1.08	6.52 ^a ± 1.85	4.39 ^{bc} ± 0.83	6.23 ^{ab} ± 1.85	7.95 ^a ± 3.10	1.89
64	0.16 ^a ± 0.07	0.09 ^b ± 0.03	0.17 ^a ± 0.07	0.10 ^b ± 0.03	0.16 ^a ± 0.06	0.22 ^a ± 0.06	0.06
65	0.32 ^a ± 0.12	0.18 ^c ± 0.04	0.29 ^{ab} ± 0.07	0.21 ^{bc} ± 0.07	0.32 ^a ± 0.08	0.34 ^a ± 0.12	0.09
66	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01
67	0.07 ± 0.05	0.06 ± 0.03	0.04 ± 0.02	0.03 ± 0.02	0.06 ± 0.04	0.06 ± 0.05	0.04
68	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
69	1.00 ± 0.68	0.96 ± 0.44	0.86 ± 0.24	1.00 ± 0.28	1.36 ± 0.63	1.24 ± 0.82	0.54
70	0.07 ^{ab} ± 0.04	0.07 ^{ab} ± 0.04	0.06 ^b ± 0.02	0.07 ^{ab} ± 0.02	0.09 ^{ab} ± 0.04	0.10 ^a ± 0.05	0.03
71	12.42 ^a ± 10.14	2.39 ^c ± 3.83	11.71 ^a ± 10.02	11.07 ^{ab} ± 11.74	3.80 ^{bc} ± 4.54	6.61 ^{abc} ± 4.81	7.74
72	0.56 ^{ab} ± 0.45	0.12 ^d ± 0.18	0.79 ^a ± 0.74	0.53 ^{abc} ± 0.53	0.17 ^{cd} ± 0.20	0.30 ^{bcd} ± 0.19	0.39
73	3.90 ^a ± 0.67	3.06 ^b ± 0.46	3.92 ^a ± 1.02	4.07 ^a ± 0.79	2.91 ^b ± 0.64	3.43 ^{ab} ± 0.52	0.65
74	0.17 ^{ab} ± 0.05	0.14 ^b ± 0.04	0.18 ^a ± 0.06	0.17 ^{ab} ± 0.05	0.14 ^b ± 0.03	0.15 ^{ab} ± 0.02	0.04
75	0.29 ^b ± 0.09	0.28 ^b ± 0.11	0.41 ^{ab} ± 0.24	0.41 ^{ab} ± 0.27	0.48 ^{ab} ± 0.57	0.74 ^a ± 0.93	0.41
76	0.08 ± 0.03	0.08 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.09 ± 0.05	0.08 ± 0.06	0.04
77	0.20 ^{ab} ± 0.03	0.19 ^{abc} ± 0.09	0.15 ^{bc} ± 0.03	0.23 ^a ± 0.02	0.14 ^c ± 0.03	0.15 ^{bc} ± 0.04	0.05
78	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01
79	0.04 ^b ± 0.02	0.04 ^b ± 0.01	0.04 ^{ab} ± 0.02	0.05 ^{ab} ± 0.02	0.06 ^a ± 0.02	0.04 ^{ab} ± 0.02	0.02
80	0.06 ± 0.03	0.06 ± 0.03	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.05	0.03
81	0.21 ^{ab} ± 0.09	0.38 ^a ± 0.35	0.17 ^b ± 0.05	0.19 ^b ± 0.08	0.18 ^b ± 0.08	0.20 ^b ± 0.15	0.17
82	0.03 ± 0.01	0.04 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.02
83	1.61 ± 2.10	1.03 ± 1.11	0.66 ± 0.57	1.08 ± 1.40	0.85 ± 0.82	1.08 ± 1.66	1.38
84	0.05 ± 0.05	0.07 ± 0.07	0.03 ± 0.03	0.04 ± 0.04	0.06 ± 0.05	0.09 ± 0.14	0.06
85	0.56 ± 0.53	0.43 ± 0.52	0.35 ± 0.25	0.21 ± 0.08	0.32 ± 0.26	0.23 ± 0.10	0.38
86	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01
87	2.34 ^a ± 1.05	1.23 ^b ± 0.30	2.33 ^a ± 1.04	2.28 ^a ± 1.06	1.73 ^{ab} ± 0.43	1.66 ^{ab} ± 0.50	0.75
88	0.11 ^{ab} ± 0.04	0.07 ^c ± 0.02	0.12 ^a ± 0.04	0.10 ^{ab} ± 0.04	0.09 ^{abc} ± 0.02	0.08 ^{bc} ± 0.02	0.03
89	10.46 ^{ab} ± 8.61	1.75 ^c ± 3.26	14.17 ^a ± 14.30	9.22 ^{abc} ± 10.04	2.87 ^c ± 3.91	5.56 ^{bc} ± 3.99	7.49
90	0.46 ^{ab} ± 0.41	0.06 ^c ± 0.13	0.65 ^a ± 0.67	0.41 ^{abc} ± 0.46	0.12 ^{bc} ± 0.18	0.24 ^{bc} ± 0.18	0.35
91	0.00 ^{ab} ± 0.01	<0.01 ^{ab}	0.01 ^a ± 0.01	<0.01 ^{ab}	<0.01 ^b	<0.01 ^{ab}	0.01
92	0.01 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01
93	0.39 ± 1.02	0.89 ± 1.50	0.08 ± 0.04	0.08 ± 0.03	0.18 ± 0.21	0.08 ± 0.04	0.86
94	0.07 ± 0.02	0.10 ± 0.09	0.07 ± 0.02	0.06 ± 0.01	0.08 ± 0.02	0.07 ± 0.03	0.04
95	0.10 ± 0.06	0.12 ± 0.06	0.09 ± 0.04	0.12 ± 0.07	0.11 ± 0.04	0.10 ± 0.06	0.05
96	0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.01
97	0.12 ± 0.06	0.13 ± 0.07	0.09 ± 0.04	0.09 ± 0.04	0.11 ± 0.06	0.12 ± 0.10	0.06
98	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01
99	0.06 ^{ab} ± 0.02	0.05 ^{ab} ± 0.02	0.05 ^b ± 0.02	0.05 ^{ab} ± 0.03	0.05 ^{ab} ± 0.02	0.07 ^a ± 0.04	0.02
100	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.01	0.01

Supplementary data

Table S8.1 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
101	0.21 ± 0.09	0.21 ± 0.09	0.23 ± 0.10	0.21 ± 0.09	0.19 ± 0.11	0.25 ± 0.24	0.11
102	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01
103	0.04 ^{ab} ± 0.02	0.04 ^{ab} ± 0.02	0.03 ^{ab} ± 0.02	0.03 ^b ± 0.01	0.05 ^a ± 0.02	0.04 ^{ab} ± 0.01	0.02
104	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.01
105	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.01
106	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.01 ± 0.02	0.01
107	0.07 ± 0.03	0.05 ± 0.03	0.05 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.04	0.03
108	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.02
109	0.24 ^{ab} ± 0.09	0.23 ^{ab} ± 0.04	0.18 ^b ± 0.04	0.26 ^a ± 0.06	0.25 ^{ab} ± 0.08	0.20 ^{ab} ± 0.13	0.07
110	0.03 ^a ± 0.01	0.03 ^a ± 0.01	0.01 ^b ± 0.01	0.02 ^{ab} ± 0.02	0.02 ^{ab} ± 0.01	0.02 ^{ab} ± 0.02	0.01
111	0.11 ± 0.05	0.10 ± 0.05	0.11 ± 0.06	0.11 ± 0.06	0.12 ± 0.07	0.14 ± 0.09	0.06
112	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01
113	0.03 ^{ab} ± 0.01	0.04 ^{ab} ± 0.01	0.05 ^a ± 0.02	0.03 ^b ± 0.02	0.05 ^a ± 0.02	0.04 ^{ab} ± 0.03	0.02
114	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01
115	0.11 ^{ab} ± 0.02	0.08 ^b ± 0.03	0.12 ^a ± 0.03	0.11 ^a ± 0.02	0.12 ^a ± 0.05	0.11 ^{ab} ± 0.04	0.03
116	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01
117	0.06 ± 0.03	0.05 ± 0.02	0.07 ± 0.05	0.05 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.03
118	0.01 ^{ab} ± 0.01	0.01 ^{abc} ± 0.01	0.01 ^{abc} ± 0.01	<0.01 ^{bc}	0.01 ^a ± 0.01	<0.01 ^c	0.01
119	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02
120	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.01
121	0.04 ^{ab} ± 0.01	0.04 ^{ab} ± 0.02	0.03 ^b ± 0.01	0.03 ^{ab} ± 0.01	0.04 ^a ± 0.02	0.04 ^{ab} ± 0.02	0.02
122	0.03 ^a ± 0.01	0.03 ^{ab} ± 0.01	0.02 ^b ± 0.01	0.02 ^{ab} ± 0.01	0.03 ^{ab} ± 0.01	0.02 ^b ± 0.01	0.01
123	0.04 ^{ab} ± 0.02	0.04 ^b ± 0.02	0.04 ^b ± 0.02	0.04 ^{ab} ± 0.03	0.04 ^b ± 0.03	0.06 ^a ± 0.03	0.02
124	0.20 ^b ± 0.04	0.21 ^{ab} ± 0.03	0.22 ^{ab} ± 0.03	0.25 ^a ± 0.05	0.22 ^{ab} ± 0.04	0.20 ^b ± 0.04	0.04
125	0.04 ± 0.03	0.05 ± 0.03	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.02
126	0.07 ± 0.03	0.06 ± 0.02	0.08 ± 0.03	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.02
127	0.07 ± 0.03	0.07 ± 0.04	0.06 ± 0.03	0.08 ± 0.03	0.09 ± 0.04	0.08 ± 0.05	0.03
128	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01
129	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.03	0.07 ± 0.02	0.10 ± 0.04	0.09 ± 0.05	0.03
130	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^b ± 0.00	0.01 ^a ± 0.01	0.01 ^{ab} ± 0.00	0.01
131	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01
132	<0.01 ^a	<0.01 ^c	<0.01 ^{bc}	<0.01 ^a	<0.01 ^{ab}	<0.01 ^{bc}	<0.01
133	0.01 ^b ± 0.01	0.02 ^a ± 0.02	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01
134	<0.01	0.01 ± 0.01	<0.01	0.01 ± 0.00	<0.01	<0.01	<0.01
135	0.04 ^{ab} ± 0.02	0.06 ^a ± 0.05	0.03 ^b ± 0.01	0.03 ^b ± 0.01	0.03 ^b ± 0.02	0.04 ^{ab} ± 0.02	0.02
136	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
137	0.12 ^{ab} ± 0.06	0.23 ^a ± 0.25	0.07 ^b ± 0.03	0.07 ^b ± 0.03	0.07 ^b ± 0.04	0.09 ^b ± 0.04	0.12
138	0.02 ^{ab} ± 0.01	0.03 ^a ± 0.03	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^{ab} ± 0.01	0.01

