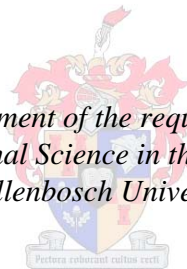


# **Effect of cottonseed oilcake meal on ostrich growth performance, meat chemical composition and sensory attributes**

by  
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## DECLARATION

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## SUMMARY

This research study consists of three investigations with regard to ostrich (*Struthio camelus* var. *domesticus*) production, meat quality and the processing of ostrich meat into a value added meat product.

The first study was conducted in order to establish whether the gradual replacement of soybean oilcake meal with cottonseed oilcake meal (CSOCM) as a protein source in the diet of slaughter ostriches would affect ostrich growth performance and meat quality. A total of 105 ostriches were divided into five feeding groups according to the CSOCM inclusion level: Control (0% CSOCM), 3%, 6%, 9% and 12% CSOCM, and fed with experimental diets from 6 to 13 months of age. As a result of feeding CSOCM, the final live weight and the average daily gain significantly increased in the 12% CSOCM group compared to the other treatment diets. The proximate composition, cholesterol content, mineral and fatty acid profile of the meat remained unaffected. Considering all the results, CSOCM may be used as an alternative protein source to soybean oilcake meal in ostrich nutrition, resulting in decreased feed costs.

Secondly, a descriptive sensory analysis, together with chemical and physical measurements, was performed to determine whether the manipulation of the fatty acid composition in the fan fillet (*Iliofibularis* muscle) as a result of feeding CSOCM would be detected on a sensory level. Two levels of CSOCM were investigated; 0% as a control and 9% CSOCM. No significant differences were found for the physical measurements (cooking loss (%) and shear force) as well as for the pH and proximate composition of the raw fan fillet. The Control group presented a higher ( $P < 0.05$ ) mono-unsaturated fatty acid (MUFA) content in the cooked fan fillet whereas the 9% CSOCM group showed a favourable increased ( $P < 0.05$ ) poly-unsaturated fatty acid (PUFA) content when compared to the cooked Control samples. As a result, the poly-unsaturated:saturated fatty acid (PUFA:SFA) ratio in the 9% CSOCM group was also higher ( $P < 0.05$ ). No differences ( $P > 0.05$ ) were found between the treatments for the n-6:n-3 (omega 6 to omega 3) ratio. The 9% CSOCM group had a more intense beef aroma, had a higher level of initial and sustained juiciness as well as increased tenderness ( $P < 0.05$ ). Inclusion of 9% CSOCM resulted in a favourable cooked ostrich fan fillet.

Finally, the effect of feeding CSOCM on a processed ostrich meat product was investigated. Fan fillet (*Iliofibularis* muscle) from 13 month old birds receiving no cottonseed oilcake meal (Control) or 9% cottonseed oilcake meal (9% CSOCM) was used. Olive oil was used as a replacement for pork fat, and warthog (*Phacochoerus africanus*) meat was used to replace commercial pork meat in the production of a semi dry sausage, cabanossi. Olive oil was included at three levels (0%, 1% and 2%). Six treatments were investigated: Control 0% olive oil, Control 1% olive oil, Control 2% olive oil, 9% CSOCM 1% olive oil, 9% CSOCM 2% olive oil en 9% CSOCM 2% olive oil. The Control and 9% CSOCM ostrich meat did not differ significantly in chemical composition nor fatty acid profile. After smoking and drying the fat content in the cabanossi containing 0%, 1% and 2%

olive oil averaged 7.2%, 7.45% and 8.65% respectively. Processed meat products containing less than 10% fat are classified as a low-fat meat product. Olive oil is a mono-unsaturated vegetable oil containing mainly Oleic acid (C18:1n9c), and low quantities of saturated fatty acids and polyunsaturated fatty acids. Total mono unsaturated fatty acids in the cabanossi increased from 47.0% to 73.0% of total fat, whilst total saturated fatty acids and total polyunsaturated fatty acids decreased from 40.6% to 19.9% and 11.6% to 6.6% respectively as olive oil increased from 0% to 2%. The inclusion of olive oil at 2% resulted in cabanossi with increased ( $P < 0.05$ ) tenderness, juiciness and cured red meat colour, all factors that appeal greatly to the consumer. Overall flavour was not adversely affected by the inclusion of olive oil.

This investigation indicated that the use of CSOCM had no negative effect on the production performance of ostriches whilst a 9% CSOCM inclusion level resulted in meat that was found to be favourable by a trained sensory panel. Furthermore, the use of CSOCM as a feed component also had no negative effect on a processed product (cabanossi) derived from the meat obtained from the birds fed this feed component. The CSOCM used in this investigation had low levels of gossypol (10 to 20ppm) and more research is required on the effect of the use of CSOCM with higher levels of gossypol on the production performance of ostriches.

## OPSOMMING

Die studie het bestaan uit drie ondersoeke met betrekking tot volstruis (*Struthio camelus* var. *domesticus*) -produksie, -vleiskwaliteit en die vervaardiging van waarde-toegevoegde geprosesseerde volstruis-vleisprodukte.

Die doel van die eerste studie was om vas te stel of die geleidelike vervanging van sojaboon-oliekoekmeel met katoensaad-oliekoekmeel (CSOCM) as 'n proteïenbron in die voeding van volstruise, die groeipersentasie en vleiskwaliteit van die *Iliofibiularis* spier (fan fillet) sal affekteer. 'n Totaal van 105 volstruise is verdeel in vyf voedingsgroepe volgens die katoensaad oliekoekmeel insluitingsvlak: Kontrole (0% CSOCM), 3%, 6%, 9% en 12% CSOCM. Die onderskeie voedingsgroepe was van ses tot 13 maande ouderdom op die eksperimentele voere geplaas. Die resultate het aangedui dat die voëls in die 12% CSOCM behandelingsgroep 'n betekenisvolle ( $P < 0.05$ ) toename in finale lewende massa asook gemiddelde daaglikse toename gehad het. Die proksimale samestelling, cholesterol-inhoud, mineraal- en vetsuursamestelling van die vleis was nie geaffekteer deur die insluiting van CSOCM nie. Die CSOCM kan dus wel as 'n alternatiewe proteïenbron in die voeding van volstruise gebruik word. Laasgenoemde bevinding kan ook lei tot verlaagde voerkostes, aangesien CSOCM heelwat goedkoper is as sojaboon-oliekoekmeel.

Die tweede deel van die studie was van 'n chemiese asook sensoriese aard. 'n Beskrywende sensoriese analiese is uitgevoer om vas te stel of die manipulering van die vetsuursamestelling in die volstruis fan fillet as gevolg van die CSOCM sensories waargeneem kan word. Die chemiese en fisiese eienskappe van die vleis is ook ondersoek. Twee vlakke van CSOCM inhoud is ondersoek; 0% (as kontrole) en 9% CSOCM. Geen betekenisvolle verskille is gevind vir die fisiese vleiskwaliteit (kookverliespersentasie en taaiheid), asook vir die proksimale samestelling en pH van die fan fillet nie. Die gekookte fan fillet van die Kontrole behandeling het 'n betekenisvolle ( $P < 0.05$ ) toename in mono-onversadigde vetsure (MUFA) getoon en die 9% CSOCM het 'n voordelige toename in poli-onversadigde vetsuur-inhoud (PUFA) gehad. Die poli-onversadigde tot versadigde vetsuurverhouding (PUFA:SFA) was as 'n gevolg ook betekenisvol hoër. Geen verskille ( $P > 0.05$ ) is opgemerk in die omega-6 tot omega-3 poli-onversadigde vetsuurverhouding (n-6:n-3) nie. Met betrekking tot die sensoriese eienskappe het die 9% CSOCM 'n meer opvallende beesvleis aroma, hoër vlakke van aanvanklike sappigheid en ook sagter vleis in vergelyking met die kontrole behandeling gehad ( $P < 0.05$ ). Insluiting van 9% CSOCM het gelei tot 'n gekookte volstruis fan fillet van voornemende kwaliteit.

Laastens is daar ondersoek ingestel op die vervanging van varkvet met olyfolie in die vervaardiging van 'n volstruis cabanossi. Chemiese asook sensoriese analyses is uitgevoer op die gedroogde en geroekte volstruis cabanossi. Vir die vervaardiging van laasgenoemde produkte is die fan fillet van 13 maande oue voëls van die Kontrole (0% CSOCM) en 9% CSOCM behandelings gebruik. Addisioneel tot die volstruisvleis is daar ook vlakvarkvleis (*Phacochoerus africanus*) gebruik om die kommersiële varkvleis te vervang. Olyfolie was ingesluit teen drie vlakke

(0%, 1% en 2% van die totale mengsel). Ses behandelings was ondersoek: Kontrole 0% olyfolie, Kontrole 1% olyfolie, Kontrole 2% olyfolie, 9% CSOCM 1% olyfolie, 9% CSOCM 2% olyfolie en 9% CSOCM 2% olyfolie. Daar was geen verskille ( $P > 0.05$ ) in die chemiese en vetsuursamestellings van die Kontrole en 9% CSOCM volstruisvleis nie. Na die droging en rooksiklus was die gemiddelde vet-inhoud van die 0%, 1% en 2% olyfolie cabanossi monsters onderskeidelik 7.2%, 7.45% en 8.65%. Geprosesseerde vleisprodukte met 'n vet-inhoud van minder as 10% word in die kommersiële vleisindustrie na lae vet vleisprodukte verwys. Olyfolie is baie ryk aan MUFA, veral Oleïensuur (C18:1n9c) en dit bevat ook lae hoeveelhede SFA en PUFA. Die totale MUFA inhoud in die cabanossi het toegeneem van 47.0% tot 73.0% terwyl die totale SFA en PUFA onderskeidelik afgeneem het van 40.6% tot 19.9% en 11.6% tot 6.6%, met 'n olyfolie toename van 0% tot 2%. Die insluiting van olyfolie teen 2% het gelei tot 'n sagter cabanossi wat meer sappig was met 'n meer opvallende rooi gekuurde vleiskleur, wat almal eienskappe is wat dié produk meer aantreklik maak vir die verbruiker.

Hierdie studie het aangedui dat CSOCM geen negatiewe effek gehad het op die produksie van volstruisvleis nie. Volstruisvleis van die behandelingsgroep wat CSOCM teen 9% van die dieet ontvang het, het wel vleis geproduseer wat as aanvaarbaar aanskou was deur 'n opgeleide sensoriese paneel. Die gebruik van CSOCM as 'n voerbestanddeel het ook geen negatiewe effek gehad op 'n geprosesseerde produk (cabanossi) gemaak van die volstruisvleis nie. Die CSOCM wat in die huidige studie gebruik is, het baie lae vlakke van gossypol (10 – 20dpm) gehad en verdere ondersoek is noodsaaklik om die effek van CSOCM met hoër vlakke van gossypol op die produksie van volstruisvleis te bevestig.

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“I can do all things through Christ who strengthens me” Philippians 4:13

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**LIST OF ABBREVIATIONS**

mg	milligram
g	gram
kg	kilogram
ml	millilitre
L	Litre
cm	centimetre
m <sup>2</sup>	Square metre
IMF	Intramuscular Fat
SFA	Saturated Fatty Acids
MUFA	Mono unsaturated fatty acids
PUFA	Polyunsaturated fatty acids
P:S	Poly-unsaturated to Saturated Fatty Acid Ratio
n-6:n-3	Omega-6:Omega-3 Fatty Acid Ratio
EPA	Eicosapentaenoic Acid
DHA	Docosahexanoic Acid
pH <sub>u</sub>	Ultimate pH at ≈24 h <i>post mortem</i>
WHC	Water Holding Capacity
WBS	Warner-Bratzler shear force
DFD	Dark firm dry
LSMeans	Least Squares Means
SD	Standard Deviation
ppm	Parts per million
dpm	Dele per miljoen
CSOCM	Cottonseed oilcake meal
SBOCM	Soybean oilcake meal
LW	Live weight
FI	Feed intake
FCR	Feed conversion ratio
ADG	Average daily gain
GE	Gross energy
MJ	Mega Joule
NDF	Neutral detergent fibre
ADF	Acid detergent fibre



## NOTES

The language and style used in this thesis is in accordance with the requirements of the Journal of Meat Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between the chapters, especially in the Materials and Methods section, was therefore unavoidable.

Part of this thesis was presented at the following symposium:

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## CHAPTER 1

### GENERAL INTRODUCTION

The ostrich industry in South Africa dates back to the 1860's when ostriches were domesticated in the Klein Karoo, Western Cape (Smit, 1963). The industry was initially focused on the feathers and hides for the fashion industry. Over the last decade however focus has shifted and is now aimed at further developing and sustaining the meat production sector of the South African ostrich industry (Cooper & Horbańczuk, 2002).

In order to ensure successful rearing of ostriches from hatchling to slaughter bird, high standards of nutrition need to be maintained and managed (Cooper et al., 2004) and according to Brand & Olivier (2011), more knowledge is required on this topic as feed costs comprise close to 80% of total production costs. Of the nutritional cost implications, the protein source presents the largest component and farmers in the industry are interested in protein sources that are less expensive but still able to produce acceptable production yields and reproduction performances in their flocks.