Supplementary data

Table S8.1 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
139	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.02
140	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	<0.01	0.01
141	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.02
142	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
143	0.09 ± 0.04	0.10 ± 0.04	0.08 ± 0.04	0.10 ± 0.02	0.09 ± 0.05	0.10 ± 0.05	0.04
144	0.02 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.01	0.02 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^b ± 0.00	0.01
145	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.01
146	<0.01	0.00 ± 0.01	<0.01	<0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
147	0.02 ^a ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.01
148	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
149	0.03 ^a ± 0.01	0.04 ^a ± 0.02	0.02 ^b ± 0.01	0.02 ^b ± 0.01	0.03 ^{ab} ± 0.01	0.03 ^{ab} ± 0.01	0.01
150	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	<0.01	<0.01	0.01
151	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.03	0.01
152	0.01 ± 0.00	0.01 ± 0.00	<0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	<0.01
153	0.04 ^a ± 0.02	0.03 ^{ab} ± 0.01	0.02 ^b ± 0.02	0.04 ^a ± 0.02	0.03 ^{ab} ± 0.01	0.03 ^{ab} ± 0.01	0.01
154	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	<0.01
155	0.03 ^{ab} ± 0.01	0.03 ^{ab} ± 0.01	0.04 ^a ± 0.01	0.02 ^{ab} ± 0.01	0.03 ^{ab} ± 0.02	0.02 ^b ± 0.01	0.01
156	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.01
157	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.02
158	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
159	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01
160	<0.01 ^b	0.01 ^b ± 0.01	0.01 ^a ± 0.00	0.01 ^b ± 0.00	<0.01 ^b	<0.01 ^b	<0.01

(SD) Standard Deviation; (PTR-MS) Proton transfer reaction-mass spectrometry; ^a Concentration is expressed as volume mixing ratio in air (parts per billion by volume). (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rùens; (SE) Semi-extensive; (LSD) Least significant difference; ^{a-d} Values in the same row for the ions with different superscripts are significantly different ($P \leq 0.05$); (nd) None detected.

Supplementary data

Table S8.2 The means (\pm SD) of the ions determined in PTR-MS analyses of the lamb fat from the different regions

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
22	0.04 \pm 0.04	0.04 \pm 0.03	0.05 \pm 0.03	0.05 \pm 0.03	0.02 \pm 0.03	0.04 \pm 0.03	0.03
23	0.02 \pm 0.02	0.01 \pm 0.02	0.00 \pm 0.01	0.01 \pm 0.01	0.01 \pm 0.02	0.01 \pm 0.01	0.02
24	nd	nd	nd	nd	nd	nd	-
25	nd	nd	nd	nd	nd	nd	-
26	0.01 ^b \pm 0.01	0.01 ^{ab} \pm 0.01	0.02 ^a \pm 0.01	0.01 ^{ab} \pm 0.01	0.01 ^{ab} \pm 0.01	0.01 ^{ab} \pm 0.01	0.01
27	0.09 ^{ab} \pm 0.05	0.07 ^{abc} \pm 0.05	0.10 ^a \pm 0.04	0.04 ^c \pm 0.02	0.03 ^c \pm 0.02	0.06 ^{bc} \pm 0.04	0.04
28	0.05 \pm 0.03	0.07 \pm 0.06	0.06 \pm 0.05	0.04 \pm 0.04	0.06 \pm 0.06	0.03 \pm 0.02	0.05
29	nd	nd	nd	nd	nd	nd	-
30	0.41 ^b \pm 0.68	1.00 ^{ab} \pm 1.65	1.34 ^{ab} \pm 1.96	1.11 ^{ab} \pm 1.41	2.06 ^a \pm 2.37	0.67 ^{ab} \pm 0.93	1.55
31	0.08 ^b \pm 0.17	0.11 ^{ab} \pm 0.16	0.25 ^a \pm 0.30	0.22 ^{ab} \pm 0.14	0.06 ^b \pm 0.11	0.14 ^{ab} \pm 0.18	0.17
33	30.91 \pm 12.44	33.36 \pm 8.51	31.27 \pm 4.82	33.85 \pm 5.61	39.48 \pm 10.80	39.51 \pm 11.08	9.47
34	0.01 \pm 0.05	nd	nd	nd	0.03 \pm 0.10	0.04 \pm 0.10	0.06
35	0.07 ^b \pm 0.05	0.09 ^b \pm 0.07	0.12 ^{ab} \pm 0.05	0.17 ^a \pm 0.09	0.10 ^b \pm 0.08	0.11 ^{ab} \pm 0.04	0.06
36	0.44 ^b \pm 0.17	0.28 ^{bc} \pm 0.29	0.15 ^c \pm 0.12	0.36 ^{bc} \pm 0.20	0.20 ^c \pm 0.15	0.87 ^a \pm 0.43	0.22
39	20.30 ^a \pm 2.00	19.42 ^a \pm 3.37	20.76 ^a \pm 3.08	18.75 ^{ab} \pm 2.18	16.75 ^b \pm 1.65	20.20 ^a \pm 3.74	2.52
40	0.17 ^a \pm 0.07	0.14 ^{ab} \pm 0.08	0.16 ^{ab} \pm 0.06	0.12 ^{ab} \pm 0.05	0.10 ^b \pm 0.06	0.16 ^a \pm 0.08	0.06
41	35.23 ^a \pm 9.48	31.33 ^{ab} \pm 17.43	32.64 ^{ab} \pm 13.41	19.81 ^c \pm 4.66	16.11 ^c \pm 3.77	23.47 ^{bc} \pm 5.67	10.64
42	7.83 ^{ab} \pm 6.72	5.13 ^b \pm 5.31	5.33 ^b \pm 2.69	5.80 ^{ab} \pm 3.57	5.92 ^{ab} \pm 4.39	10.40 ^a \pm 3.97	4.85
43	49.86 ^a \pm 11.50	31.94 ^{bc} \pm 19.74	53.74 ^a \pm 23.76	34.36 ^b \pm 14.07	18.38 ^c \pm 4.79	31.93 ^{bc} \pm 9.14	14.48
44	1.52 ^a \pm 0.40	1.00 ^b \pm 0.67	1.66 ^a \pm 0.68	1.05 ^b \pm 0.40	0.51 ^c \pm 0.17	0.83 ^{bc} \pm 0.31	0.46
45	14.11 \pm 5.17	17.77 \pm 13.97	17.55 \pm 11.97	14.39 \pm 6.38	18.94 \pm 11.63	13.92 \pm 6.49	9.85
46	0.09 \pm 0.13	0.21 \pm 0.30	0.17 \pm 0.20	0.15 \pm 0.16	0.20 \pm 0.23	0.19 \pm 0.19	0.21
47	4.83 ^{cd} \pm 2.92	2.94 ^d \pm 3.08	13.14 ^{ab} \pm 14.41	17.66 ^a \pm 8.88	9.50 ^{bc} \pm 4.93	6.65 ^{cd} \pm 5.61	6.29
48	0.15 ^b \pm 0.09	0.09 ^b \pm 0.08	0.40 ^a \pm 0.36	0.48 ^a \pm 0.27	0.21 ^b \pm 0.11	0.17 ^b \pm 0.15	0.17
49	0.11 ^b \pm 0.09	0.20 ^{ab} \pm 0.19	0.24 ^a \pm 0.15	0.19 ^{ab} \pm 0.09	0.18 ^{ab} \pm 0.09	0.15 ^{ab} \pm 0.06	0.12
50	0.02 ^{abc} \pm 0.02	0.03 ^{abc} \pm 0.04	0.00 ^c \pm 0.01	0.05 ^a \pm 0.05	0.01 ^{bc} \pm 0.02	0.04 ^{ab} \pm 0.07	0.04
51	0.48 \pm 0.18	0.44 \pm 0.13	0.46 \pm 0.10	0.46 \pm 0.08	0.50 \pm 0.13	0.55 \pm 0.11	0.13
52	0.02 \pm 0.02	0.01 \pm 0.02	0.03 \pm 0.02	0.02 \pm 0.02	0.03 \pm 0.03	0.01 \pm 0.02	0.02
53	0.06 ^{ab} \pm 0.05	0.11 ^a \pm 0.11	0.04 ^b \pm 0.02	0.05 ^{ab} \pm 0.02	0.04 ^b \pm 0.03	0.05 ^b \pm 0.05	0.06
54	0.02 ^{ab} \pm 0.02	0.02 ^{ab} \pm 0.02	0.01 ^b \pm 0.01	0.03 ^a \pm 0.03	0.02 ^{ab} \pm 0.02	0.04 ^a \pm 0.02	0.02
55	10.57 ^{ab} \pm 2.79	13.50 ^a \pm 5.75	9.27 ^b \pm 2.09	9.87 ^b \pm 1.68	9.06 ^b \pm 1.08	9.48 ^b \pm 1.46	3.06
56	0.15 ^{ab} \pm 0.19	0.29 ^a \pm 0.30	0.07 ^b \pm 0.05	0.05 ^b \pm 0.04	0.09 ^b \pm 0.11	0.10 ^b \pm 0.08	2.01
57	16.09 ^b \pm 2.60	27.51 ^a \pm 17.37	9.24 ^b \pm 1.79	11.87 ^b \pm 2.72	12.92 ^b \pm 3.05	14.08 ^b \pm 2.67	7.92
58	0.71 ^b \pm 0.15	1.19 ^a \pm 0.79	0.42 ^b \pm 0.15	0.44 ^b \pm 0.20	0.51 ^b \pm 0.15	0.53 ^b \pm 0.17	0.37
59	158.38 ^{bc} \pm 87.56	141.61 ^{bc} \pm 88.27	214.73 ^{ab} \pm 111.67	269.11 ^a \pm 109.51	105.04 ^c \pm 29.29	97.14 ^c \pm 43.57	78.89
60	5.34 ^{bc} \pm 3.01	4.78 ^{bc} \pm 3.03	7.47 ^{ab} \pm 3.88	9.11 ^a \pm 3.71	3.52 ^c \pm 0.89	3.40 ^c \pm 1.63	2.71
61	0.52 ^{bc} \pm 0.84	0.55 ^{bc} \pm 0.96	1.23 ^{ab} \pm 0.90	0.51 ^{bc} \pm 1.17	0.08 ^c \pm 0.19	1.80 ^a \pm 1.83	0.95
62	0.11 ^b \pm 0.06	0.36 ^a \pm 0.26	0.42 ^a \pm 0.24	0.27 ^{ab} \pm 0.20	0.12 ^b \pm 0.11	0.11 ^b \pm 0.09	0.16

Supplementary data

Table S8.2 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
63	0.16 ^{ab} ± 0.22	0.16 ^{ab} ± 0.18	0.11 ^b ± 0.07	0.11 ^b ± 0.13	0.08 ^b ± 0.13	0.30 ^a ± 0.23	0.17
64	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01
65	0.09 ^c ± 0.04	0.07 ^c ± 0.06	0.27 ^{ab} ± 0.30	0.31 ^a ± 0.13	0.15 ^{bc} ± 0.08	0.11 ^c ± 0.10	0.12
66	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	<0.01	0.01
67	0.19 ^{ab} ± 0.14	0.26 ^a ± 0.24	0.05 ^c ± 0.03	0.07 ^{bc} ± 0.04	0.11 ^{bc} ± 0.04	0.06 ^c ± 0.04	0.13
68	0.03 ^{ab} ± 0.02	0.06 ^a ± 0.06	0.02 ^b ± 0.01	0.02 ^b ± 0.02	0.04 ^{ab} ± 0.02	0.02 ^b ± 0.01	0.03
69	3.52 ^b ± 2.39	6.52 ^a ± 5.03	1.80 ^b ± 0.89	2.15 ^b ± 0.83	3.32 ^b ± 1.33	2.69 ^b ± 0.77	2.62
70	0.40 ^{ab} ± 0.29	0.56 ^a ± 0.47	0.18 ^{bc} ± 0.08	0.14 ^c ± 0.05	0.27 ^{bc} ± 0.12	0.19 ^{bc} ± 0.05	0.26
71	8.49 ± 5.25	7.81 ± 8.46	8.09 ± 7.28	6.85 ± 6.17	3.99 ± 2.36	4.37 ± 1.50	5.58
72	0.43 ± 0.24	0.32 ± 0.28	0.39 ± 0.33	0.33 ± 0.31	0.22 ± 0.12	0.21 ± 0.10	0.23
73	4.24 ± 1.17	4.13 ± 1.08	4.17 ± 1.11	3.80 ± 1.03	3.47 ± 0.81	3.58 ± 0.95	1.00
74	0.16 ^{ab} ± 0.08	0.18 ^{ab} ± 0.05	0.21 ^a ± 0.06	0.17 ^{ab} ± 0.06	0.14 ^b ± 0.05	0.18 ^{ab} ± 0.09	0.06
75	0.12 ± 0.12	0.16 ± 0.16	0.28 ± 0.13	0.16 ± 0.09	0.18 ± 0.22	0.27 ± 0.15	0.15
76	0.03 ^b ± 0.03	0.05 ^b ± 0.04	0.17 ^a ± 0.09	0.01 ^b ± 0.01	0.05 ^b ± 0.03	0.02 ^b ± 0.02	0.04
77	0.08 ^{bc} ± 0.07	0.14 ^b ± 0.05	0.24 ^a ± 0.10	0.13 ^b ± 0.07	0.09 ^{bc} ± 0.04	0.06 ^c ± 0.04	0.06
78	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.02	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.02	0.01
79	0.07 ^{abc} ± 0.04	0.08 ^{ab} ± 0.07	0.04 ^{bc} ± 0.04	0.03 ^{bc} ± 0.04	0.09 ^a ± 0.05	0.06 ^{abc} ± 0.03	0.05
80	0.05 ^{ab} ± 0.02	0.07 ^a ± 0.04	0.04 ^{ab} ± 0.02	0.03 ^b ± 0.03	0.05 ^{ab} ± 0.02	0.07 ^a ± 0.05	0.03
81	4.21 ^{ab} ± 3.85	8.63 ^a ± 10.97	0.12 ^b ± 0.05	0.74 ^b ± 0.38	0.19 ^b ± 0.07	0.77 ^b ± 0.88	5.16
82	0.30 ^{ab} ± 0.26	0.60 ^a ± 0.73	0.01 ^b ± 0.01	0.06 ^b ± 0.03	0.02 ^b ± 0.01	0.07 ^b ± 0.07	0.35
83	2.59 ^{ab} ± 3.52	5.78 ^a ± 6.82	0.47 ^b ± 0.42	0.53 ^b ± 0.72	0.66 ^b ± 0.36	0.69 ^b ± 0.25	3.46
84	0.18 ^{ab} ± 0.23	0.36 ^a ± 0.46	0.04 ^b ± 0.03	0.04 ^b ± 0.04	0.05 ^b ± 0.02	0.05 ^b ± 0.05	0.23
85	0.61 ^b ± 0.26	1.42 ^a ± 1.22	0.28 ^b ± 0.06	0.40 ^b ± 0.19	0.49 ^b ± 0.14	0.48 ^b ± 0.13	0.56
86	0.05 ^b ± 0.03	0.09 ^a ± 0.06	0.02 ^b ± 0.01	0.03 ^b ± 0.03	0.05 ^b ± 0.02	0.03 ^b ± 0.02	0.03
87	2.83 ± 1.49	2.56 ± 1.52	3.16 ± 1.38	3.14 ± 0.89	2.89 ± 0.96	2.65 ± 1.01	1.24
88	0.15 ± 0.09	0.12 ± 0.08	0.15 ± 0.08	0.16 ± 0.03	0.13 ± 0.06	0.12 ± 0.07	0.07
89	5.07 ^a ± 4.62	0.66 ^b ± 0.99	3.75 ^{ab} ± 2.74	4.78 ^a ± 5.21	1.17 ^b ± 1.44	2.36 ^{ab} ± 1.40	3.15
90	0.22 ^{ab} ± 0.23	0.03 ^c ± 0.03	0.29 ^a ± 0.27	0.18 ^{abc} ± 0.20	0.06 ^{bc} ± 0.09	0.10 ^{bc} ± 0.05	0.17
91	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.03	nd	0.00 ± 0.02	0.00 ± 0.01	0.02
92	0.04 ^{ab} ± 0.03	0.07 ^a ± 0.07	0.01 ^b ± 0.01	0.03 ^b ± 0.02	0.04 ^{ab} ± 0.03	0.01 ^b ± 0.00	0.04
93	1.64 ^{bc} ± 1.54	3.91 ^a ± 3.68	0.11 ^c ± 0.13	0.72 ^{bc} ± 0.97	2.78 ^{ab} ± 2.57	0.55 ^c ± 1.07	2.15
94	0.16 ^{bc} ± 0.09	0.38 ^a ± 0.28	0.06 ^c ± 0.04	0.09 ^c ± 0.06	0.29 ^{ab} ± 0.24	0.09 ^c ± 0.09	0.17
95	0.22 ^b ± 0.22	0.64 ^a ± 0.84	0.03 ^b ± 0.03	0.03 ^b ± 0.03	0.05 ^b ± 0.04	0.08 ^b ± 0.08	0.38
96	0.04 ^b ± 0.04	0.09 ^a ± 0.05	0.02 ^b ± 0.02	0.03 ^b ± 0.02	0.04 ^b ± 0.03	0.03 ^b ± 0.04	0.04
97	0.28 ^{ab} ± 0.24	0.53 ^a ± 0.67	0.03 ^b ± 0.03	0.07 ^b ± 0.06	0.10 ^b ± 0.10	0.11 ^b ± 0.07	0.32
98	0.02 ^b ± 0.01	0.05 ^a ± 0.04	0.02 ^b ± 0.01	0.02 ^b ± 0.02	0.02 ^b ± 0.01	0.02 ^b ± 0.02	0.02
99	0.09 ^a ± 0.05	0.09 ^a ± 0.04	0.03 ^b ± 0.02	0.04 ^b ± 0.02	0.07 ^{ab} ± 0.04	0.06 ^{ab} ± 0.04	0.04
100	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01

Supplementary data

Table S8.2 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
101	0.35 ^b ± 0.36	0.61 ^{ab} ± 0.39	0.40 ^b ± 0.30	0.34 ^b ± 0.24	0.43 ^{ab} ± 0.33	0.79 ^a ± 0.70	0.37
102	0.03 ± 0.03	0.05 ± 0.04	0.03 ± 0.02	0.03 ± 0.03	0.03 ± 0.03	0.05 ± 0.04	0.03
103	0.09 ± 0.10	0.14 ± 0.12	0.10 ± 0.09	0.15 ± 0.13	0.12 ± 0.11	0.16 ± 0.09	0.10
104	0.02 ± 0.03	0.01 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02
105	0.09 ^a ± 0.04	0.02 ^b ± 0.02	0.02 ^b ± 0.03	0.02 ^b ± 0.01	0.03 ^b ± 0.02	0.11 ^a ± 0.04	0.03
106	0.02 ^{ab} ± 0.03	0.01 ^{ab} ± 0.01	0.03 ^a ± 0.03	0.00 ^b ± 0.01	0.03 ^{ab} ± 0.03	0.00 ^b ± 0.01	0.02
107	0.04 ^a ± 0.04	0.04 ^a ± 0.03	0.03 ^{ab} ± 0.03	0.00 ^b ± 0.01	0.03 ^{ab} ± 0.03	0.04 ^a ± 0.04	0.03
108	0.01 ^{ab} ± 0.01	0.00 ^b ± 0.01	0.01 ^a ± 0.02	0.01 ^b ± 0.00	0.01 ^{ab} ± 0.01	0.00 ^b ± 0.01	0.01
109	0.23 ^b ± 0.10	0.35 ^a ± 0.20	0.16 ^b ± 0.08	0.18 ^b ± 0.08	0.20 ^b ± 0.10	0.18 ^b ± 0.08	0.12
110	0.02 ^{ab} ± 0.02	0.03 ^a ± 0.02	0.01 ^b ± 0.01	0.01 ^{ab} ± 0.02	0.02 ^{ab} ± 0.01	0.02 ^{ab} ± 0.02	0.02
111	0.16 ± 0.07	0.13 ± 0.07	0.11 ± 0.07	0.11 ± 0.06	0.16 ± 0.09	0.13 ± 0.05	0.07
112	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.01
113	0.04 ^{bc} ± 0.03	0.05 ^{ab} ± 0.04	0.01 ^c ± 0.02	0.04 ^{bc} ± 0.02	0.08 ^a ± 0.04	0.05 ^b ± 0.02	0.03
114	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01
115	0.11 ± 0.05	0.11 ± 0.08	0.10 ± 0.04	0.11 ± 0.06	0.07 ± 0.04	0.12 ± 0.08	0.06
116	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01
117	0.07 ± 0.08	0.07 ± 0.06	0.12 ± 0.12	0.12 ± 0.12	0.09 ± 0.12	0.15 ± 0.18	0.10
118	0.01 ^{ab} ± 0.01	0.00 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.01	0.01 ^{ab} ± 0.02	0.01 ^{ab} ± 0.01	0.01
119	0.08 ^a ± 0.05	0.04 ^{ab} ± 0.05	0.02 ^b ± 0.02	0.03 ^b ± 0.03	0.04 ^b ± 0.04	0.05 ^{ab} ± 0.05	0.04
120	0.01 ^b ± 0.02	0.04 ^a ± 0.03	0.02 ^{ab} ± 0.02	0.01 ^b ± 0.01	0.01 ^b ± 0.02	0.01 ^b ± 0.02	0.02
121	0.04 ^{ab} ± 0.05	0.07 ^a ± 0.05	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.02 ^b ± 0.02	0.01 ^b ± 0.01	0.04
122	0.01 ^b ± 0.01	0.03 ^a ± 0.02	0.03 ^{ab} ± 0.02	0.02 ^{ab} ± 0.01	0.02 ^{ab} ± 0.02	0.02 ^{ab} ± 0.02	0.02
123	0.03 ^{ab} ± 0.03	0.06 ^a ± 0.06	0.02 ^b ± 0.03	0.01 ^b ± 0.02	0.02 ^b ± 0.02	0.03 ^{ab} ± 0.03	0.03
124	0.25 ± 0.08	0.23 ± 0.09	0.22 ± 0.11	0.23 ± 0.07	0.18 ± 0.08	0.21 ± 0.08	0.08
125	0.04 ± 0.03	0.04 ± 0.03	0.03 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.02
126	0.07 ± 0.04	0.05 ± 0.04	0.05 ± 0.05	0.05 ± 0.05	0.07 ± 0.04	0.05 ± 0.05	0.04
127	0.03 ^b ± 0.03	0.05 ^{ab} ± 0.04	0.04 ^{ab} ± 0.03	0.04 ^{ab} ± 0.03	0.07 ^a ± 0.05	0.06 ^{ab} ± 0.02	0.04
128	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
129	0.06 ^b ± 0.06	0.06 ^b ± 0.06	0.07 ^{ab} ± 0.07	0.08 ^{ab} ± 0.05	0.12 ^a ± 0.06	0.09 ^{ab} ± 0.02	0.05
130	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01
131	0.03 ^{ab} ± 0.03	0.03 ^{ab} ± 0.03	0.05 ^a ± 0.05	0.05 ^{ab} ± 0.03	0.02 ^b ± 0.02	0.05 ^{ab} ± 0.05	0.03
132	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	<0.01	0.00 ± 0.01	0.01
133	0.04 ^{ab} ± 0.08	0.09 ^a ± 0.12	0.00 ^b ± 0.01	nd	0.01 ^b ± 0.01	0.03 ^{ab} ± 0.05	0.07
134	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.02	0.00 ^b ± 0.01	0.00 ^b ± 0.00	0.00 ^b ± 0.00	0.00 ^b ± 0.01	0.01
135	0.17 ^{ab} ± 0.17	0.40 ^a ± 0.52	0.01 ^b ± 0.01	0.03 ^b ± 0.03	0.01 ^b ± 0.01	0.06 ^b ± 0.05	0.24
136	0.02 ^{ab} ± 0.02	0.03 ^a ± 0.03	0.01 ^b ± 0.01	0.02			
137	3.13 ^{ab} ± 2.84	6.13 ^a ± 7.82	0.06 ^b ± 0.03	0.52 ^b ± 0.33	0.08 ^b ± 0.06	0.55 ^b ± 0.73	3.70
138	0.34 ^{ab} ± 0.28	0.72 ^a ± 0.89	0.02 ^b ± 0.01	0.05 ^b ± 0.04	0.01 ^b ± 0.02	0.04 ^b ± 0.06	0.41