Whole cottonseeds are a product of cotton (*Gossypium*) production for the textile industry. The processing of cottonseeds is a major industry resulting in the extraction of the oil almost exclusively for the use in human consumption and the cottonseed meal as animal feed or fertilizer (Adams et al., 1960). Cottonseed oilcake has been successfully used in ruminant nutrition as it has a relatively high energy and protein content (26% crude protein and 25% oil, respectively) (Clawson et al., 1975). In monogastric nutrition however, its utilization has been greatly limited due to a toxic polyphenolic compound, generally occurring in the pigment glands but also other parts of the plant, named gossypol. Controversial results have been obtained during scientific studies of the utilization of gossypol containing cottonseed oilcake products in monogastric nutrition (Ikurior & Fetuga, 1988; Rhule, 1995; Fombad & Bryant, 2004; Winterholler et al., 2009; Nunes, et al., 2010; Wanapat et al., 2012). Aganga et al. (2003) have alleged that gossypol has toxic effects on ostriches. No studies have however been conducted on the effect of cottonseed oilcake meal (CSOCM) on the production and meat quality of ostriches.

Ostrich meat has gained much appreciation and attention as a healthy red meat alternative. This health label is because of a favourable fatty acid profile (intramuscular ostrich fat (IMF) contains 16.50% poly-unsaturated (PUFA) n-3 fatty acids), low intramuscular fat content (Mellet, 1985), low sodium content and richness in heme-iron in the meat (Sales & Hayes 1996; Sales et al., 1999). The majority of ostrich meat produced in South Africa is usually sold as fresh meat, vacuum packed and exported (Hoffman, 2008). Since March 2011 when *Avian influenza* (H5N2 strain) was reported for the first time in Klein Karoo, Oudtshoorn, the European Union (EU) regulations have banned the export of South African ostrich meat to European countries (Cooper et al., 2004). This restriction has necessitated the expansion of the local South African fresh meat market.

Consumer preference has however changed drastically over the last decade and there is an emphasis on nutrition and health, specifically with regards to saturated fat and cholesterol content of meat products (Resurreccion, 2003). Dietary guidelines are urging consumers to reduce the amount of total fat and SFA (saturated fatty acids) intake as a means of reducing the risks of coronary heart disease (Williams, 2000). In this regard, ostrich meat presents itself as a very attractive alternative to other generally consumed red meats. In order to satisfy consumer demand for a greater variety of products, value added meat products, such as processed meats, should be developed. In processed meat products, increasing the mono-unsaturated fatty acid (MUFA) content is of importance. The objective of increasing MUFA in processed meat products is due to its association with decreasing coronary heart disease in humans (Bloukas & Paneras, 1993) similar to PUFA. Furthermore, by increasing MUFA in processed meat products, the latter is less susceptible to oxidation than products with a high PUFA content which could lead to unfavourable sensory properties.

A strategy to enhance the nutritional value of processed meat products by increasing MUFA content intake is to replace animal fat with certain vegetable oils (Rodríguez-Carpena, Morcuende & Estévez, 2012). Olive oil is one of the most mono-unsaturated oils available and has been used in a variety of value added meat products as a replacement for animal fat (Rodríguez-Carpena et al., 2012; Ansorena & Astiasaran, 2004; Pappa, Bloukas & Arvaritoyannis, 2000; Bloukas & Paneras, 1993). It has also proven to be very successful with regards to nutritional value as well as sensory quality. Several value added ostrich products have already been manufactured but these are mainly based on established technologies used on other red meat types (Hoffman, 2008).

The objectives of this study was threefold:

- (1) To establish whether and to what extent the gradual replacement of soybean oilcake meal with CSOCM would affect ostrich growth performance and meat quality.
- (2) To determine whether the CSOCM can be detected on a sensory level due to fatty acid profile manipulation through feeding practices and finally
- (3) To investigate whether olive oil can be used as replacement for saturated animal fat, in the production of a value added ostrich meat product made from the meat of ostriches that had consumed CSOCM.

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## CHAPTER 2

### LITERATURE REVIEW

#### EFFECT OF DIETARY PROTEIN ON THE QUALITY OF FRESH AND PROCESSED OSTRICH MEAT

##### 2.1 HISTORY

Domestication and commercial breeding of the ostrich (*Struthio camelus australis*) started in Algeria and in Italy in 1859 (Mellet, 1985). The history of the ostrich as a livestock production unit in South Africa dates back to the 1860's, when they were captured and domesticated (*Struthio camelus* var. *domesticus*; Swart, 1988) in the Klein Karoo, Western Cape, for the production of feathers and fashion items from the hide (Smit, 1963). In 1865 there were 80 tame birds and this number increased dramatically over the following years to approximately a million birds in 1913 (Gertenbach, 2011), with ostrich feathers being the fourth largest export commodity of South Africa. During the 1880's birds were exported to New Zealand, South America, Australia and Europe (Deeming & Ayres, 1994; Gertenbach, 2011).

During World War 1 in 1914 there was a serious collapse in the industry and only South Africa of all the countries were able to maintain, to a certain extent (23 500 birds), their ostrich industry (Deeming & Ayres, 1994). In 1945, the KKLK (Klein Karoo Landboukoöpersie) was established in Oudtshoorn in order to regain the status of the ostrich industry, and in 1965 and 1970 the abattoir and leather tanning facilities were respectively established (Gertenbach, 2011). It was only in the 1980's that the meat and leather products from the ostrich industry became of higher importance, specifically a demand for the meat in the European countries as it was seen as a healthier alternative to beef and lamb due to the low fat content of the meat (Gertenbach, 2011).

Mellet (1993) stated that even though the ostrich industry is well established, the productivity was still not close to other domestic livestock productions in South Africa. On average 50% of all eggs hatch and only 40% of hatchlings survive to slaughter age (Mellett, 1993). Since the seventies however, the Department of Agriculture, Western Cape has been assisting in increasing the productivity of this industry by numerous research products, specifically focusing on nutrition and breeding.

The ostrich industry in South Africa is concentrated in the southern (25%) and western (65% in the Little Karoo) parts of the country with 80% of the country's ostrich products being produced here mainly for export purposes to the EU countries (Brand & Gous, 2006). The current income generated from ostrich products, meat, leather and feathers in South Africa are 60%, 30% and 10% respectively. The fluctuation in demand for the feathers and leather is largely dependent on

the fashion industry, which according to Gertenbach (2011) was probably the reason for the drastic decline in demand for feathers.

When Namibia achieved independence in 1990 it was possible for the ostrich industry to reach international levels, with South Africa still remaining the major contributor to the industry. Other countries with fairly well established ostrich industries are the US and Israel. Due to the lack of infrastructure and knowledge on slaughter procedures the main ostrich market in the EU was producing breeding stock for export only. Birds from the UK are being exported to other countries of the world. The transition from mainly breeding, over to a slaughter market in Europe only took place over the last year or so (Adams & Revell, 2003).

As the number of ostriches being produced increases across the world, the need to increase the efficiency of the production system becomes very important. In South Africa, the need to place ostrich meat as a healthy red meat alternative on the shelves of the supermarket and not as an exotic meat product is being realized (Adams & Revell, 2003). According to Mellett (1992) South African consumers do regard ostrich meat as a healthier alternative due to the favourable fatty acid profile (intramuscular ostrich fat contains 16.50% poly-unsaturated n-3 fatty acids), as well as the lower intramuscular fat content of the meat.

The majority of ostrich meat produced in South Africa is usually sold as fresh meat, vacuum packed and exported. With regards to processed ostrich meat products, they are limited and are comprised of mainly burger patties and sausages manufactured from only the trimmings (Hoffman, 2008). Other processed products, such as biltong, are also a general value added product produced from ostrich meat in South Africa. The importance of not only marketing ostrich meat as a healthy red meat alternative but to develop value-added meat products is evident.

## **2.2 OSTRICH FARMING**

The natural habitat of the ostrich seems to be Africa, Assyria and Arabia but they only appear to be naturally occurring in Africa and of the four subspecies of ostrich (Somalian Blue, Kenyan Red, Zimbabwean Blue and North African line), the South African black ostrich is likely a crossbreed (*Struthio camelus* var. *domesticus*; Swart, 1988), bred to have improved feather quality (Gertenbach, 2011).

The primary concern in farming with ostriches is the rearing of chicks up to the age of three months, as the mortality rate in South Africa can be as high as 40-50%, as mentioned earlier, but will not be discussed in further detail as it is not of relevance in this study. In any production unit, feed costs comprise close to 60 or even 75% of the total costs and care should be taken to ensure a profitable unit. After three months of age the feeding and nutrition of ostriches become the point of concern in the industry.



## **2.3 FEEDING AND NUTRITION OF OSTRICHES**

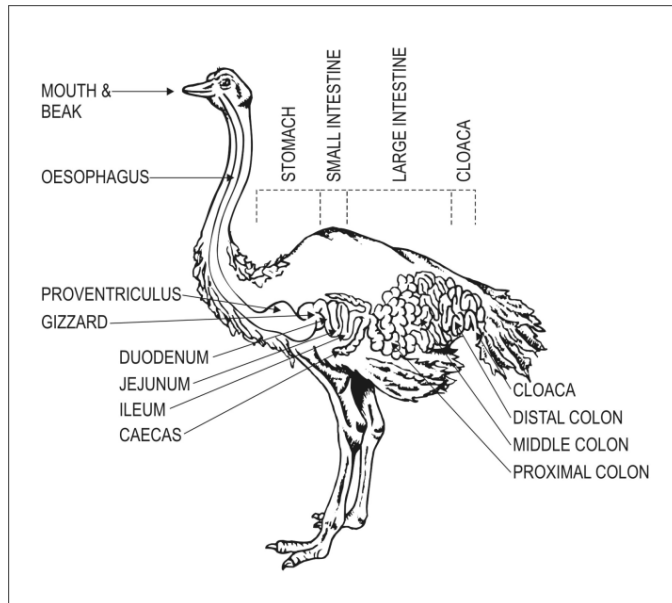
### ***2.3.1 Natural feeding behaviour and preference***

In their natural environment ostriches will vary their diet according to the natural plant life that is available and ostriches have been classified as selective grazers (Sauer & Sauer, 1966 as cited by Angel, 1996). Preferred plants are green annual grasses and forbs, which are low in phenolics and high in fibre, but they also consume leaves, flowers, succulent fruits and woody plants (Williams et al., 1993). In the modern ostrich industry ostriches are also provided with formulated feeds and concentrates, but these birds can be seen consuming the natural vegetation whenever it is available, sometimes even preferring this above the concentrated ration.

To ensure successful growth and reproduction in the ostrich, good nutrition, and the ability of the bird to utilize the nutritional supplements are important. As almost 75% of the costs involved in an intensive ostrich production unit are dedicated to the feed (Brand & Olivier, 2011), understanding the nutrient requirements and digestibility thereof is important. Historically, the nutritional requirements for ostriches were being met by formulating feeds based on poultry or even turkey requirements (Ullrey & Allen, 1996) and have led to difficulties and unrealistic nutrient values (Deeming, 1999; Cilliers et al., 1998). In order to acquire a better understanding of ostrich nutrition, further research is needed as ostriches have been seen as having a unique tolerance to what they are willing to ingest, based on GIT contents (Cooper, et al., 2004).

### ***2.3.2 Digestion in the ostrich***

The ostrich is a monogastric animal with a rather large digestive tract consisting of a beak, mouth, oesophagus, proventriculus (granular stomach), gizzard (smooth muscle stomach), small intestine (duodenum, jejunum, ileum), large intestine (two caeca and the proximal, middle and distal colon) and cloaca.



**Figure 2.1.** Graphic illustration of the digestive system of the ostrich (adopted from Brand & Gous, 2006).

According to Angel (1996), the ostrich has developed unique characteristics in the gastro intestinal tract in order to survive in their natural environments. In contrast to other avian species, the ostrich lacks a crop, but is equipped with a rather large proventriculus and gizzard that fulfils the role as a storage organ. The gizzard aids in mechanically grinding the feed to a finer form to aid in digestion and to assist in this action, stones and pebbles should be made available for the bird to ingest. According to Brand & Olivier (2011) the stones should be 50-70% of the size of the toenail of the bird.

Another characteristic of the ostrich is the large intestine, which enables the bird to utilize fibrous plant material (Brand et al., 2000) and Table 2.1 shows a comparison of the lengths of intestines of different birds and from this it is evident that the ostrich is equipped with a uniquely large hindgut (57% of the total intestinal length).

**Table 2.1.** Comparative lengths of the intestine of different birds (adapted from Angel, 1996; )

	Ostrich <sup>a</sup>		Emu <sup>a</sup>		Rhea <sup>a</sup>		Chicken <sup>a</sup>	
	cm	%	cm	%	cm	%	cm	%
Small intestine	512	36	351	90	140	61	61	90
Cecum	94	6	7	2	48	21	5	7
Colon	800	57	28	7	40	17	2	3

<sup>a</sup> Age of the animals: Adult ostrich, 100 day old emu, adult rhea and adult chicken

The slow rate of passage of digesta in ostriches aids in creating a favourable environment for fermentation of fibrous plant components in the hindgut (Deeming, 1999). The retention time in ostrich is 48 hours with a neutral detergent fibre digestibility of 63% (Swart, 1988; Swart et al., 1993a,b,c). This characteristic also contributes to the ostrich's ability to obtain more energy from the same feed when compared to other animals such as pigs, poultry and even ruminants (Brand et al., 2000; Brand & Olivier, 2011; Aganga et al., 2003). In a study by Brand et al. (2000) ostriches, pigs, poultry and ruminants were fed three different diets varying in fibre content, therefore also varying in energy content and the results showed ostriches obtained significantly ( $P < 0.01$ ) higher metabolisable energy (ME) values for all diets.

### 2.3.3 Nutrient requirements

As is the case in any livestock unit, the dietary requirements of ostriches are determined by the stage of growth at any given time (Brand & Olivier, 2011). Body composition in terms of protein:fat ratio change over time and the gastrointestinal tract also changes drastically to become similar to that of a hindgut fermenter (Aganga et al., 2003). Nutrient requirements will therefore change accordingly. Normally, different feeds would be supplied during the growing period up to maturity and these different feeding stages are depicted in Table 2.2 (Brand, 2008).

**Table 2.2** Commercial feeding stages of growing ostriches (Brand & Gous, 2006).

Feeding Stages	Age (month)	Live mass (kg)	Growth-rate (g/bird/day)	Proposed energy value of the feed (ME ostrich/kg feed)
Pre-Starter	0 – 2	0.8 – 10	150	14.5
Starter	2 – 4.5	10 – 40	400	13.5
Grower	4.5 – 6.5	40 – 60	330	11.5
Finisher	6.5 – 10.5	60 – 90	250	9.5
Maintenance	10.5 – 12.0	90 – 100	200	8.5
Breeder	20 +	110 +	-	9.5

A relationship exists between the energy value of the feed, feed intake and dietary nutrient composition, as the level of intake will vary according to energy level of the feed therefore determining the dietary nutrient composition; high density feed lead to decreased feed intake and a low density feed will lead to increased intake (Brand et al., 2000; Brand & Olivier, 2011). Table 2.3 indicates the predicted feed intake of ostriches receiving three different levels of dietary ME (i.e. 80%, 100% and 120% of proposed dietary energy values).

**Table 2.3** Predicted feed intake (g/bird/day) of growing ostriches at different ages up to maturity (Brand & Jordaan, 2006).

<b>Age (months)</b>	<b>80% of ME</b>	<b>100% of ME</b>	<b>120% of ME</b>
0 – 1	0.36	0.29	0.24
1 – 2	0.68	0.54	0.45
2 – 3	0.99	0.79	0.66
3 – 4	1.38	1.10	0.92
4 – 5	1.72	1.38	1.15
5 – 6	2.05	1.64	1.36
6 – 7	2.53	2.03	1.69
7 – 8	2.77	2.21	1.84
8 – 9	2.95	2.36	1.97
9 – 10	3.09	2.47	2.06
10 – 11	3.19	2.55	2.13
11 – 12	3.27	2.61	2.18
12 – 13	3.32	2.66	2.21
13 – 14	3.36	2.69	2.24
14 – 15	3.39	2.71	2.26
15 – 16	3.41	2.73	2.28
16 – 17	3.43	2.74	2.29

In 1998 Cilliers et al. determined the protein and amino acid requirements for maintenance and growth in ostriches and Table 2.4 shows the requirements obtained. However, according to Brand & Olivier (2011) the growth rate and feed intake will once again determine the exact dietary amino acid requirements.

**Table 2.4** Predicted mean dry matter intake with accompanied protein and amino acid requirements for ostriches calculated from values published by Cilliers et al. (1998).

Parameter predicted	Production stage				
	Pre-starter	Starter	Grower	Finisher	Maintenance
Live mass (kg)	0.85 – 10.0	10 – 40	40 – 60	60 – 90	90 – 120
Age (months)	0 – 2	2 – 5	5 – 7	7 – 10	10 – 20
Feed intake (g/day)	275	875	1603	1915	2440
Protein (g/100g feed)	22.89	19.72	14.71	12.15	6.92
Lysine (g/100g feed)	1.10	1.02	0.84	0.79	0.58
Methionine (g/100g feed)	0.33	0.33	0.29	0.28	0.24
Cystine (g/100g feed)	0.23	0.22	0.18	0.17	0.14
Total SAA (g/100g feed)	0.56	0.55	0.47	0.45	0.38
Threonine (g/100g feed)	0.63	0.59	0.49	0.47	0.36
Arginine (g/100g feed)	0.97	0.93	0.80	0.78	0.63
Leucine (g/100g feed)	1.38	1.24	0.99	0.88	0.59
Isoleucine (g/100g feed)	0.70	0.65	0.54	0.51	0.38
Valine (g/100g feed)	0.74	0.69	0.57	0.53	0.36
Histidine (g/100g feed)	0.40	0.43	0.40	0.40	0.37
Phenylalanine (g/100g feed)	0.85	0.79	0.65	0.61	0.45
Tyrosine (g/100g feed)	0.45	0.44	0.38	0.38	0.31
Phenylalanine and tyrosine (g/100g feed)	1.30	1.23	1.03	0.99	0.76

### 2.3.4 Feed management

In modern day practices, ostriches are either kept extensively (completely dependent on the natural habitat or cultivated pastures) or semi-intensively, grazing on veld or cultivated pastures but also receiving a concentrate formulated feed supplement. The other alternative is raising the birds on a full balanced feed being provided in a feedlot (intensive rearing) (Brand & Gous, 2006). In any of these circumstances the objective would be to ensure adequate nutrients being provided for the birds. Any shortage in nutrients in the grazing is provided through supplemented feed.

Clean and good quality water should always be provided, especially for newly hatched chicks. In semi intensive or extensive conditions where succulent plants are available ostriches would rarely need to drink water (Brand, 2011).

According to Brand (2010) ostriches are rarely kept on natural veld as it has been seen that these birds can easily destroy the natural habitat, especially if the stocking density is too high. In South Africa the general practice is to keep birds on zero grazing and supply mechanically packed lucerne as green feed or as the hay portion to a completely balanced ration (Brand, 2011). In the arid Little Karoo region of South Africa, 80% of birds bred for meat production are kept under intensive feedlot conditions and 20% on grazing conditions. In the Eastern and Southern Cape 60% are intensive feedlot reared and 40% graze (Brand, 2011). Feed costs comprise the majority of the total costs in any livestock production unit and must be formulated by a qualified animal scientist. Formulations for feed are based on a least cost analysis.

### **2.3.5 Sources of feed**

Cultivated pastures generally consist of lucerne, but ostriches can also be successfully reared on medics, seradella or canola pastures (Brand, 2011). Table 2.5 taken from the Department of Agriculture, Western Cape, depicts the main and most important feed sources used in South Africa.

**Table 2.5** Most important sources of feed in ostrich feeding (Brand, 2011).

<b>Concentrates</b>	<b>Roughages</b>	<b>Protein</b>	<b>Mineral</b>
Maize	Lucerne hay	Soya bean oilcake	Feed lime
Barley	Barley hay	Canola oilcake	Dicalcium phosphate
Wheat	Oat hay	Sunflower oilcake	Monocalcium phosphate
Triticale	Oat bran	Fishmeal	Salt
Oats	Wheat bran	Full fat soya	Mineral and Vitamin premix
Brewers grain	Oat straw	Full fat canola	
	Wheat straw	Sunflower seeds	
	Silage	Lupins	
		Beans	
		Gluten	
		Peas	

## 2.4 COTTONSEED AND GOSSYPOL

Whole cottonseeds are a product of cotton (*Gossypium*) production. The processing of cottonseeds is a major industry resulting in the extraction of the oil almost exclusively for the use in human consumption and the cottonseed meal as animal feed or fertilizer (Adams et al., 1960).

Cottonseed is a less expensive source of protein and energy supplement, and has relatively high values in terms of protein and energy (26% crude protein and 25% oil respectively) (Clawson, et al., 1975). The use of cottonseed in livestock diets is complicated by the presence of a toxic compound, gossypol.

Gossypol is a polyphenolic compound naturally occurring in the pigment glands of the plant. The presence of this compound in the feed leads to decreased growth and depending on the level of inclusion, increased rates of mortality (Clawson, et al., 1975). In the whole seed, gossypol is present in the free form, contained in the gelatinous capsule, which according to Martin (1990) is the toxic form. Mechanical rupturing of the gland wall is impossible, but water, alcohols and ketones will lead to swelling and rupturing of the gland wall (Kuiken & Trant, 1952). Heating, for example during the oil extraction procedure of cottonseed, will also rupture the pigment gland releasing the gossypol over the proteins (Clawson et al., 1961). As a result the gossypol binds to the proteins, forming large molecules and rendering the gossypol non-toxic. A part of the free gossypol binds especially to the lysine component of the protein, lowering the protein content of the processed seeds (Martin, 1990). Subsequent developments in reducing the toxic effects of free gossypol, is with the addition of Calcium and Iron salts in the feed. Jarquin et al. (1966) showed that with the addition of 1%  $\text{Ca(OH)}_2$  and 0.1%  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in swine feed it completely eliminates the effects of gossypol poisoning among the pigs without seriously reducing the protein quality. These positive effects are according to Jarquin et al. (1966) most likely due to the formation of an insoluble iron compound of gossypol and the oxidation of gossypol thereby lowering the concentration. Increased levels of protein in the diet also leads to decreased detrimental effects from gossypol (Jarquin et al., 1996).

The content of gossypol in whole cottonseed ranges from 0,02% to 6,64% and is thought to provide resistance to insects (Adams et al., 1960). Many factors influence gossypol content such as: species of cotton plant, climatic conditions, soil conditions, fertilizer, etc. although cottonseed oil-cake meal (CSOCM) has long been recognized as an economical protein source for dairy cow diets, it is potentially toxic when fed to certain animals.

This component is of great importance with relation to the rations allowable to use in livestock feed. The form in which it is supplied as an animal feed source is cottonseed meal, wherein the gossypol is transformed into its bound form, rendering it almost completely devoid of its toxic effects in some species.

### **2.4.1 Implications of cottonseed in diets of monogastric animals**

The first published evidence of cottonseed leading to injuries under livestock was as early as 1859 (Adams et al., 1960), but not all were in accordance with the fact that gossypol was the poisoning component. According to Smith (1956) the confusion was due to the frequent occurrence of Vitamin A deficiency in the diets used. It is now accepted that gossypol is toxic, especially in swine as they are most susceptible.

According to Adams et al., 1960, in gossypol poisoning an intense burden is placed on the heart and lungs as gossypol prevents oxygen from being released by oxyhemoglobin as well as it having a hemolytic effect on erythrocytes (red blood cells), decreasing the amount of protein available for binding to oxygen as well as plasma fluid (Menaul, 1923 as cited by Adams et al., 1960).

The pathological changes occurring due to gossypol poisoning, is described by Smith (1956) through examining 18 pigs that died during the course of receiving different levels of free gossypol in their feed. Gossypol inhibits the release of oxygen from haemoglobin and also has a haemolytic effect on erythrocytes resulting in decreased available oxygen. The result is the lack of sufficient supplies of oxygen to the heart and lungs as well as decreased protein molecules for plasma fluid to bind to. The secondary symptoms occurring most frequently were laboured breathing (dyspnoea), brought on by serious infection of the lungs or heart. According to Smith (1956), as weakness and emaciation progressed, appetite remained good among the pigs up until death. Microscopically, congestion and oedema of the heart and liver were prominent features.

One reason for avoiding cottonseed (gossypol) with monogastrics is the restriction it places on lysine utilisation, one of the first limiting amino acids (proteins) in monogastric animals. Ostriches are able to produce microbial protein in the hindgut but can only be fermented as an energy source in the form of volatile fatty acids such as acetate. Amino acids can not be absorbed from the hind gut or utilized as a protein source.

The effect of cottonseed inclusion on the chemical composition of the meat needs to be determined in order to establish whether cottonseed can be utilized as a less expensive alternative to current sources of protein. As the need for value added ostrich products are increasing, the effect of cottonseed on the processed ostrich meat is also of importance. The effect of gossypol in the feed of ostriches have not yet been determined.

## **2.5 PHYSICAL MEAT CHARACTERISTICS**

Ostrich meat is seen as a healthy alternative to red meat consumers. Physical meat quality is determined by the following characteristics: pH, tenderness, colour and water holding capacity.



### **2.5.1 pH**

Living muscle generally has a pH around 7.2, but after the animal dies anaerobic glycolysis takes place causing a drop in the pH of the muscle due to the production of lactic acid (Sales, 1999). Ostrich meat has a relatively high, final pH (pH reached 24 hours post mortem;  $pH_U$ ) (Sales, 1999) and can be classified as intermediate, being between normal ( $pH < 5.8$ ) and extreme dark firm dry (DFD) meat ( $pH > 6.2$ ) (Cooper & Horbańczuk, 2002). Dark firm dry (DFD) meat is the result of a decreased rate of pH decline post mortem, often caused by a depletion of muscle glycogen (Lawrie, 1998) ante mortem brought on by stress (Sales, 1996).