Supplementary data

Table S8.2 (Continued)

Ions	Volatile compound headspace concentration (ppbv) ^a						LSD
	CK	HK	NK	BL	RU	SE	P = 0.05
139	0.06 ^{ab} ± 0.09	0.12 ^a ± 0.18	0.00 ^b ± 0.01	0.02 ^b ± 0.02	0.01 ^b ± 0.02	0.01 ^b ± 0.01	0.09
140	0.01 ^{ab} ± 0.01	0.01 ^a ± 0.02	0.00 ^b ± 0.00	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01
141	0.02 ^b ± 0.02	0.11 ^a ± 0.11	0.03 ^b ± 0.02	0.03 ^b ± 0.02	0.02 ^b ± 0.02	0.04 ^b ± 0.02	0.05
142	0.01 ^b ± 0.01	0.02 ^a ± 0.03	0.01 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01
143	0.07 ± 0.07	0.10 ± 0.08	0.05 ± 0.04	0.08 ± 0.08	0.09 ± 0.05	0.09 ± 0.03	0.06
144	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.01
145	0.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	0.03 ± 0.03	0.04 ± 0.04	0.04 ± 0.03	0.03
146	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.00 ^b ± 0.01	0.02 ^a ± 0.02	0.01
147	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.02	0.01
148	0.01 ^{ab} ± 0.01	0.00 ^b ± 0.01	0.00 ^{ab} ± 0.01	nd	0.00 ^{ab} ± 0.01	0.01 ^a ± 0.01	0.01
149	0.03 ^{ab} ± 0.02	0.04 ^a ± 0.05	0.01 ^b ± 0.03	0.00 ^b ± 0.01	0.02 ^{ab} ± 0.02	0.02 ^{ab} ± 0.02	0.03
150	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	<0.01	0.00 ± 0.01	0.01 ± 0.01	0.01
151	0.02 ^{ab} ± 0.02	0.02 ^{ab} ± 0.01	0.01 ^{ab} ± 0.01	0.02 ^a ± 0.02	0.01 ^b ± 0.01	0.02 ^{ab} ± 0.01	0.01
152	0.01 ± 0.01	0.01 ± 0.02	<0.01	0.01 ± 0.01	0.01 ± 0.01	nd	0.01
153	0.04 ^{ab} ± 0.03	0.05 ^a ± 0.05	0.01 ^b ± 0.01	0.02 ^{ab} ± 0.03	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.03
154	0.01 ± 0.01	0.00 ± 0.01	<0.01	0.00 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.01
155	0.01 ^c ± 0.01	0.04 ^a ± 0.03	0.01 ^c ± 0.01	0.03 ^{ab} ± 0.03	0.02 ^{bc} ± 0.02	0.03 ^{abc} ± 0.02	0.02
156	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01
157	0.01 ^b ± 0.01	0.04 ^a ± 0.03	0.02 ^b ± 0.01	0.02 ^b ± 0.03	0.02 ^b ± 0.01	0.03 ^{ab} ± 0.03	0.02
158	0.00 ^b ± 0.00	0.02 ^a ± 0.02	0.00 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.00 ^b ± 0.01	0.01 ^{ab} ± 0.01	0.01
159	0.01 ^b ± 0.01	0.02 ^{ab} ± 0.01	0.01 ^{ab} ± 0.02	0.02 ^a ± 0.02	0.01 ^{ab} ± 0.02	0.01 ^{ab} ± 0.01	0.01
160	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	nd	<0.01	nd	<0.01

(SD) Standard Deviation; (PTR-MS) Proton transfer reaction-mass spectrometry; ^a Concentration is expressed as volume mixing ratio in air (parts per billion by volume). (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern Karoo; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive; (LSD) Least significant difference; ^{a-d} Values in the same row for the ions with different superscripts are significantly different ($P \leq 0.05$); (nd) None detected.

Chapter 9

General discussion and conclusions

The significance of food authentication is related to the need to verify the exact nature of a product (Dennis & Ashurst, 1996). The authentication of meat products is particularly important to enforcement bodies and food producers or traders who want to ensure that consumers are not misled (Dennis & Ashurst, 1996; Sentandreu & Sentandreu, 2014). Unfortunately, fraudulent activities occur on a regular basis as entities alter the nature of a product or misuse the name for capital gain. In the meat industry, the main areas susceptible to fraud are region of origin meat products, substitutions of meat ingredients by other animal species and/or non-meat components and changes to the processing and/or production method of meat products (Dennis & Ashurst, 1996; Ballin, 2010). Globally species-specific meat types, traditionally produced in a defined origin, are gaining popularity. The identification of a product in terms of its geographical origin and production or processing parameters linked to it, forms part of a successful marketing strategy (Perini *et al.*, 2009). It allows producers to obtain market recognition as the products are recognised and appreciated by consumers for their “holistic”, “natural”, and “superior” quality. These characteristics are utilised through marketing and they may even be sold at a premium price. In effect, food fraud can occur through the mislabelling of products not even related to the designated origin. Hence, there is a need that the labelling of regional products is supported by analyses which confirm the origin (Dennis & Ashurst, 1996).

According to the European Union (Chapter 2), protected lamb meat types and regional products are increasingly receiving consumer attention, yet there is still a lack of scientific evidence to verify the quality claims (i.e. the unique aroma and flavour attributes related to diet/origin) and the authentic nature of the products. The lack of scientific evidence is also true for the authenticity of regionally unique South African lamb, such as Karoo lamb. Karoo lamb is recognised as a *Protected Geographical Indication* (PGI), i.e. where its name is linked to quality (Bramley *et al.*, 2013). In fact, it is the first red meat product to receive PGI status in South Africa. The quality is associated with its extensive production system, where animals are raised on the natural veld which consists of unique Karoo plants species – believed to be responsible for the unique aroma and flavour of Karoo lamb (Estler *et al.*, 2006). Given that Karoo lamb is linked with a quality meat product, the risk exists that its name may be commercially exploited, especially since origin-based claims are becoming increasingly important in the marketing of meat (Kirsten *et al.*, 2012). Therefore, to ensure enforcement and better policing it is critical to establish methods to determine the authenticity of meat.

To confirm the origin of regional products, more attention is given to the development of analytical tools for the authentication of food products. These tools are very important as they provide a means by which producers of unique food products can differentiate their products. The authenticity of food products is largely confirmed through the measurement of multiple markers and/or complex chemical or physical profiles/fingerprints supported by multivariate statistical analysis opposed to the use of a single tool (Capuano *et al.*, 2013). In the past, various reviews have been published on the techniques used for this purpose. For

instance, Franke *et al.* (2005) reviewed analytical developments with respect to the determination of the geographic origin of raw meat. Analytical techniques are used to determine the authentic nature of meat with special emphasis being placed on methods which allow fast, cost-effective and non-destructive monitoring of such products at either farm, abattoir or supermarket level. The need for an instrumental technique that can link the composition (i.e. chemical compound or fingerprint) of lamb meat to its characteristic qualities and origin would improve the detection of fraud on various commercial levels (Dennis & Ashurst, 1996). A broad range of analytical techniques are currently used for the authentication of food products. These consist of DNA based techniques, enzymatic methods, spectroscopy, histology and image analysis, bio-imaging, multi-element analyses, lipid profiling, volatile organic compound profiling, sensory profiling methods, proteomic methods and metabolomics. This study focussed on a few of these techniques to determine the origin of regionally unique South African lamb and characterise the sensory and chemical profiles.

The crucial factor defining the authenticity of extensively produced South African lamb is the diet of the sheep. Diet is a key factor as it causes variation in meat composition which affects the type and amount of precursors formed. The precursors are responsible for the ultimate development of volatile aroma compounds influencing the flavour and aroma of the meat and fat. For the production of Karoo lamb, the extensive grazing of sheep on the natural Karoo veld is fundamental to the authentic nature of the meat. In effect, lamb meat is not permitted to be sold as Karoo lamb if it has been raised in a feedlot or a Non-Karoo region. In this study, the feeding regimes of the lambs were traced through fingerprinting techniques using the spectral properties, sensory attributes, fatty acids, volatiles and stable isotopes of the meat. The analytical techniques were selected in order to validate the origin-based link and identify potential biomarkers to confirm the origin. For an accurate verification of geographic origin of meat, the best approach is to apply a combination of parameters and techniques. In the event that labelled region of origin lamb meat is not falsely classified, producers should be monitored to confirm that the requirements for certification are met.