In a study by Hoffman (2007), it was evident that ostriches that were more resistant to handling, or more nervous, leading to higher levels of stress experienced showed higher pH values at 24 hours post mortem. The relatively high,  $pH_U$  of ostrich muscle may be favourable in terms of water holding capacity (WHC) when looking into value-added meat product development from this species (Fisher et al, 1999).

### **2.5.2 Tenderness**

Tenderness usually refers to the ease of shearing or cutting during mastication (Cooper & Horbańczuk, 2002; Sales, 1999), and is probably one of the most sought after qualities for consumers. Ultimately tenderness will be determined by the consumer but due to a large variety of regional preferences instrumental methods are used (Sales, 1999). Warner-Bratzler Shear (WBS) force is used to record objective tenderness values and works on the principles that a higher force is required to cut a core of meat in half as the meat becomes tougher (Lawrie, 1998). According to Sales (1996) ostrich muscles can be classified according to WBS force into the following categories: (i) most tender: *Femorotibialis medius*, *Gastrocnemius*, *Iliofemoralis*; (ii) tender: *Iliotibialis lateralis*, *Ambiens*; and (iii) least tender: *Iliofibularis*, but are subject to many variables, including cooking duration.

### **2.5.3 Colour**

Colour is very important, as it is the first visible indication of the quality of the product for consumers (Cooper & Horbańczuk, 2002; Sales, 1999). Raw ostrich meat has a darker red appearance in comparison to beef (Sales, 1999), and the darker appearance may be due to the relatively higher  $pH_U$  reached after 24h. According to Lawrie (1998), the  $pH_U$  might be responsible for the darker colour as it leads to muscle fibres being tightly packed together, creating a barrier for light diffusion. This quality in ostrich meat leads to a recommendation by Sales (1996) that different colour groups needs to be grouped together not only in the marketing of whole raw muscles but also in value added products to avoid variation in the visual appearance of the final processed products.

#### **2.5.4 Water holding capacity**

Water holding capacity (WHC) is defined as the capacity of the meat to retain water during application of external forces, cutting or mincing. Water holding capacity influences the appearance before cooking, cooking ability, juiciness during chewing and the total amount of saleable meat (Sales & Horbanczuk, 1998). Ostrich meat may give an impression of being dry in the mouth, especially if the cooking time is too long and the lower level of intramuscular fat of ostrich meat accelerates this dryness, resulting in lowered sustainable juiciness when chewing (Cooper & Horbańczuk, 2002; Sales, 1999). The juiciness of a cut of ostrich meat or the capacity to hold moisture when processed is affected by the fact that ostrich meat loses moisture when vacuum packed and stored in refrigerated (2°C for 14 days) or frozen (20°C for 4 months) conditions (Sales, 1999). This characteristic should definitely be researched more thoroughly in order to improve ostrich meat's viability as an export product as well as an option to use in processed products.

### **2.6 NUTRITIONAL QUALITY OF OSTRICH MEAT**

To increase the demand for ostrich meat, the modern, health conscious consumer's knowledge on the nutrient composition of ostrich meat must be increased and so marketing these characteristics is a necessity. Consumers are mostly interested in the fat, cholesterol, fatty acid (FA) compositions, protein and minerals. Of these, fat is most likely one of the deciding factors when purchasing fresh meat.

#### **2.6.1 Fat**

Ostrich meat presents an exceptionally low intramuscular fat (IMF) content (0.92% and 0.70%; Lanza et al., 2004; Sales & Hayes, 1996) when compared to other livestock species (Sales, 1996; Sales, 1999; Cooper & Horbańczuk, 2002), which in terms of marketing is ideal in the modern day, but with regards to sensory attributes, for example sustained juiciness, is critical as fat has a stimulatory effect on saliva excretion (Lawrie, 1998). Ostrich meat should not be cooked to the point of being well done (80°C) as it creates a feeling of dryness in the mouth (Sales, 1999). As mentioned before, ostrich meat has a relatively high pH<sub>U</sub> leading to increased WHC, which is a characteristic also seen in meat that has a higher level of IMF; higher IMF tends to loosen up the fibre structures allowing more water to be retained within the muscle (Lawrie, 1998), but this effect is lost in ostrich meat that has a high WHC due to its low IMF (Sales, 1995).

#### **2.6.2 Cholesterol**

Initially it was thought that ostrich meat was almost devoid of cholesterol (Cooper & Horbańczuk, 2002), this was due to the fact that ostrich meat has such a low IMF content, but the correlation between IMF and cholesterol is poor (Sales, 1999). Further research showed however that the

cholesterol content of ostrich meat is in the same range as turkey and beef (Cooper and Horbańczuk, 2002) and has been reported to be around 57mg/100g tissue (Sales, 1996).

Cholesterol is mainly situated as a structural component in cell membranes and is also built into nervous tissue and is therefore an important component for life. Reports have shown that the amount of synthesized cholesterol in the body is much higher than the daily intake. Cholesterol exchange however, is necessary to avoid a build-up of cholesterol in the bloodstream, which is one of the causes of arteriosclerosis (Cooper & Horbańczuk, 2002; Sales, 1999).

### **2.6.3 Fatty Acids**

Preached across the world was the importance of decreasing intake of saturated fatty acid (SFA) and increasing intake of polyunsaturated fatty acids (PUFA) in order to prevent coronary heart disease (Sales, 1999). Ostrich meat has a good fatty acid profile and higher levels of PUFA, than beef and chicken; 30%, 5% and 19% respectively (Cooper and Horbańczuk, 2002). Sales (1996) states that of great importance is the knowledge that feeding regime can affect the fatty acid profile of poultry and fowl. Current diets of people are deficient of omega 3 (n-3) fatty acids, and is detrimental as omega 3 fatty acids are important as it lowers the incidences of coronary disease, is essential for growth and development in man throughout life, and omega 3 fatty acids have more effective antithrombotic and antiatherogenic properties than the corresponding omega 6 PUFA (Cooper & Horbańczuk, 2002). In 2001 these same authors reported that the current ratio of omega 3:omega 6 (n-3:n-6) of human diets range between 1:10 and 1:20, but the optimum ratio is in reality 1:1. Therefore, by feeding oils containing omega 3 fatty acids to ostrich, the result could be omega 3 fatty acid enriched ostrich meat, which is a character that has to be addressed in combined feeding and meat studies (Sales, 1996).

## **2.7 NUTRITION AND MEAT QUALITY**

Three major contributors to meat quality are flavour, tenderness and juiciness all of which are attributes that add to the attractiveness of meat to consumers and can also be influenced by nutrition, mainly through the effects on the amount and type of fat in the meat (Wood et al., 1999).

Wood et al. (1998) describes the correlation between amount of fat and tenderness in the following manner. Fat accumulation takes place firstly in subcutaneous and intermuscular fat depots possibly insulating the muscles against the effects of cold air. Secondly it accumulates in muscle forming the so called intramuscular fat (IMF) or marbling. As intramuscular fat increases it possibly dilutes the fibrous protein, making it less resistant to shearing. Another result of fat increase is that fat cell expansion in the perimysial connective tissue leads to muscle fibres being forced apart and resulting in an opened-up muscle structure. Feeding a high-energy diet, above that which is required for maintenance and muscle deposition will lead to increased IMF and possibly increased tenderness (Wood et al., 1999).

Consumers have however become even more aware of not only the amount of fat but also the type of fat present in meat products, specifically fatty acids and their effect on human health.

### **2.7.1 Fatty acids and meat quality**

Various aspects of meat quality (colour, firmness or softness of meat, appearance (fat colour), shelf life and flavour development during cooking or processing of meat) are affected by fatty acids (Wood et al., 2004).

Due to different melting points of fatty acids, the variation in composition of fatty acids will affect the firmness or softness of fat. As unsaturation increases melting point decreases, for example, of the 18C fatty acid series, Stearic acid (C18:0) melts at 69.6°C and Oleic acid (C18:1n-9c) melts at 13.4°C and Linoleic acid (C18:2n-6c) at -5°C (Wood et al., 2004).

Appearance of the fat will be affected by level of saturation, as groups of fat cells containing fatty acids with a high melting point, solidified fat, will appear whiter than fat cells with a lower melting point, liquid fat. Back fat of pigs containing Linoleic acid (C18:2n-6c) at levels exceeding 15mg/100mg of total fatty acids produce soft fat leading to reduced shelf life (Wood et al., 1999). Rancidity and colour deterioration (shelf life parameters) is affected by the amount of double bonds in unsaturated fatty acids as these are more easily or rapidly oxidized. The products from lipid oxidation catalyses the oxidation reactions causing dark brown metmyoglobin (Wood et al., 1999). According to Wood et al. (2003) this characteristic of UFA is important for flavour development during cooking or processing meat. During cooking volatile, odourous, lipid oxidation products are released and are involved in reactions with products produced from the Maillard reaction that all contribute to the flavour and odour of cooked meat.

### **2.7.2 Manipulating the fatty acid profile**

Recently the interest in manipulating the fatty acid composition of meat has increased as meat is seen as a major source of fat in the diet of man (Wood et al., 2004). Of great concern is the level of saturated fatty acids and the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (P:S) as well as the balance in the diet between the PUFA n-3 and n-6. In recent years, great interest has been shown in the long chain omega-3 PUFA, eicosapentaenoic acid (EPA-C20:5n-3) and docosahexaenoic acid (DHA-C22:6n-3) and their beneficial cardiovascular and anti-inflammatory properties (Williams, 2000). According to Williams (2000) the reason for the low level of omega-3 PUFA in the modern man's diet is due to the low consumption of fish and fish products. Focus is placed on meat as a natural supplier of omega-3 PUFA to the diet of man.

Monogastric animals, like pigs (Wood et al., 1999) and poultry and fowl (Sales, 1996) absorb intact fatty acids through the small intestine and incorporate them unchanged into tissue lipids. PUFA Linoleic (C18:2n-6c) and  $\alpha$ -linolenic (C18:3n-3) acids are not synthesized in the body and concentrations of these fatty acids in tissue are more readily changed with a change in diet than

SFA and MUFA that are synthesized in the body (Wood et al., 1999). This quality makes it possible to modify the fatty acid composition of meat and fat tissue in these animals through means of diet modification. The hydrogenating effect of ruminant bacteria makes the incorporation of feed FA into tissue FA in ruminants less direct by converting PUFA into SFA or unsaturated fatty acids (UFA) with fewer double bonds (Wood et al., 1999).

Riley et al. (2000) investigated inclusion of linseed in the diets of boar and gilt pigs and the effect on nutritional value of the pork meat. Linseed is rich in the n-3 fatty acid  $\alpha$ -linolenic acid, which acts as the precursor for EPA (C20:5n-3) and DHA (C22:6n-3) in vivo. Linseed was either fed at high concentrations over a short period of time (0g or 114g linseed/kg food provided to 16 pigs of 87kg live weight for 20 or 27 days) or at lower concentrations for a longer period of time (0g, 10g, 20g or 30g linseed/kg food provided to 64 pigs of 46kg live weight for 54, 62, 68 or 75 days). The n-6:n-3 ratio in muscle and fat tissue were reduced in the long-term (30g/kg) diet just as successfully as in the short-term (114g/kg) diet but only required 0.73 as much linseed. Thus, the nutritional value was improved with no changes in organoleptic characteristics, as measured by a trained taste panel and no loss in shelf life stability was observed in the appearance of the product (Riley et al., 2000).

In 2005, Hoffman et al. tested the effect of dietary inclusion of fish oil (0, 10, 20 and 30 grams per day) rich in omega-3 fatty acids on the sensory, fatty acid and physicochemical characteristics of ostrich meat. Fatty acid profile of both muscle and fat were altered showing increased SFA, decreased PUFA and unchanged MUFA. Even though the general amount of PUFA decreased, increases in the essential long chained (C20:4n-6, C20:5n-3 and C22:6n-3) PUFA was observed leading to the conclusion that the inclusion of fish oil shows a positive effect on the fatty acid profile of ostrich meat even if there was an increase in the SFA content. Saturated fatty acid levels in ostrich meat (37.24% of total FA (Horbanczuk et al., 1998)) are low when compared to other livestock species and an increase therefore does not automatically lower the quality of ostrich meat. The ultimate pH ( $pH_u$ ) (pH reached after 24 hours) and lightness ( $L^*$ ) was reduced in the muscle, which can be explained by increased IMF values as energy content of the feed was increased by the addition of 6.7% fish oil. Flavour and aroma of the abdominal fat pads were affected by the inclusion of the fish oil, fishiness increased ( $P < 0.05$ ), but the aroma and flavour in muscle only showed a slight tendency to increased fishiness when fish oil levels were increased. Further studies on the effect of fatty acids in the diet on the development of flavour and aroma in fresh as well as processed meat will be useful as ostrich meat has a low IMF level, therefore decreasing the level of flavour development due to dietary manipulation of the fatty acid content.

Different sources of dietary fat inclusion with the aim of increasing PUFA in muscle and fat tissue of meat animals would be beneficial for the health of the consumer but could be detrimental for flavour and aroma compounds PUFA are more susceptible to protein and lipid degradation as well as oxidation due to chemical structure stability and could therefore lead to reduction of shelf life, off

flavours or rancidity (Warnants et al., 1999).

## **2.8 FLAVOUR AND AROMA DEVELOPMENT IN DRY CURED MEAT PRODUCTS**

According to Toldrá (1998) the process of flavour development is very complex as flavour compounds could be the result of numerous biochemical reactions. These reactions are regulated by enzymes and the biochemical reactions include lipid oxidation, Maillard reactions or Strecker degradations.

The main biochemical reactions involved in flavour development are proteolysis (Figure 2.2) and lipolysis (Figure 2.3). The degree to which these endogenous enzymes or microbial enzymes, naturally occurring or added as starter cultures, are involved will depend on the processing procedures under which the specific product is manufactured (Toldrá, 1998). These biochemical reactions then generate volatile and non-volatile compounds directly contributing to the final flavour of the product (Toldrá et al., 1997). The degree to which endogenous enzymes are responsible for final flavour formation is dependent on the type of product. The endogenous enzymes in salami for example are greatly altered during mixing and homogenizing where a cured Iberian ham for example remains intact and a lower level of micro-organisms are present in the inner part of the ham.

Proteolysis also contributes to the texture of the final product whereas lipolysis greatly contributes to the aroma and final sensory quality of the product (Toldrá, 2006).

### **2.8.1 Proteolysis**

The proteolytic enzyme system in muscle is quite complex and comprises of endopeptidases (calpains and cathepsins B, L, H and D) and exopeptidases (tri-peptidylpeptidases I and II, di-peptidylpeptidases I, II, III and IV as well as alanyl, arginyl, methionyl, leucyl and pyroglutamyl aminopeptidases) and is quite complex (Toldrá et al., 2000).

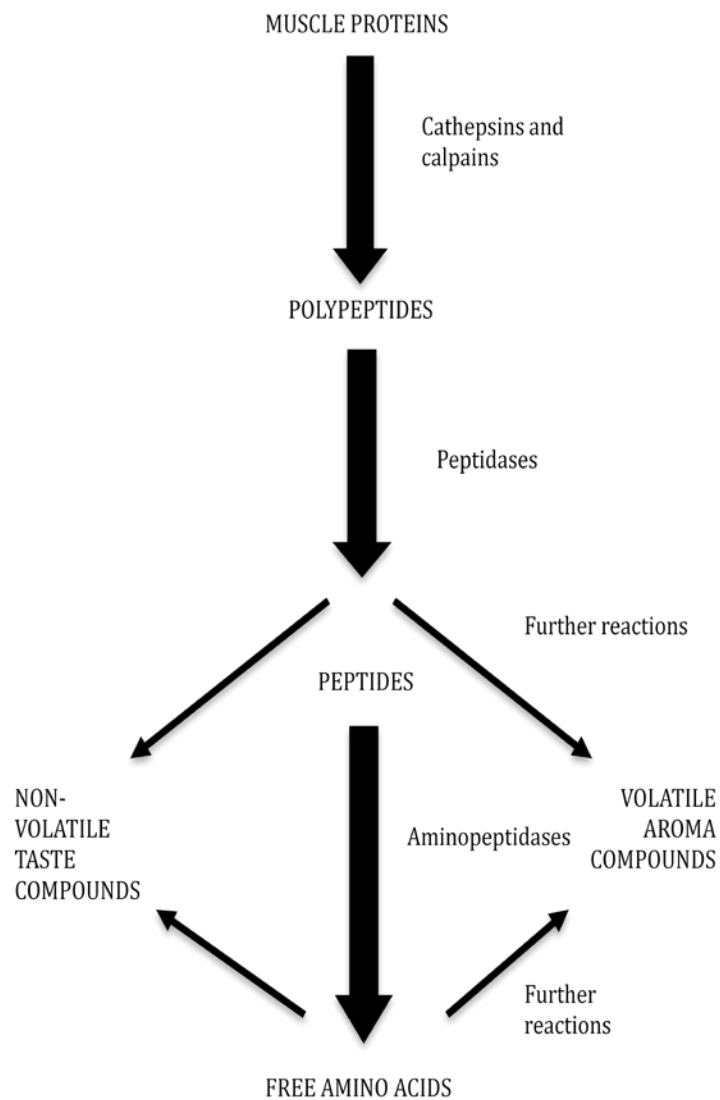
#### *2.8.1.1 Endopeptidase*

##### *2.8.1.1.1 Cathepsins (Lysosomal proteinase)*

Cathepsins are small enzymes and show activity at acidic pH values and the ability to degrade different myofibrillar proteins when in in vitro environments (Toldrá et al., 1997).

Cathepsins remain quite stable throughout processing, i.e. they show prolonged activity. This becomes especially evident during increased curing times like during processing of Iberian cured hams; assessed during production of a dry cured ham Toldrá et al. (1997) found cathepsins B, H and L to show peaked activity during the 6<sup>th</sup> and 10<sup>th</sup> month of production. Profiles of muscle sarcoplasmic proteins and myofibrillar proteins structures change during processing, giving rise to

numerous fragments or compounds affecting the quality of the end product in terms of flavour and structure.



**Figure 2.2** Flow chart showing the major steps in post-mortem muscle proteolysis (adapted from Toldrá, 1998).

**Table 2.6** Endopeptidase active degradation on myofibrillar proteins (Schwartz & Bird, 1977; Toldrá et al., 1997)

<b>Proteinase</b>	<b>Yes</b>	<b>No</b>	<b>Slow</b>
Cathepsin B	Myosin Actin Troponin T	Myosin light chains Troponin C	
Cathepsin D	Myosin heavy chains Actin Alpha-actinin Titin Tropomyosin Troponin I and T		Actin
Cathepsin H		Endo- and amino-peptidase activity	
Cathepsin L	Myosin heavy chains Actin Tropomyosin Alpha-actinin Troponins I and T	Troponin C	
$\mu$ - and m-calpain (Calpain I and II)	Troponin T and I Tropomyosin C-protein Filamin Desmin Vinculin Titin and nebulin	Myosin Actin Alpha-actinin Troponin C	

#### 2.8.1.1.2 Calpains (*Sarcoplasmic proteinase*)

Known in the literature as calcium activated neutral proteinases, calcium dependant proteases or calcium-activated sarcoplasmic factor (CASF), calpains are a group of cystein endopeptidases requiring calcium in order to function (Toldrá et al., 1997). An inhibitor of  $\mu$ -calpain and m-calpain, calpastatin, and three proteases,  $\mu$ -calpain, m-calpain and calpain 3, make up the calpain system found in skeletal muscle (Koochmariaie & Geesink, 2006).

Unlike cathepsins, calpains show maximal activity around a slightly higher pH of around 7.5 (neutral pH) and are widely distributed in the cytosol and Z disc region of the muscle fibre (Toldrá et al., 1997). Even though calpains contribution to proteolysis is limited, Koochmariaie & Geesink (2006) reported that calpains degrade troponin T and I, tropomyosin, C-protein, filamin, desmin



and vinculin as well as titin and nebulin but has no effect on myosin, actin, alpha-actinin and troponin C. Calpains are limited in their action as they are pH sensitive, due to the presence of the endogenous inhibitor calpastatin and the fact that they autolyse as soon as they are activated (Koochmaraie & Geesink, 2006). In co-ordination with cathepsins however, calpains assist in the initial breakdown of myofibres by hydrolyzing of muscle proteins during the salting and post-salting stages, producing fragments or compounds (Toldrá et al., 1997) as will be discussed in further detail later in this chapter.

## **2.8.2 Exopeptidases**

### *2.8.2.1 Aminopeptidases*

According to Toldrá et al. (1997), these enzymes appear to be metallo-proteins, with complex structures and are present in a wide variety of molecular weights. Naming of these enzymes, are based upon the requirement or preference for a specific N-terminal amino acid (Toldrá et al., 1997). The degree of preference is however not similar in all muscle aminopeptidases (Toldrá et al., 2000). For example, alanyl aminopeptidases has a broad range of specificity, hydrolyzing phenylalanine, lysine, methionine, alanine and leucine (Flores et al., 1996) whereas arginyl aminopeptidase activity is restricted to a few terminal amino acids such as arginine and lysine (Flores et al., 1993).

Except for leucyl aminopeptidase, the remaining aminopeptidase are all active at neutral pH (Flores et al., 1996) and are generally found in the cytosol (Toldrá et al., 1997) where alanyl aminopeptidase is responsible for as much as 86% of the total aminopeptidase activity and is therefore of great importance (Toldrá et al., 2000). Stability of these aminopeptidase is good. Alanyl aminopeptidase shows the highest exopeptidase activity during the curing process, arginyl and leucyl to a lesser extent and then pyroglutamyl aminopeptidase that presents rather poor stability, negligible activity by day 40 of processing a dry cured ham (Toldrá et al., 2000).

Table 2.7 indicates the factors or circumstances under which aminopeptidase activity is reduced.

**Table 2.7** Aminopeptidases and factors that influence activity (adapted from Toldrá et al., 2000; Flores et al., 1997).

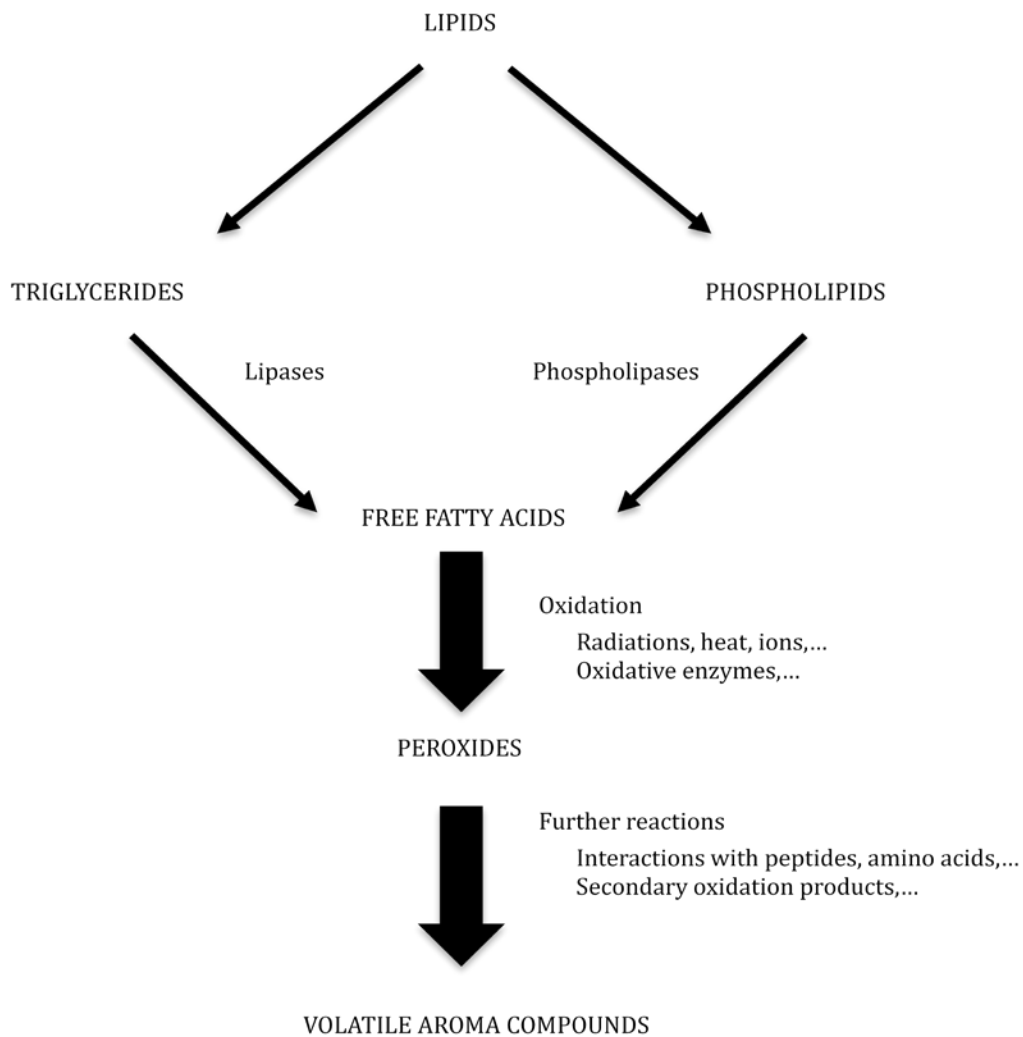
<b>Aminopeptidases</b>	<b>Salt</b>	<b>Water activity</b>
Alanyl	Reduced	Reduced
Pyroglutamyl	Reduced	Reduced
Leucyl	No reduction	Reduced
Arginyl	No reduction	Reduced

A factor with less effect is pH as the range of variation is narrow during longer curing times, around 5.6 - 5.8 to 6.4 (Toldrá et al., 2000). Lastly, aminopeptidases activity is greatly reduced through the accumulation of free amino acids producing a negative feedback inhibition (Flores et al., 1998). Alanyl, more so than arginyl aminopeptidases were both affected by the increased concentrations of free amino acids in dry cured meat, being inhibited via the competitive mode (Flores et al., 1998).