This study spanned over a period of 4 years and included two datasets. The dataset of the first two years (phase 1) consisted of 70 lambs sourced from 7 farm (10 animals per farm). The dataset of the last two years (phase 2) consisted of 201 lamb sourced from 67 farms (3 animals per farm). The aim of phase 1 was to compare the sensory and chemical profiles of South African lamb meat from different geographical origins and determine the dietary effect thereupon. Phase 2 focussed on the use of analytical techniques to authenticate the meat with the prospect of developing a rapid and effective origin based testing method and the first baseline database for regionally unique South African lamb. When the results of the first and second phase of the study are explored and mapped, several trends within the different regions can be seen (Fig. 9.1 to 9.6).

Sensory profile and key volatiles

The sensory research performed in Chapter 3 laid the foundation for the study. The variation in the sensory profiles of lambs from different farms confirmed the effect of diet. The key sensory attributes were the

herbaceous aroma and flavour attributes. In fact, their significance is related to a diet rich in fragrant plants. As a result, the subsequent chapters focused on determining which chemical components are responsible for the sensory variation. Terpenes are the volatiles of importance for the authentication of Karoo vs. Non-Karoo lamb. Characteristic terpenes were detected in Chapter 4 and 8. The importance of terpenes did not come as a surprise given that they are plant secondary metabolites, transferred from forage to meat (Larick *et al.*, 1987). The transfer therefore links the diet to the meat and fat. Terpenes have been suggested as suitable biomarkers or indicators of the geographical origin (Cornu *et al.*, 2001). Although biomarkers have been used for the indication of grass feeding as opposed to concentrate feeding, several hurdles exist for its use as a tool for the authentication of Karoo lamb. Biomarkers characteristic of plants may be found in the meat and fat of animals raised on various types of vegetation. Identification of biomarkers unique to Karoo bushes is important and these can only be linked to that region.

In Chapter 4 the terpenes of importance were α -pinene, β -pinene and limonene. These volatiles are known to have typical aromas associated with pine trees, rosemary (*Rosmarinus officinalis*), lemon, turpentine and citrus (Calkins & Hodgen, 2007). They were also detected in the Karoo bushes (Chapter 4). The Karoo bushes were collected from the different regions and their fatty acid, as well as, volatile profiles were determined (Chapter 4). The Karoo lamb meat samples contained more terpenes than the Non-Karoo lamb meat samples with none detected for Rûens (RU) and only two detected for Free State (FS) (Chapter 4). Hantam Karoo/Calvinia (HK/CAL) had the highest ($P \leq 0.05$) concentrations of α -pinene, β -pinene and limonene (Chapter 4). These findings were confirmed with proton-transfer reaction mass spectrometry (PTR-MS) using a larger sample set of lambs from different farms within the defined regions. Ions m/z 81 and m/z 137, indicative of terpenes, were predominant in the meat and fat of Karoo lamb samples and fragrant Karoo bushes (Chapter 8). Thus, the dominant volatiles in the lamb fat and herbaceous plants were linked. In agreement with the results of Chapter 3, the concentration of ions m/z 81 and m/z 137 were higher in the fat compared to the meat. The herbaceous flavour of the meat (Chapter 3) combined with m/z 81 and m/z 137 of the lamb meat samples (Chapter 8) are shown in Figure 9.1, while that of the fat are shown in Figure 9.2. It is evident that for both datasets (phase 1 and phase 2), the Hantam Karoo contained a greater concentration of terpenes and had a more prominent herbaceous aroma and flavour. Although it may appear that the concentrations were lower in Figure 9.4, when the values according to the legends are taken into account the fat had higher values than the meat. It is also apparent that the difference between Karoo and Non-Karoo in concentration of the ions was greater for the fat compared to the meat (Fig. 9.1 and 9.2). As a result, the fat is more discriminative in terms of origin classification and also a better sample to source for analysis with the terpenes as markers for Karoo origin.

Another terpene biomarker identified was caryophyllene (Chapter 4). In a different study, this volatile was never detected in the fat of animals raised and finished on concentrates (Priolo *et al.*, 2004). Its importance as a tracer compound is linked to the fact that it is hypothetically metabolised or eliminated during the stall-feeding period, its concentration lowered with new fat formation or only deposited in the period before

slaughtering (Priolo *et al.*, 2004). In the study trans-caryophyllene was detected in all the samples, except for RU (Chapter 4). Again the highest ($P \leq 0.05$) concentration was detected in HK/CAL meat. The reason for its absence in RU meat is again linked to diet as caryophyllene is not a main volatile for lucerne (*Medicago sativa*), while it is a predominant volatile in some of the Karoo and Free State plants' samples (Chapter 4).

In Figure 9.3 and 9.4 the herbaceous aroma of the fat (Chapter 3), relative concentration of α/β -pinene (Fig. 9.3) or caryophyllene (Fig. 9.4) of the meat (Chapter 4) and m/z 137 of the lamb fat samples (Chapter 8) are shown. Although the concentration of caryophyllene was lower (Fig. 9.4) than that of α/β -pinene (Fig. 9.3), both maps show the same trend. Overall the Karoo samples have a higher rating for herbaceous aroma and a higher concentration of terpenes. Within the Karoo region, lamb meat sourced from the Hantam Karoo had a prominent herbaceous profile, while that of the other Karoo regions were less prominent in this flavour attribute. This is as a result of the vegetation and typical diet of the animals within the regions where that of Hantam Karoo consists mostly of herbaceous Karoo bushes and shrubs, while more grass species are present in the other regions. It can be postulated that the grass acts as a "diluting agent" or modulator, thereby reducing the herbaceous effect of the bushes.

An aspect not explored in the study is the consumer's liking towards Karoo and Non-Karoo lamb meat. Depending on the consumer, the herbaceous notes might be perceived as being negative or positive. Also, given the clear distinction between the Rûens and Karoo lamb, can the consumer distinguish Karoo lamb from Non-Karoo lamb? It would be of value to explore the consumer's degree of liking towards Rûens lamb. If there is a defined market for Rûens lamb, the possibility for its certification and protection (like Karoo lamb) would be created. These aspects should be explored in future studies using advanced statistical regression techniques..

Furthermore, it is essential to establish the required period of grazing on Karoo veld in order for the volatiles to be detected in the meat and fat. According to the regulations (DTI, 2013), Karoo lamb should originate from the Karoo (born in) or, the ones born outside the Karoo, should remain in the Karoo (on the natural veld) for a continuous period of 6 months before slaughter. It would be of value to determine how the level of volatiles derived from the Karoo plants in the meat and fat, and the typical sensory profile of the meat and fat varies within the 6 months. If the typical sensory quality is reached after a shorter period, the possibility exist that the 6-month period could be reduced. The effect of feed supplementation should also be explored. Currently, additional feeding supplements may be given to Karoo lamb during the reproductive cycle and dry spells, provided that it does not exceed 30% of the daily requirements of the lamb and is given when the lambs are grazing in the Karoo veld (DTI, 2013). The effect of the amount of supplementation given, the period it is given and the type of feed supplementation given should be investigated. Not only would the effect of supplementation on the sensory quality be determined, but also transition from Karoo to Non-Karoo lamb

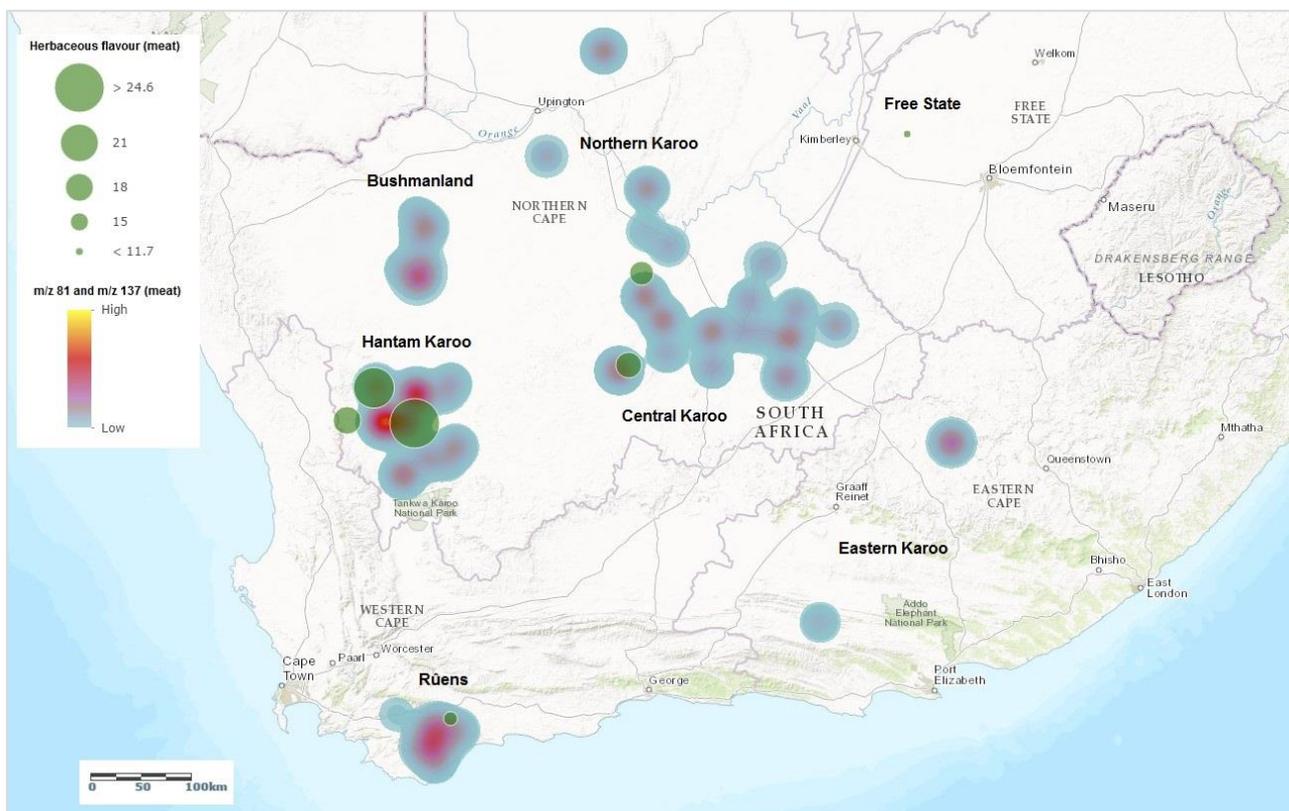


Figure 9.1 Herbaceous flavour of the meat (Chapter 3) combined with m/z 81 and m/z 137 of the lamb meat samples (Chapter 8).

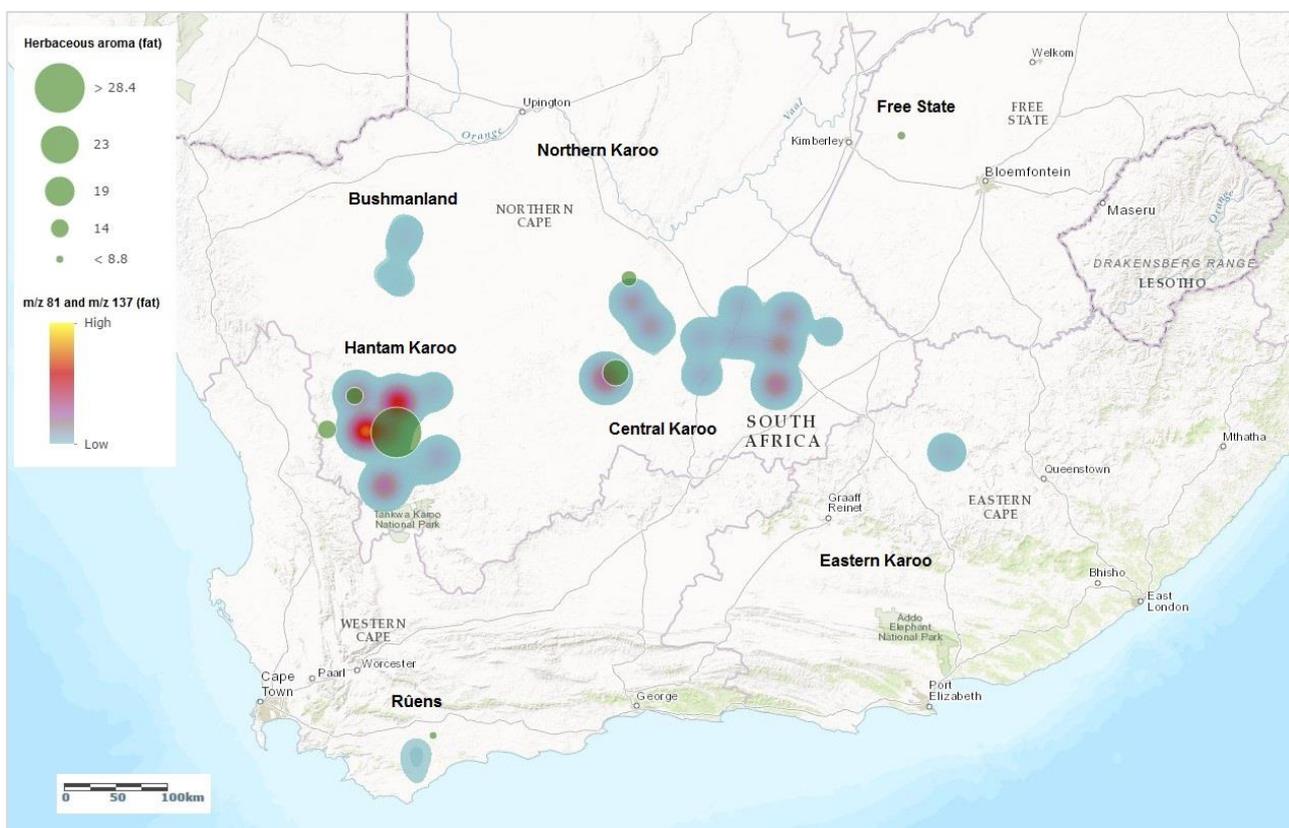


Figure 9.2 Herbaceous aroma of the lamb fat (Chapter 3) combined with m/z 81 and m/z 137 of the lamb fat samples (Chapter 8).

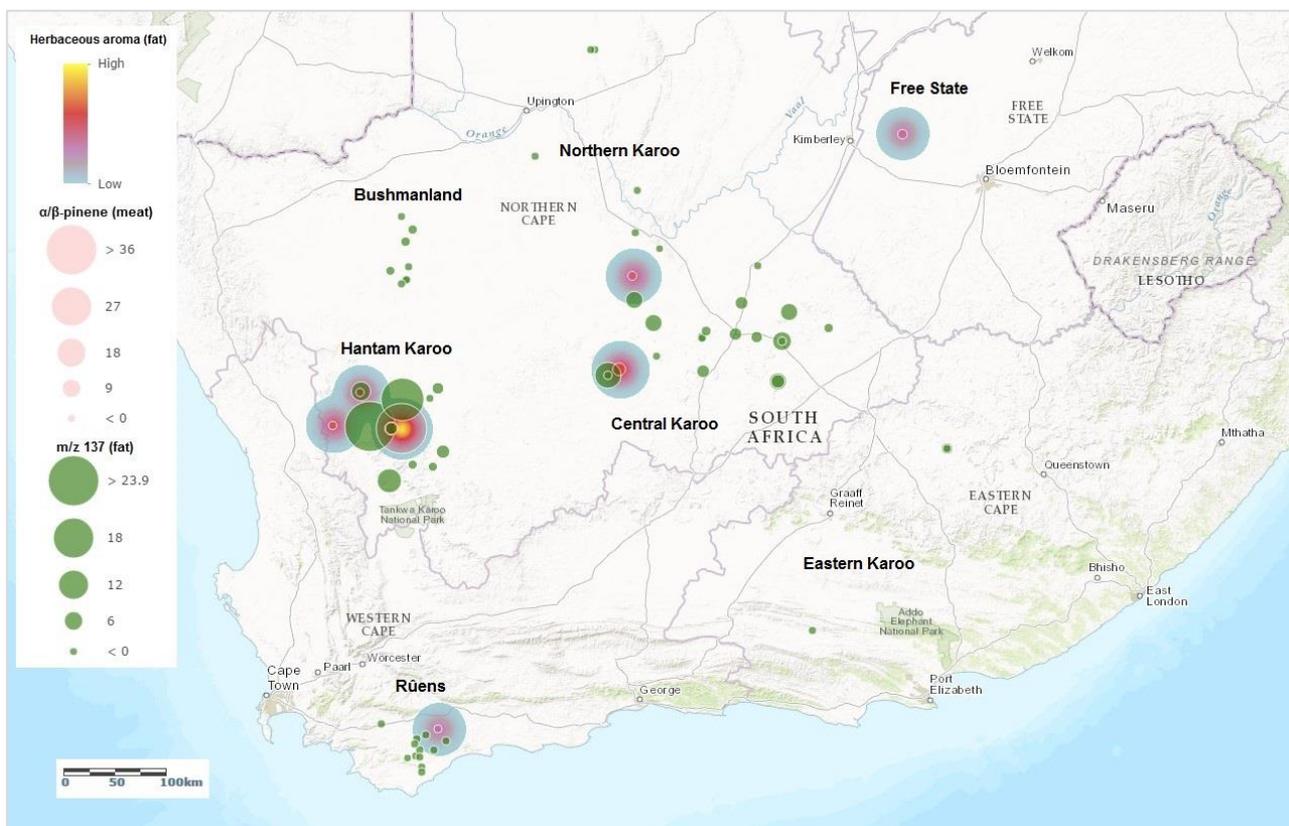


Figure 9.3 Herbaceous aroma of the lamb fat (Chapter 3), relative concentration of α/β -pinene of the lamb meat (Chapter 4) and m/z 137 of the lamb fat samples (Chapter 8).

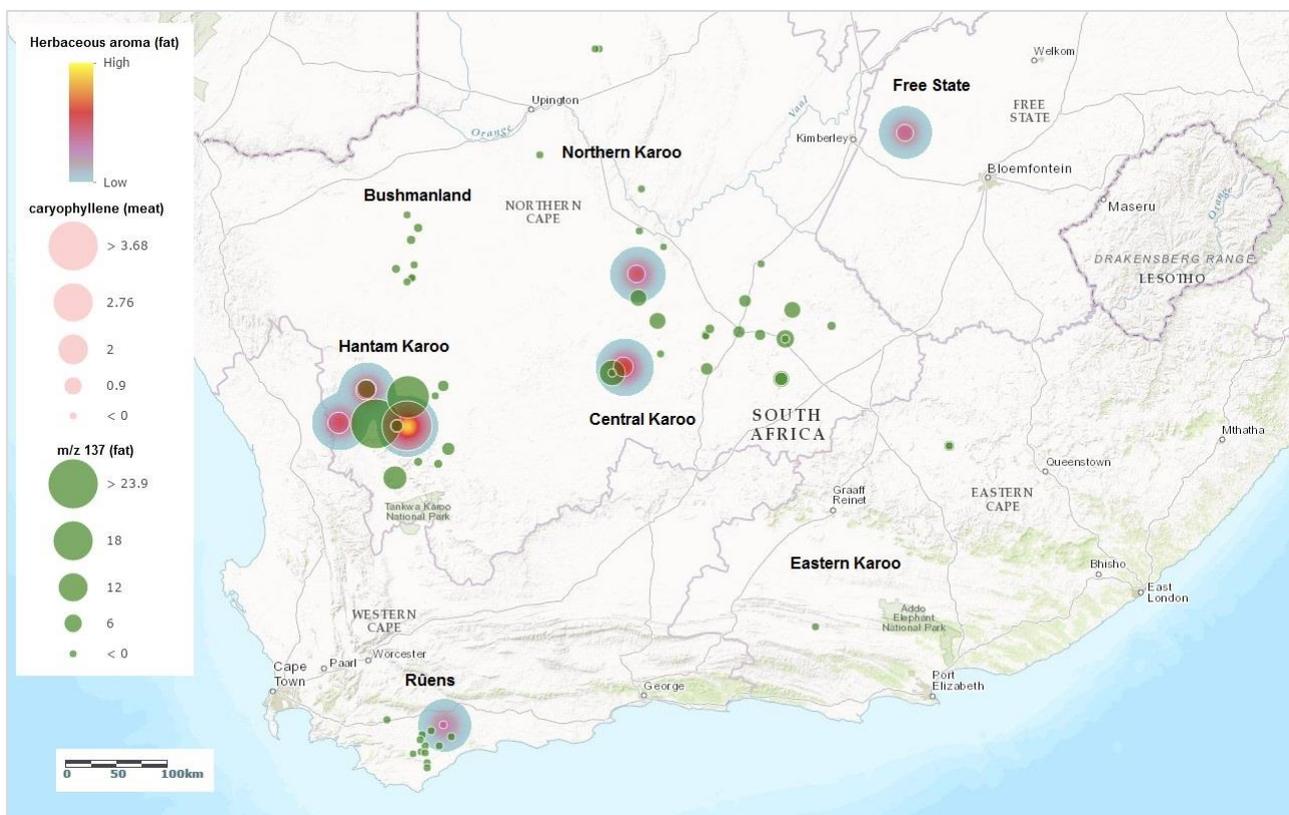


Figure 9.4 Herbaceous aroma of the lamb fat (Chapter 3), relative concentration of caryophyllene of the lamb meat (Chapter 4) and m/z 137 of the lamb fat samples (Chapter 8).