The result of the activity of these aminopeptidase, especially alanyl aminopeptidase, is the generation of free amino acids, as reported by Aristoy & Toldrá (1991) by the increase of free amino acids during the processing duration of dry-cured ham. Of the amino acids produced, glutamic acid, lysine, alanine, leucine and arginine were generated in highest amount when processing dry cured ham (Toldrá et al., 2000) and contribute to the volatile compounds producing characteristic flavours in cured meat products (Toldrá, 2006).

### ***2.8.3 Lipid degradation***

The main degradation mechanisms affecting lipids during dry curing is lypolysis and lipid oxidation (Martin et al., 1998) causing large changes which could affect the sensory properties of dry cured products, specifically colour and flavour (Coutron-Gambotti & Gandemer, 1999).



**Figure 2.3** Flow chart indicating the major steps in post mortem muscle lipolysis and oxidation to flavour compounds (Toldrá, 1998).

### 2.8.3.1 Lipolysis

During the initial stages of producing dry cured products, muscular and adipose tissue undergo extreme lypolysis (Toldrá et al., 1997). This is evident through the major increase of free fatty acids (FFA) and diacylglycerols and a decrease of triacylglycerol concentrations (Toldrá et al., 1997; Toldrá, 1998; Martin et al., 1998). These changes are of great importance as the resulting compounds serve as precursors to volatile compounds attributing to the characteristic flavour and appearance of dry cured products such as Spanish Serrano dry-cured ham (Flores et al., 1997) or Iberian dry-cured ham (Martin et al., 1998).

In adipose tissue, there are three important enzymes involved, namely lipoprotein lipase, hormone sensitive lipase and monoacylglycerol lipase, all of which are active under neutral or basic pH conditions (Toldrá et al., 1997). Lipoprotein lipase shows preference for primary esters, leading to fatty acids at position one to have preference over fatty acids at position three (Toldrá et al., 1997). Hormone sensitive lipase is responsible for hydrolysis of the ester-bond in triacylglycerols and results in the release of diacylglycerols under neutral pH conditions (Toldrá, 2006). According to Coutron-Gambotti & Gandemer (1998), lypolysis in adipose tissue support a hypothesis of non-specific hydrolysis of triglycerides.

Muscle lipase constitutes lysosomal acid lipase, which is responsible for hydrolyses of tri-, di- and monoacylglycerols, and shows a preference for the primary ester bonds of triacylglycerols (Toldrá et al., 1997). Another very important enzyme in the muscle lipase group is acid phospholipase, that is responsible for degrading the phospholipid components of adipose tissue (Yuan et al., 1990; Toldrá, 2006). Vestergaard et al. (1999) reported that there is a positive relationship between neutral and acid lipase proposing that the same factors inhibit and induce their activity. Several factors exist, all contributing to the amount or level of activity of enzymes involved in lipolysis; processing conditions, raw material, salt level and water activity (Martin et al., 1998; Vestergaard et al., 1999).

### 2.8.3.2 Products of Lipolysis

The evidence of the enzyme activity lies in the concentration of the products released from their actions. Fatty acids, tri-, di- and monoglycerides are released in different quantities and at different rates during the processing stages.

During the first few months of dry-curing the concentrations of triglycerides decrease sharply and the di- and monoglycerides increase accordingly (Coutron-Gambotti & Gandemer, 1998). The amount of diglycerides remain higher than the monoglycerides, making it evident that a limiting factor of lipolysis is the activity of triglycerol lipase (Coutron-Gambotti & Gandemer, 1998). Free fatty acids (FFA) concentrations seem parallel to lipolytic activity; FFA increase as acid lipase activity increase making it evident that the enzymes are active (Vestergaard et al., 1999).

From the hydrolysis of triglycerides, myristic, heptadecanoic, linolenic and arachidonic acids were present at the highest levels during processing of a dry-cured ham (Toldrá et al., 1997). Coutron-Gambotti & Gandemer (1998) postulate that the physical composition of the triglycerides, affect the rate of lipolysis, as triglycerides that contain two saturated fatty acids (SFA) are solid at temperatures during processing and therefore limit the lipase activity.

Martin et al. (1998) also postulates that the fatty acids (FA) released from phospholipid hydrolysis is of greater importance when evaluating the total FA content of a dry-cured ham. The specific FA released from phospholipid hydrolysis were Linoleic (C18:2), Arachidonic (C20:4), Oleic (C18:1), Palmitic (C16:0) and Stearic acids (C18:0) (Martin et al., 1998).

The saturation of the fatty acids released will affect the concentrations of FFA during production as saturation determines the susceptibility to oxidation as mentioned earlier. According to Toldrá (2006), oxidation of FFA, mostly unsaturated, readily take place leading to the formation of volatile compounds contributing to the characteristic flavour development of dry-cured meat products.

## **2.9 FLAVOUR COMPOUNDS**

### **2.9.1 Volatile**

All the changes in muscle and adipose tissue as discussed above give rise to volatile compounds that directly contribute to the flavour and aroma of the final product.

According to Huan et al. (2005), these volatile compounds can be clustered into chemical families as follows; alkanes and alkenes, aromatic and cyclic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, terpenes, oxygenous heterocycle compounds, sulphur compounds, chloride compounds, amides and others. The concentrations of the specific compounds and the variety of compounds will vary according to the stage of production as well as the methods used in producing a specific product (Huan et al., 2005). The initial lipid and protein composition of the muscle and fat, directly related to the feed composition will also contribute to the volatile compounds generated during processing and flavour formation and this emphasizes the importance of feed formulation especially in processed meat products.

What is of importance is the correlation of these volatile compounds and the sensory characteristics and Barbieri et al., (1992) states that it is most likely that a flavour and aroma of a specific product relies on a balance of various components and not a specific compound.

### **2.9.2 Non-volatile**

Non-volatile compounds such as taste active peptides and free amino acids can also contribute to the characteristic flavour and aroma of a product (Toldrá, 1998) even more so during longer curing times (Toldrá et al., 1997). In fact, Flores et al. (1997) shows several amino acids strongly correlated with the length of the drying process as well as the cured and pork flavour attributes. These amino acids include glutamic acid, aspartic acid, histidine, arginine, valine, methionine,

isoleucine, leucine, phenylalanine, tryptophan and lysine. Tastes of individual amino acids were characterized as sweet, salty, sour, bitter or monosodium glutamate (MSG)-like and peptides as being sour, bitter or practically tasteless, but in general these tastes are complex and need to be described by more than one characteristic (Kirimura et al., 1969).

According to Mottram (1998) the Strecker degradation of amino acids by dicarbonyl compounds formed in the Maillard reaction is an important reaction associated with flavour development. Each compound, volatile or non-volatile comes from a specific reaction and some compounds may be of multiple origins (Huan et al., 2005).

Factors that could possibly affect the flavour quality of a meat product, independent from the ante mortem factors, could be, among others, the raw meat properties, additives and processing conditions (Toldrá, 1998). Another controversy with regards to flavour and aroma of a product is the fact that every person will use different precursors in order to describe a flavour, making it difficult to pinpoint one or a combination of compounds to one or more flavours (Flores & Toldrá et al., 1997).

## 2.10 VALUE ADDED OSTRICH MEAT PRODUCTS

Value added Ostrich products currently produced in South Africa are listed in Table 2.8 whilst Table 2.9 shows the typical chemical composition of some of these products.

**Table 2.8** Various ostrich products available commercially in South Africa (Hoffman, 2005)

<b>Fresh products</b>	<b>Processed products</b>
Skinpack fillet	Ostrich bacon
Skinpack steak	Ostrich ham
Skinpack kebab	Vienna
Skinpack goulash	Russians
Skinpack sausage	Smoked fillet
Skinpack burger	French polony
Skinpack mince	Ostrich mince

**Table 2.9** The chemical composition of various processed ostrich meat products sold in retail outlets in South Africa (Hoffman, 2005).

<b>Chemical component (%)</b>	<b>French Polony</b>	<b>Ostrich Ham</b>	<b>Ostrich Bacon</b>	<b>Smoked Russian</b>	<b>Smoked Vienna</b>	<b>Smoked Fillet</b>
Dry matter	29.31	32.32	26.60	33.91	36.41	26.90
Protein	12.36	17.87	20.45	17.73	13.35	20.85
Fat	6.93	1.75	1.92	10.78	14.85	2.28
Ash	7.66	11.54	11.55	6.60	5.77	8.87
Cholesterol (mg/100g)	36.6	32.9	50.7	39.5	43.7	51.0
<b>FATTY ACIDS</b>						
C14:0	0.60	1.38	1.30	1.69	0.67	0.86
C16:0	25.79	21.97	27.65	27.30	24.31	19.84
C18:0	7.94	12.65	10.20	12.53	8.36	13.38
C20:0	0.11	0.12	0.20	0.22	0.21	0.15
C22:0	0.01	0.00	0.08	0.00	0.02	0.11
C24:0	0.01	0.00	0.35	0.00	0.02	0.11
SFA	34.46	36.11	39.78	41.74	33.59	34.44
C16:1n-7	5.61	2.97	5.03	2.96	5.50	3.80
C18:1n-9	37.60	46.65	28.95	44.61	43.04	32.22
C20:1n-9	0.33	0.09	0.00	0.16	0.28	0.21
C24:1n-9	0.04	0.00	0.00	0.00	0.27	0.19
MUFA	43.58	49.70	33.97	47.73	49.09	36.41
C18:2n-6	15.91	8.20	14.78	7.94	12.92	17.99
C18:3n-6	0.06	0.25	0.72	0.06	0.04	0.06
C18:3n-3	4.47	1.98	2.90	1.63	3.36	2.28
C20:2n-6	0.17	0.00	0.13	0.00	0.19	0.22
C20:3n-6	0.08	0.19	0.20	0.00	0.11	0.55
C20:4n-6	0.84	2.23	5.64	0.43	0.53	5.63
C20:3n-3	0.06	0.12	0.00	0.00	0.00	0.00
C20:5n-3	0.11	0.56	0.90	0.00	0.06	1.08
C22:2n-6	0.00	0.00	0.00	0.00	0.00	0.00
C22:4n-6	0.10	0.00	0.46	0.48	0.05	0.46
C22:5n-3	0.14	0.37	0.42	0.00	0.06	0.43
C22:6n-3	0.06	0.30	0.10	0.00	0.00	0.43
PUFA	22.00	14.18	26.25	10.53	17.32	29.15

As seen above, there is already quite a variety of ostrich products on the market. Cabanossi however is one of South Africa's many delicacies but very little research has been done on the chemical processes taking place during processing as well as factors affecting the quality of the finished product.

### **2.10.1 Cabanossi**

Cabanossi is a type of dry sausage that is lightly seasoned and then dried and smoked. Traditionally it comes in the form of a long, thin sausage, about 30cm long and 2cm in diameter made up of beef and pork meat although some variations in the meat type has been seen. The origin of this sausage is Polish where the name is derived from 'kaban' which means hog (young male pig, fattened on potatoes) in eastern Poland.

A typical recipe for producing cabanossi is 35% beef, 30% pork (meat 80%, fat 20%), 25% bacon and 10% spices stuffed into a natural casing followed by drying and smoking. The end product is a semi dry sausage (50-60% moisture) showing 40-50% weight loss during processing with a favourable smoky flavour. It is evident from the recipe that cabanossi is not a very healthy product as the majority of the mixture consists of animal fat. Consumer demand is changing and one of the most important factors affecting this changes is the increase of health concerns (Resurreccion, 2003) leading to consumers not only being interested in the amount of fat in their products but also the quality fat present in their meat. The need for product development and innovation are necessary for growth of available food products for the discerning consumer. Ostrich meat is known as a healthy red meat alternative due to its low IMF and favourable fatty acid profile and can therefore be used in creating and developing new products available for this type of consumer. For example, by using ostrich meat as the main meat source in preparing cabanossi it not only increases the quality of the product it also supplies the market with a product that meets the need of the health conscious consumer.

This study is consists of three parts. Firstly, the effect of inclusion of cottonseed oilcake as a dietary protein source for ostriches and the effect thereof on the live performance as well as the chemical composition of ostrich meat. Secondly, the consumer perception of the meat from birds receiving cottonseed oilcake is analyzed. And thirdly, ostrich and warthog cabanossi is prepared with the aim of targeting an upmarket group. Warthog meat is added to add to the exotic meat flavour of the product and simultaneously replacing the pork meat component of the product. Olive oil is added at increasing levels replacing the pork back fat component further adding to the quality of the value added product and supporting the increase in health awareness.



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## CHAPTER 3

### EFFECT OF COTTONSEED OILCAKE INCLUSION ON THE GROWTH PERFORMANCE AND CHEMICAL COMPOSITION OF OSTRICH MEAT

#### ABSTRACT

This study investigated the effect of replacing dietary soybean oilcake meal with increasing levels of cottonseed oilcake meal (CSOCM) on the growth performance and meat (*Iliofibularis* muscle) chemical composition of ostriches in order to decrease total feed costs. A total of 105 ostriches were divided into five feeding groups according to the CSOCM inclusion level: Control (0% CSOCM), 3%, 6%, 9% and 12% CSOCM, and fed with the experimental diets from 6 to 13 months of age. As a result of feeding CSOCM, the final live weight and the average daily gain significantly increased in the 12% CSOCM group. The proximate composition, cholesterol content, mineral and fatty acid profile of the meat remained unaffected. Considering all the results, CSOCM may be used as an alternative protein source to the more expensive soybean oilcake meal in ostrich nutrition.

*Keywords:* Ostrich, Feeding, Cottonseed oilcake, Growth performance, Meat quality

### 3.1 INTRODUCTION

Ostrich (*Struthio camelus* var. *domesticus*) farming as a commercial enterprise began in South Africa between 1857 and 1864 (Smit, 1963) and has made a major contribution to the agricultural economy of South Africa over the last 150 years (Brand & Cloete, 2009). Although ostriches were originally domesticated for the harvesting of their feathers, which was followed by high prices being paid for their skins, nowadays they are farmed to provide a healthy red meat (Cooper & Horbańczuk, 2002). As stated in many studies (Sales & Hayes 1996; Sales et al., 1999), ostrich meat has high PUFA content, low total fat, low sodium content, and is rich in heme-iron, and for these reasons can be considered a healthy red meat to be appreciated by modern consumers.

The ostrich is mainly herbivorous and, in natural conditions, its diet consists of a variety of plants and succulents (Jamroz, 2000). However, Nitzan et al., (2002) noticed that under grazing conditions, ostriches prefer forb-type pastures, and alfalfa, for example (Strydom et al., 2009), to grasses. In South Africa, ostriches are reared mainly under intensive feedlot (80%) or semi-intensive grazing (20%) conditions (Brand & Gous, 2006), and therefore most of the nutrition is provided by formulated feeds and concentrates. In feedlots, feed should be supplied *ad libitum* with clean water available at all times in order to aid digestion because ostriches should be fed continuously rather than separate meals (Aganga, et al., 2003). The successful rearing of ostriches from hatching to grower to breeder birds requires high standards of nutrition (Cooper et al., 2004) and because nutrition accounts for nearly 80% of total ostrich production costs (Brand & Olivier, 2011), more knowledge on this topic is required. The protein source represents a large part of these costs, and therefore the discovery of alternatives at lower prices that do not affect ostrich growth performance and are preferably not used in human nutrition would be beneficial for the industry and its farmers alike.

Cottonseed oilcake, a textile industry by-product, offers an alternative to the commonly-used but more expensive soybean. The main problem that has limited its utilization in animal nutrition thus far is the presence of gossypol, a toxic polyphenol naturally found in the pigment glands of the cottonseed. Gossypol is a reactive compound that rapidly binds minerals and amino acids when it is in its free form (Guedes & Soto-Blanco, 2010). Gossypol content varies between species of cotton plant and also depends on differences in cottonseed processing (Schroeder et al., 1995; Nagalakshmi et al., 2007; Kakani et al., 2010); moreover, animal species react differently to this particular polyphenol. For all these reasons, scientific studies concerning its utilization in animal nutrition, especially monogastrics, have provided controversial results (Ikurior & Fetuga, 1988; Rhule, 1995; Fombad & Bryant, 2004; Winterholler et al., 2009; Nunes et al., 2010; Wanapat et al., 2012). Although Aanga et al. (2003) stated that gossypol has also been shown to have toxic effects on ostriches, no studies on the effect of cottonseed oilcake meal (CSOCM) on the production and meat quality of ostrich meat have been conducted until now.



This study aimed to establish whether and to which extent the gradual replacement of soybean oilcake meal with CSOCM could affect ostrich growth performance and meat quality. The aim of the study therefore also included verifying whether CSOCM might be considered a viable alternative protein source in ostrich feeding or not.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Experimental design and diets

#### 3.2.1.1 Birds, experimental design and diets

A total of 105 ostriches (*Struthio camelus* var. *domesticus*) were used to study the effect of the inclusion of cottonseed oilcake on growth performance and meat (*Iliofibularis* muscle) chemical composition of ostriches from 6 months to 13 months of age. Hatching and rearing of the 105 ostriches took place on Kwessie farm, in Calitzdorp, South Africa. Ostriches remained on this farm until 6 months of age before being transported to the Kromme Rhee experimental farm outside Stellenbosch, South Africa, where they were kept for the duration of the trial (7 months; March to October). The birds were divided into five groups containing both sexes, however, as it was not possible to sex birds at this young age, the genders were not balanced. Ostriches were then subdivided into three replicate pens, resulting in 15 different paddocks, 7 ostriches per paddock (approx. 200 m<sup>2</sup>/bird) and fed five experimental diets (Tables 3.1 and 3.2) *ad libitum* with free access to drinking water. Diets were formulated on an iso-nitrogenous and iso-energetic basis allowing for the gradual replacement of soybean oilcake meal (SBOCM) with cottonseed oilcake meal (0% CSOCM/Control, 3% CSOCM, 6% CSOCM, 9% CSOCM and 12% CSOCM) (Table 3.1). CSOCM used in this study had 30.9% crude protein, 0.6% crude fat, 26.2% crude fibre and 9.1% moisture, respectively. Body weights were recorded individually on a monthly basis for the 7 month duration of the trial. Feed intake was also determined per month for each of the replicates. Therefore, feed conversion rate (FCR) as well as average daily gain (ADG) were determined as an average for the replicate. During the trial, 5 animals died: 2 in the Control group, 1 in the 3%, 1 in the 6%, and 1 in the 12% CSOCM groups. Birds were kept in roofed pens for two weeks prior to slaughter so as to undergo the mandatory blood tests for avian influenza. During this period they still received their standard experimental diets.

Slaughtering of ostriches took place at Ostriswell, a commercial abattoir in Swellendam, South Africa, using standard procedures as described by Hoffman (2012). The birds were transported for 4 hours and stood in lairage overnight where they had access to drinking water but no feed. Electrical head stunning (90-110 V, 400-600 mA, 4-6 s) was applied. After stunning the ostriches were suspended by both legs and exsanguinated by a neck cut to the aortic vein (thoracic stick). Bleeding was allowed for 10-15 min followed by plucking, skinning, evisceration and a health

inspection. Carcasses were chilled for 24 hours at 0-4°C, after which the fan fillet was excised for chemical analysis. Meat samples were vacuum packed and frozen at -20°C until further analyses.

Proximate composition (of both feed and meat samples), fibre fractions and gross energy (GE, MJ/kg) of the diets were analysed at Stellenbosch University, South Africa, whereas Ca and P of the diets were analysed at the Elsenburg Institute for Animal production, Stellenbosch.

Free, bound and total gossypol content in the CSOCM raw material and free gossypol content in the experimental diets were measured at CENTROLAB<sub>cc</sub>, Durbanville, South Africa. The fatty acid (FA) profile of the experimental diets and the meat, iron content of the diets as well as the mineral and cholesterol content of the meat were analysed at the Animal Medicine, Production and Health (MAPS) Department of the University of Padova, Italy. Meat samples were freeze-dried before transportation.

### *3.2.1.2 Chemical analysis of feed samples*

The finely ground feed samples were analyzed to determine dry matter (procedure 934.01), ash (procedure 967.05) and crude protein (2001.11) content according to AOAC (2002) methods. Ether extract was determined using ether extraction with diethyl ether reagent (AOAC 2002, procedure 920.39). Crude fibre was analyzed according to AOAC (2002, procedure 962.09). Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were also determined (Goering & Van Soest, 1970; Robertson & Van Soest, 1981 respectively). Gross energy was measured with an adiabatic bomb calorimeter (CP 500).

**Table 3.1** Chemical composition, mineral and free gossypol content of the cottonseed oilcake meal (CSOCM) and the experimental diets (g/100g as fed)

	Experimental Diets					
	CSOCM	Control	CSOCM 3%	CSOCM 6%	CSOCM 9%	CSOCM 12%
DM	91.9	89.8	89.9	88.7	89.1	89.7
Crude protein	30.9	17.3	16.3	16.2	15.7	16.0
Crude fat	0.60	2.74	2.84	2.87	2.83	3.04
Crude fibre	26.2	12.6	13.1	11.8	12.3	12.8
Ash	6.03	11.2	10.6	9.63	10.1	10.6
NDF	49.2	21.4	23.3	22.3	23.4	26.0
ADF	33.4	15.7	16.3	15.1	15.4	16.6
Ca	0.24	2.69	2.44	2.14	2.36	2.37
P	0.79	0.62	0.63	0.64	0.67	0.75
Fe	0.024	0.037	0.039	0.037	0.039	0.036
GE <sup>a</sup> (MJ/kg)		15.35	15.35	15.30	15.23	15.33
ME <sup>b</sup> (MJ/kg)		11.32	11.32	11.32	11.32	11.32
Free Gossypol (ppm)	82	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>	10 - 20	10 - 20

<sup>a</sup>Gross energy; <sup>b</sup>Metabolisable energy; <sup>c</sup>Not Detected; Chemical analysis performed in triplicate

Experimental diets were formulated to be equal in Metabolisable energy (ME), protein and amino acid contents (Mixit-Win, Agricultural Software Consultants Incorporated, San Diego, California, USA). The ME values of the raw ingredients were converted from pig ME-values to ostrich ME values as described by Brand, van der Merwe, Salih & Brand (2000b).

Calcium and phosphorus content of the diets was analyzed according to Palic et al. (2007; method 6.1.1). The feed sample was ashed at 460-480 °C for 6 h and, after cooling, 5 ml of 6 M HCl were added. The sample was then placed in an oven for 30 minutes at 50 °C. Subsequently, 35 ml distilled water were added and the solution was filtered into a brown bottle and made up to a final volume of 50 ml with distilled water. Elements were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Element concentrations were calculated using iTEVA Analyst software. Instrument settings were as follow: camera temp -27 °C, generator temp 24 °C, optics temp 38 °C, RF (radio frequency) power 1150 W, pump rate 50 rpm, aux gas flow 0.5 L/min, nebulizer 0.7 L/min, coolant gas 12 L/min and normal purge gas flow. Wavelengths for the elements were as follows: Ca 317.933 nm and P 177.495 nm. After 11 samples, standards with a high, medium and low range were analyzed for quality control.

Iron content of the diets was determined through inductively coupled plasma-optical emission spectroscopy (ICP-OES) by SPECTRO Ciros Vision EOP, with auto-sampling SPECTRO ADS500 and software SPECTRO Smart Analyzer Vision 1.50.534 (SPECTRO Analytical Instruments GmbH., Kleve, Deutschland). The following absorption line was used: 259.940. Iron concentration is expressed as g/100 g as fed.

Free, bound and total gossypol content of CSOCM and free gossypol content of experimental diets was determined using high performance liquid chromatography (HPLC) according to the method described by Hron, Kuk & Abraham (1990). The liquid chromatograph (LC) was an IBM/Nicolet LC/9560 equipped with a Waters NOVA-PAK 3.9 mm x 15 cm, 4  $\mu$ , C<sub>18</sub> reverse-phase column and a 2 cm disposable C<sub>18</sub> Supelguard column. The LC was operated isocratically with a mobile-phase flow rate of 1.0 ml/min. Samples were injected into a Rheodyne 1725 injector equipped with a 50  $\mu$ l sample loop. Gossypol was detected as the gossypol-aminopropanol (GA) complex by an IBM/Nicolet model LC/9563 variable wavelength UV detector at 254 nm. Peak areas were determined with an IBM 9000 computer. A three dimensional chromatographic scan of the GA complex was obtained, using the above columns and conditions, on a HP 1090 LC equipped with a diode array detector and HP 300 work station (Hron et al., 1990).

### **3.2.2 Meat quality measurements**

#### *3.2.2.1 Proximate composition*

Thawed and homogenised fan fillet samples were analysed according to the AOAC (2002) methods to determine moisture (procedure 934.01) and ash (procedure 942.05) whereas protein content was determined according to the AOAC (1992) method, procedure 992.15. The total lipid content of the meat was analysed using the chloroform/methanol (1:2) fat extraction method (procedure 920.39).

#### *3.2.2.2 Cholesterol content*

Cholesterol content of the freeze-dried fan fillets was determined through absolute quantitative analysis by high performance liquid chromatography (HPLC) (Indyk, 1990; Casiraghi et al., 1994). A 500 mg meat sample was transferred into a polypropylene tube and then into a glass tube of 50 ml with Teflon cap. To this 50 ml glass tube, 4 ml of 96% ethanol and 2 ml of KOH (50% w/v) was added and then placed in a water bath (TMR 740) at 70°C for 20 minutes. Tubes were removed and allowed to cool down to room temperature. After cooling, 1 ml of internal standard (solution of pregnenolone 1 mg/ml) was added to each tube. To each tube, 25 ml of hexane-diethyl ether (ratio of 1/1 v/v) was added and then shaken vigorously for 5 minutes. Subsequently, 20 ml of water was added to the samples and stirred for 1 minute, then stored overnight at 4 °C. Samples were centrifuged at 2000 rpm for 20 minutes. From the upper part, 1 ml of this organic phase was then removed with a pipette and placed in a Pyrex tube. The sample was then dried in a centrifugal

vacuum for 30 minutes and re-suspended in 1 ml of mobile phase (7% isopropyl alcohol in n-hexane (v/v) solution) for the HPLC. High performance liquid chromatography conditions were as follows: injection volume: 20 $\mu$ L, analysis time: 9 minutes, retention time of cholesterol: 4 minutes, internal standard retention time: 3.5 minutes, mobile phase flow: 0.8 ml/min, UV detection: 208 nm, chromatography column: CLONE BOND 10 x 3.9 mm SILICA 300 micron Phenomenex.