Fatty acids

According to anecdotal data, claims are regularly made stating that Karoo lamb has a “better” or “healthier” fatty acid profile compared to Non-Karoo lamb meat. For a healthy diet the recommended values for polyunsaturated:saturated fatty acids (P:S) are 0.45 or above and 4.0 or below for *n*-6:*n*-3 fatty acids ratios (Wood & Enser, 1997). In Chapter 4 the fatty acids profiles were determined for lamb meat of phase 1. The ratios for P:S and *n*-6:*n*-3, respectively were: 0.35 and 3.7 for Central Karoo (CK); 0.30 and 6.0 for Northern Karoo (NK); 0.30 and 3.3 for Hantam Karoo/Loeriesfontein (HK/LO); 0.34 and 3.9 for Knersvlakte (KV); 0.32 and 3.6 for Hantam Karoo/Calvinia (HK/CAL); 0.41 and 2.9 for Rûens (RU); 0.25 and 3.2 for Free State (FS). None of the treatments had a P:S ratio of 0.45 or above, while only RU (Non-Karoo) had the closest ratio and FS (Non-Karoo) the lowest. For the *n*-6:*n*-3, all the treatments except for NK had ratios below 4.0. Therefore, according to the high *n*-6:*n*-3, NK could be seen as being less healthy to consume. Based on the findings and recommended fatty acid ratios, it is evident that more research should be done on the nutritional properties of Karoo lamb to substantiate any claim made in this regard. The fatty acids ratios should also be validated for South African lamb. Furthermore, the variation in fatty acid profiles could also impart a different sensory quality (Sañudo *et al.*, 2000).

Pasture-finished lamb with high concentrations of α -linolenic acid (C18:3 *n*-3) and other *n*-3 PUFA, have high sensory scores for lamb flavour and liking, and low scores for abnormal flavour (Fisher *et al.*, 2000). Conversely, concentrate-finished lamb with high concentrations of linoleic acid (C18:2 *n*-6) and its major product, arachidonic acid (C20:4 *n*-6), have low scores for lamb flavour and liking, and high scores for abnormal flavour. In this study variation in fatty acids, as well as lipid-derived volatile compounds were found. The Karoo treatments (NK, HK/LO, KV and HK/CAL) contained more aldehydes than the Non-Karoo (RU and FS) treatment – which were related and showed a similar trend to the PUFA composition of the raw meat. Given that fatty acids are the main source of aroma precursors in meat, it was important to establish whether or not there is a relationship between the regional forage and chemical compounds derived from the fatty acids. Although this study has been successful in showing the relationship between diet and volatiles from the fatty acids, there is still a number of inconsistencies compared to previous findings in literature. It is recommended that more research should be done to determine the full fatty acid profile of Karoo lamb. Future research should include the effect of season on the fatty acid profiles, as well as how the fatty acids could be linked to specific volatile compounds.

Near-infrared spectroscopy (NIRS)

The meat of the different regions was classified using a MicroNIR device, an apparatus which is cost-effective, small, portable and easy to use. This device could easily be used in an abattoir or meat processing unit as a rapid test for the authentication of lamb meat. Regardless of the size and specifications of the device, the results show that the spectral data obtained from fresh meat samples can be used to successfully determine the origin of the meat (being Karoo or Non-Karoo) (Chapter 6). The principle behind this technique is based

on the generation of a chemical spectral fingerprint which is compared to the fingerprint of a reference spectra where the characteristic being determined, in this study origin, is known (Downey, 1996). Samples were classified according to their characteristic spectra generated through the absorption of radiation by the chemical compounds of the product.

NIRS was also used to determine if the meat had been frozen (Chapter 6). Differences were detected due to a change in the physical structure and the gross chemical composition of the meat. During the freezing process ice crystals form which rupture the cell membranes and consequently, an increase in the amount of drip loss together with a reduction in the moisture content is seen (Leygonie *et al.*, 2012). Such variation allows discrimination between samples. In the case of meat adulteration, differences in composition (moisture, protein and fat content) of meat between species enables detection (Prieto *et al.*, 2009). Therefore, when NIRS is used to classify samples according to a certain property that property can sometimes be measured indirectly. For instance, determining the origin of meat is related to the effect that origin has on the composition of the meat. When origin is defined according to a specific production system for example, extensive grazing compared to the feedlot, lamb meat with different fatty acid profiles are produced. Hence, the meat can be distinguished based on different fatty acid profiles, which would also result in different spectral fingerprints.

The NIRS results are the first baseline database for Karoo lamb. Future research should aim at analysing more samples in order to improve the calibration model. An issue of concern in the Karoo is the supplementation of concentrates during periods of draught and the finishing of sheep in the feedlot. Therefore, an important question is how the spectral data changes over time when an extensively raised animal is moved to a feedlot? In addition, how long does it take to notice changes in the sensory and chemical profile of the meat? Another observation in the study was that some of the semi-extensive (SE) lamb meat were classed as being from the Karoo in some cases and Non-Karoo in other cases (Chapter 6, 7 and 8). Again, the effect of diet on the rate of successful classification comes into play. The SE lamb types are raised extensively and fed concentrates. Unfortunately, the extent and period of concentrate feeding was unknown, however, when taking the classification results into account, it is evident that in some cases the concentrate did not have a major effect on the meat quality or the provision thereof must have been lower than the animals from the other semi-extensive farms. In addition, using NIRS (Chapter 6) only one SE sample was classified as Non-Karoo, while the rest were classified as Karoo. However, these misclassifications were related to the small sample size and the high level of variation of the additional farms sourced. The NIRS calibration model also only included variation of one Non-Karoo region (RU) where the lambs are raised on lucerne (Chapter 6). Therefore, the different vegetation of the additional Non-Karoo regions [Namibia (NAM), feedlot (FL) and semi-extensive (SE)] were not included in the model and the samples were incorrectly classified. The calibration model should be extended with sufficient samples of NAM, FL and SE, so as to include their characteristic production parameters, for future prediction.

Stable isotope ratios

The carbon and nitrogen stable isotopic ratios are shown in Figure 9.5 and 9.6, respectively. The trends of the findings of phase 1 and phase 2 are confirmed with the maps (Fig. 9.5 and 9.6). The mean $\delta^{13}\text{C}$ values are the lowest for RU lamb [-22.7‰ (phase 1) and -23.6‰ (phase 2)], increasing with movement towards the Northern parts of South Africa – indicative of an increase in C_4 grass species (resulting in an increase of $\delta^{13}\text{C}$ values) (Fig. 9.5). The Hantam Karoo (HK/CAL) lamb meat has low mean $\delta^{13}\text{C}$ values (-22.9‰), due to the prevailing presence of Karoo bushes in the region (Chapter 5). The aridity of the Northern Cape province had a great effect on the mean $\delta^{15}\text{N}$ values of lamb meat – resulting in higher $\delta^{15}\text{N}$ values (Fig. 9.6). Low mean $\delta^{15}\text{N}$ values are typical of the Rûens region due to the nitrogen fixation of legumes, such as lucerne (*Medicago sativa*). It is recommended that future studies explore the use of additional isotopes such as, hydrogen, oxygen and sulphur (^1H , ^{18}O and ^{34}S). Another aspect to explore is the determination of the uranium content of the lamb meat. Parts around Beaufort West are known for their uranium mining activities. Uranium could therefore be transferred through the environment (i.e. water) and deposited into the meat of the sheep. Determination of uranium content could provide a better discrimination between lamb meat within the Karoo region.

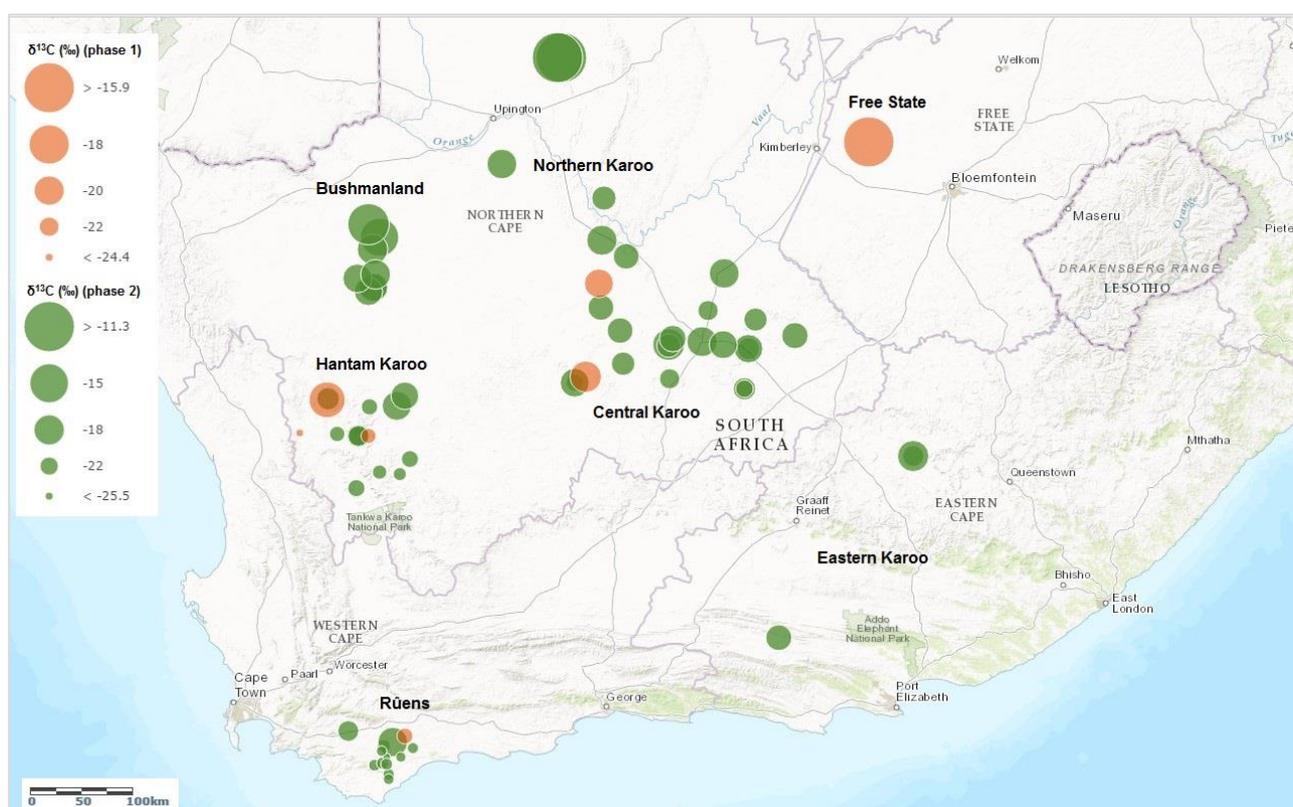


Figure 9.5 The mean $\delta^{13}\text{C}$ values of the lamb meat from the different farms (Chapter 5) and regions (Chapter 7).

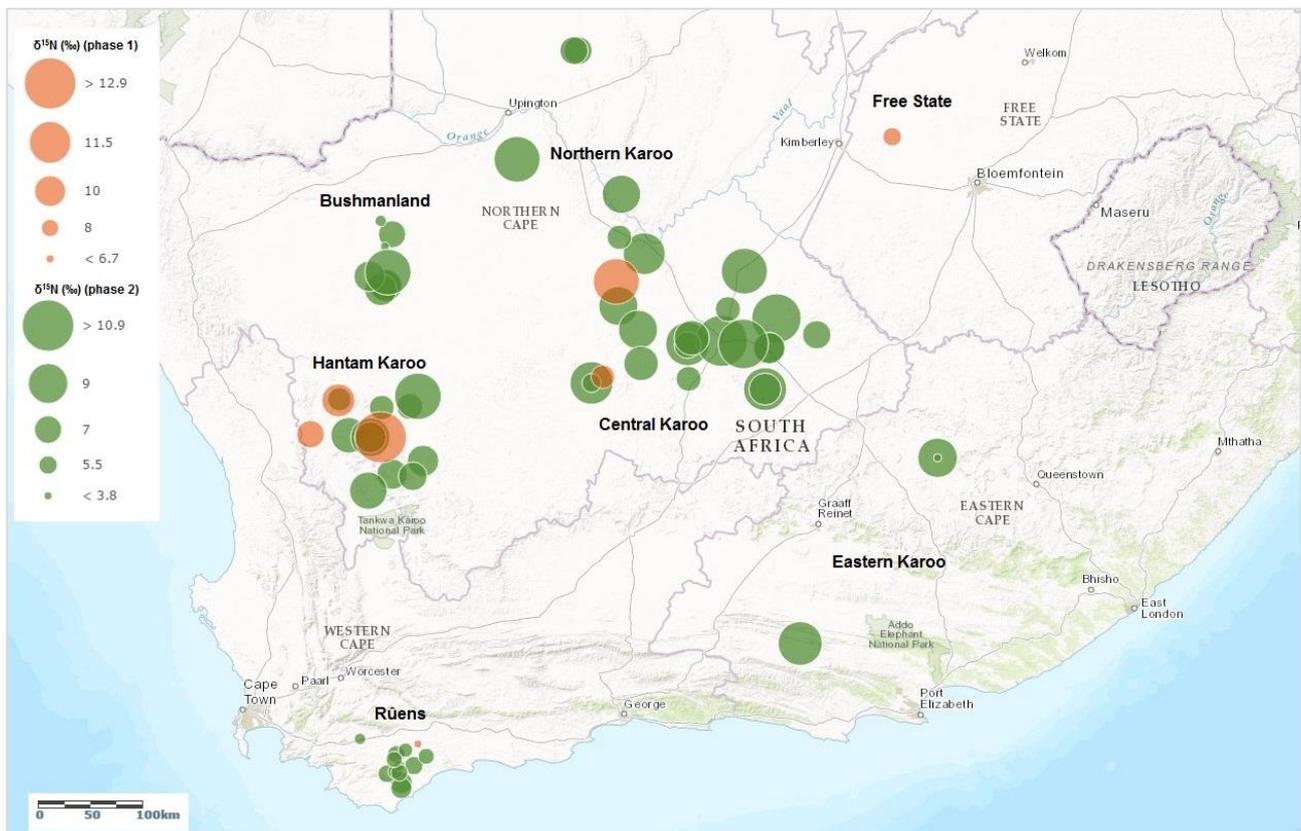


Figure 9.6 The mean $\delta^{15}\text{N}$ values of the lamb meat from the different farms (Chapter 5) and regions (Chapter 7).

Databases

A key finding of the study is the establishment of databases which are vital for the control of food authenticity (Dennis & Ashurst, 1996). Databases should not be corrupted as it would wrongfully classify authentic products. In the study it was found that using the different techniques for the classification of origin, some farms were classified differently/incorrectly. This was particularly evident using NIRS (Chapter 6) and stable isotope analysis (Chapter 7). CK samples were often classified as HK, HK as CK, RU as HK, HK as RU and lastly SE as CK. These misclassifications could suggest that the lambs were not raised extensively or that they received a large amount of supplementary feed. The CK and HK misclassification could be due to the similarities of Karoo lamb within the Karoo regions. It is recommended to expand the calibration model with sufficient samples of Non-Karoo origin for predictions in the future. It is also recommended to use the rapid NIRS test as a screening technique so that more expensive techniques can be undertaken on borderline cases.

What makes the determination of geographic origin a challenging task, when working with live animals, is that several factors may influence the quality of the meat (Chapter 2). Therefore, the database must be sufficiently flexible and large enough to account for variations such as season, breed, age, etc., as well as variation in vegetation within the defined region of origin. The high level of variation was evident in the study. There was a clear difference in the sensory quality of Hantam Karoo (HK/CAL) lamb when compared to Karoo lamb (CK, NK and KV) (Chapter 3). Although stable isotope analysis provided classification of Dorper lambs

from seven farms (Chapter 5), extension of the sample size and including more variation (i.e. different breeds, ages, farms) the isotopic ratios still proved to be a successful authentication technique (Chapter 7). Hence, in agreement with the results of other researchers: that isotope ratio mass spectrometry (IRMS) is one of the most promising analytical tools to use for the determining the geographical origin of foods (Franke *et al.*, 2005; Kelly *et al.*, 2005; Roßmann, 2007; Drivelos & Georgiou, 2012). However, a major limitation to this technique is the high cost and time of the analysis and the specialised equipment and skills required to perform the analysis.

The data generated through the study is of value to the South African meat industry as it is the first for Karoo lamb and forms baseline databases which can be used as a reliable starting point for testing the authenticity of lamb. The databases and prediction models link the chemical composition of Karoo lamb to its origin for the purpose of classification. However, the databases would require continual updating, and validation to an external sample set, to ensure that the databases remains up-to-date and relevant to the current sheep production systems. It is also important that the different regulatory bodies and institutions use the same methodology and authentic databases so that the parties operate on the same level.

Concluding remarks

The study has demonstrated that authentication is an interdisciplinary science, involving the use of several techniques to verify accuracy of results. The study is the first of its kind describing the specific sensory attributes of Karoo lamb and using analytical techniques to authenticate the meat and fat. It is the first scientific results available to confirm the unique sensory quality of Karoo lamb which links the meat to the typical diet of the sheep consisting of fragrant indigenous Karoo plants. The study has also shown and measured the aroma and flavour differences between different areas within the Karoo lamb region and Karoo lamb compared to lamb from other regions. Not only does the findings serve as evidence for the certification of Karoo lamb, but the results also justify the lamb meat's unique sensory characteristics – supporting it as a geographical indication. The new findings will assist in preventing fraudulent trading activities in which abattoirs, traders and restaurants falsely claim to sell Karoo lamb. The methodology can be implemented in the future to test the origin of the lamb. It is recommended that a state-of-the-art and rapid techniques such as PTR-MS together with the use of NIRS be implemented at abattoirs or meat processing facilities to assist with the authentication of regionally unique South African lamb meat. Given that key volatiles were higher in the fat compared to the meat, small fat samples could easily be taken and analysed on-line.

References

- Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Science*, **86**, 577-587.
- Bramley, C., Bienabe, E. & Kirsten, J. (2013). *Developing Geographical Indications in the South: The Southern African Experience*. Dordrecht, the Netherlands: Springer.
- Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, **77**, 63-80.

- Capuano, E., Boerrigter-Eenling, R., Van der Veer, G. & Van Ruth, S. M. (2013). Analytical authentication of organic products: an overview of markers. *Journal of the Science of Food and Agriculture*, **93**, 12-28.
- Cornu, A., Carnat, A.-P., Martin, B., Coulon, J.-B., Lamaison, J.-L. & Berdague, J.-L. (2001). Solid-phase microextraction of volatile components from natural grassland plants. *Journal of Agricultural and Food Chemistry*, **49**(1), 203-209.
- Dennis, M. J. & Ashurst, P. R. (1996). An introduction to food authentication. In: *Food Authentication* (edited by P. R. Ashurst & M. J. Dennis). 1st ed. Pp. 1-14. London, UK: Blackie Academic & Professional.
- Department of Trade and Industry (DTI). (2013). Merchandise Marks Act (Act No.17 of 1941). *Proposed prohibition on the use of certain words*. Pretoria, South Africa: Government Printer.
- Downey, G. (1996). Authentication of food and food ingredients by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, **4**, 47-61.
- Drivelos, S. A. & Georgiou, C. A. (2012). Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union. *Trends in Analytical Chemistry*, **40**, 38-51.
- Estler, K. J., Milton, S. J. & Dean, W. R. J. (2006). *Karoo Veld Ecology and Management*. p. 30. Cape Town, South Africa: Briza Publications.
- Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., Kurt, E., Sinclair, L. A. & Wilkinson, R. G. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science*, **55**(2), 141-147.
- Franke, B. M., Gremaud, G., Hadorn, R. & Kreuzer, M. (2005). Geographic origin of meat – elements of an analytical approach to its authentication. *European Food Research and Technology*, **221**, 493-503.
- Kelly, S., Heaton, K. & Hoogewerff, J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends in Food Science & Technology*, **16**, 555-567.
- Kirsten, J. F., Vermeulen, H., Van Zyl, K., Du Rand, G., Du Plessis, H. & Weissnar, T. (2012). The economic potential for an origin based marketing and certification system for a meat product in South Africa: perceptions, preferences and experiments. Poster presentation at the *International Association of Agricultural Economists (IAAE) Triennial Conference*, Foz do Iguacu, Brazil, August 18-24, 2012.
- Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., Garner, G. B. & Morrow, R. E. (1987). Flavor constituents of beef as influenced by forage- and grain-feeding. *Journal of Food Science*, **52**(2), 245-251.
- Leygonie, C., Britz, T. J. & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, **91**(2), 93-98.
- Perini, M., Camin, F., Bontempo, L., Rossmann, A. & Piasentier, E. (2009). Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions. *Rapid Communications in Mass Spectrometry*, **23**, 2573-2585.
- Prieto, N., Roehe, R., Lavín, P., Batten, G. & Andrés, S. (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science*, **83**, 175-186.

- Priolo, A., Cornu, A., Prache, S., Krogmann, M., Kondjoyan, N., Micol, D. & Berdagué J.-L. (2004). Fat volatile tracers of grass feeding in sheep. *Meat Science*, **66**, 475-481.
- Roßmann, A. (2007). Stable isotope databases for European food products. *Proceedings of the international workshop: Fingerprinting methods for the identification of timber origins*, 39-46, Bonn, Germany.
- Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., Maria, G., Sierra, I. & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, **54**, 339-346.
- Wood, J. D. & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, **78**(Suppl.1), S49-S60.