### *3.2.2.3 Mineral profile analysis*

The mineral profile of 15 fan fillet freeze-dried samples (3 samples per experimental group) was determined by ICP-OES. All samples were also scanned for mineral content determination by X-Ray Fluorescence (XRF) with a Bruker AXS S2 Ranger, an energy dispersive XRF with XFlash® Technology (Marguí et al., 2009). In preparation for the analysis, the freeze-dried samples were pressed under high pressure into pill form. The mineral profile obtained from ICP-OES was used for the XRF calibration curve.

### *3.2.2.4 Fatty acid analysis*

Experimental diets and homogenised freeze-dried fan fillets were analysed for FA composition. For this purpose, fat was extracted from the samples with accelerated solvent extraction (ASE®, Dionex, Sunnyvale, CA, Application Note 334). The procedure consisted of two extraction cycles with petroleum ether as a solvent at a temperature of 125 °C and a pressure of 10.3 MPa, with a heating phase of 6 min and an extraction phase of 2 min. First, transmethylation was performed on the extracted lipids to determine fatty acid methyl esters (FAMES) using a solution of 1 M sodium methoxide in methanol (1 vol.) and a solution of oxalic acid in diethyl ether (Christie, 1982). An internal standard (19:0 methyl ester) was added to the extracts prior to methylation. After centrifugation, the supernatant was injected into the split/splitless system of an 8000 Top CE gas chromatograph (ThermoQuest Italia S.p.A., Milan, Italy) with a Restek (Bellefonte, PA) Rtx-2330 capillary column (70 m  $\times$  0.18 mm internal diameter, 0.10  $\mu$ m film thickness). Hydrogen at 1.55 mL/min was used as the carrier. An oven temperature of 50 °C was held 1 min, raised to 100 °C at the rate of 50 °C/min, held 1 min, raised to 150 °C at the rate of 3 °C/min and raised to 220 °C at 2 °C/min. The injector and the detector temperatures were both set at 250 °C. The FAs were identified by comparing their retention times with a standard mixture of 37 FAMES (F.A.M.E. Mix C4–C24, Supelco, Bellefonte, PA). The concentration of individual FAMES was expressed as a percentage of the total area of known FAMES.

### **3.2.3 Statistical procedures**

Data were analyzed using the General Linear Model procedures of the statistical analysis software SAS 9.1.3 for windows (SAS, 2006). A One-way Anova was used to determine the effect of CSOCM on the feed intake (FI) and feed conversion rate (FCR). Average cumulative FI and FCR

were calculated in order to analyse the effect in each group over the complete study. Least square means were obtained using the Tukey Post Hoc test. In order to determine the effect of CSOCM on the weight gain, a linear regression model was fitted to the data and the average daily gain (ADG) was determined from the slope of the model. The significance level was calculated at a 5% confidence level and normality was analysed with a Shapiro-Wilk confidence level of 85%. Logarithmic transformations of the fatty acids data were calculated because they did not meet the assumption of normality and then further analysed. Outliers in the fatty acids data were discarded.

### **3.3 RESULTS AND DISCUSSION**

#### **3.3.1 Dietary requirements**

In order to rear any livestock species successfully, all the animal's nutrient requirements must be met. In 1996, information on the nutrient requirements for growing ostriches was still limited (Angel, 1996) and the requirements estimated were based on other poultry species. Numerous scientific studies have been conducted since then to gain more knowledge on the subject and permit more accurate calculations (Brand et al., 2000b; Cloete et al., 2012), but the newly acquired information still pales in comparison to other monogastric species, such as pigs and poultry (Brand & Olivier, 2011), however.

According to Brand & Gous (2006), protein requirements for grower and finisher ostriches are 14.71 and 12.15 g/100g feed, respectively, and decrease with age. In the formulated diets presented in Table 3.1, the crude protein content was slightly higher than the requirements. Despite this, no health problems occurred during the experimental trial. The crude fat content of CSOCM was low (0.6%) due to the oil extraction process used, the CSOCM is a source of protein and not of energy for livestock feeding (Nagalakshmi et al., 2007; Winterholler et al., 2009). Crude fibre is an important ingredient in ostrich feeds and can be supplied at levels of between 6 – 18% depending on age (Cooper et al., 2004), and these requirements were in line with the values measured in the experimental diets presented in Table 3.1.

Although the ability of the ostrich to utilize the fibrous fractions of the diet has been well documented (Aganga et al., 2003; Sales, 2006), no specific values in terms of ADF and NDF requirements have been provided.

Information on the vitamin and mineral requirements of ostriches are extremely rare, and data from other species are commonly used to calculate and formulate premixes. Calcium and Phosphorus levels should, however, be supplied in the range of 0.8–1.8% and 0.5–0.6%, respectively (Brand & Gous, 2006; Brand & Olivier, 2011).

Even though CSOCM contained 82 ppm free gossypol, 4790 ppm bound gossypol and 4872 ppm total gossypol, the free gossypol (the toxic form) found in the experimental diets was lower than 20 ppm and was thus unable to provide a negative effect on the birds' growth.

From the comparison made between the nutritional requirements found in literature and the results obtained by analysing the experimental diets presented in Table 3.1, the CSOCM diets formulated for this study could be considered as satisfying the nutritional needs of growing ostriches.

### **3.3.2 Fatty acids of experimental diets**

Table 3.2 reports the fatty acid profile of the experimental diets. CSOCM dietary inclusion led to an increase of PUFA content, mainly C18:2 $n$ -6, and to a slight reduction of SFA, mainly C18:0. The CSOCM inclusion increased both n-6 and n-3 FA, thus the ratio remained the same.

**Table 3.2** Fatty acid profile of raw materials and of experimental diets (% of total identified FAME)

	Experimental Diets				
	Control	CSOCM 3%	CSOCM 6%	CSOCM 9%	CSOCM 12%
<i>Saturated fatty acids</i>					
C10:0	0.10	0.06	0.05	0.02	0.03
C12:0	0.23	0.20	0.15	0.10	0.14
C14:0	0.68	0.48	0.42	0.40	0.46
C15:0	0.30	0.20	0.14	0.13	0.14
C16:0	23.3	24.5	20.8	22.0	24.3
C17:0	0.31	0.28	0.22	0.21	0.22
C18:0	7.00	4.01	3.48	3.08	3.85
C20:0	0.45	0.74	0.58	0.51	0.58
C22:0	0.00	0.00	0.00	0.00	0.00
C23:0	0.00	0.14	0.12	0.07	0.11
Total SFA	32.4	30.6	25.9	26.5	29.9
<i>Mono unsaturated fatty acids</i>					
C14:1	0.04	0.04	0.05	0.02	0.00
C15:1	0.00	0.00	0.00	0.00	0.00
C16:1	2.94	0.49	0.35	0.31	0.32
C17:1	0.28	0.09	0.08	0.08	0.08
C18:1n-9ct	24.8	23.6	26.2	20.9	24.8
C18:1n-11t	2.04	1.13	0.87	0.99	0.89
C20:1n-9	0.11	0.10	0.09	0.11	0.11
C22:1n-9	0.55	0.15	0.31	0.16	0.32
Total MUFA	30.8	25.6	27.9	22.5	26.5
<i>Polyunsaturated fatty acids</i>					
C18:2n-6ct	25.4	31.5	34.7	40.4	31.7
C18:3n-6	0.04	0.03	0.02	0.06	0.01
C18:3n-3	3.16	3.62	3.92	3.42	3.94
C18:2c9t11	0.06	0.11	0.12	0.11	0.13
C20:2	0.06	0.10	0.11	0.11	0.13
C20:3n-6	0.07	0.07	0.05	0.06	0.06
C20:4n-6	0.56	0.11	0.06	0.05	0.04
C20:3n-3	0.00	0.01	0.00	0.00	0.00
C20:5n-3	0.41	0.49	0.64	0.53	0.49
C22:6n-3	0.00	0.00	0.00	0.00	0.00
Total PUFA	29.7	36.0	39.6	44.8	36.5
n-6	26.0	31.7	34.9	40.6	31.8
n-3	3.57	4.12	4.56	3.95	4.43
n-6/n-3	7.29	7.70	7.64	10.30	7.17
UFA/SFA	1.87	2.01	2.61	2.54	2.11
MUFA/PUFA	1.04	0.71	0.70	0.50	0.73



### 3.3.3 Growth performance

The effect of increasing levels of CSOCM on the live performance of growing ostriches is presented in Table 3.3. Ostriches in the 12% CSOCM group showed higher final live weight (LW), and consequently higher average daily gain (ADG) than the Control group (112.4 vs 100.5kg and 273 vs 208 g/bird/day, respectively;  $P < 0.05$ ). No differences ( $P > 0.05$ ) were found among the dietary groups for FI and FCR. Guidelines for feeding and managing ostriches present average daily ostrich FI at this stage of growth (6 to 13 months of age) at between 1.1 and 2.9kg/d and FCR between 5 and 15, which was in agreement with this study. Birds at this stage of growth should generally have an ADG of between 200 and 340g/bird/day, which decreases as their age increases (Brand & Gous 2006). In this study, all the birds showed satisfactory growth performance. Other studies on ostrich growth performance (Brand et al., 2000a; Strydom et al., 2009) showed similar ADG values to those found here: 285–313g/d and 117.9–195.1g/d, respectively. In the study by Brand et al. (2000a) however, the birds were fed diets with low, medium, and high energy levels, and therefore showed slightly higher values for this parameter.

**Table 3.3** Growth performance of ostriches fed increasing dietary levels of CSOCM

	Experimental diets					RSD <sup>(1)</sup>
	Control	CSOCM	CSOCM	CSOCM	CSOCM	
		3%	6%	9%	12%	
Birds, n.	19	20	20	21	20	
Initial LW, kg	59.2	59.6	59.5	59.9	60.5	5.1
Final LW, kg	100.5 <sup>a</sup>	107.4 <sup>ab</sup>	108.3 <sup>ab</sup>	108.8 <sup>ab</sup>	112.4 <sup>b</sup>	10.2
ADG (g/bird/day)	208 <sup>a</sup>	245 <sup>ab</sup>	258 <sup>b</sup>	251 <sup>ab</sup>	273 <sup>b</sup>	0.1
FI (kg/bird/day) <sup>(2)</sup>	2.7	2.5	2.6	2.4	2.5	0.3
FCR (kg feed/kg weight gain) <sup>(2)</sup>	14.2	10.4	10.2	9.5	9.4	2.2

FI = Feed intake; FCR = Feed conversion rate; ADG = Average daily gain;

<sup>a, b</sup> Rows with different letters differed significantly ( $P < 0.05$ ); <sup>(1)</sup> Residual Standard Deviation;

<sup>(2)</sup> FI and FCR calculated as an average for the paddock

### 3.3.4 Chemical composition of fan fillet meat

Results presented in Table 3.4 show that the diet did not affect the proximate composition of fan fillet meat. Sales & Hayes (1996) noted similar values in the proximate composition of the *Iliofibularis* muscle. Lipid content of the fan fillet, however, was much higher in this study (on

average 3.1%) than noted by Sales & Hayes (1996) and by Lanza et al. (2004) (0.92% and 0.70%, respectively), whereas Cooper & Horbanczuk (2002) observed intermediate values (1.29%).

A study that compared ostrich, turkey and bovine meat in terms of physical-chemical composition (Paleari et al., 1998) reported a very low cholesterol content of 33.8mg/100 g, whereas this study (63.3mg/100 g meat; Table 3.4) provided a value comparable to that of beef (59mg/100 g) and chicken (57mg/100 g) (Cooper & Horbanczuk, 2002). Other studies, however, found ostrich cholesterol levels between 60 and 83mg/100 g meat (Sales & Horbanczuk, 1998; Cooper, 1999; Girolami et al., 2003), which were similar to the levels found in this study.

**Table 3.4** Proximate composition and cholesterol content of the fan fillet meat from ostriches receiving increasing dietary levels of CSOCM

	Experimental Diets					<i>P</i> -	
	Control	CSOCM	CSOCM	CSOCM	CSOCM	Value	RSD <sup>(1)</sup>
		3%	6%	9%	12%		
Samples, No.	16	18	17	21	19		
Dry matter, %	24.9	25.2	24.9	24.7	25.2	ns	0.73
Protein, %	21.1	21.3	21.4	20.9	21.3	ns	0.78
Lipids, %	3.09	3.30	2.88	2.94	3.23	ns	0.60
Ash, %	1.05	1.08	1.14	1.04	1.03	ns	0.21
Colesterol, mg/100 g	62.3	63.8	62.3	63.7	64.2	ns	4.82

<sup>(1)</sup> Residual standard deviation

Fan fillet mineral content showed slight differences when compared to those of Sales & Hayes' (1996) study; in particular, K, P, Fe, Zn were higher (by 12, 12, 11 and 44%, respectively) and Ca was lower (by -48%) in this study, whereas Na content was identical (40.6mg/100 g meat).

Iron content was also higher (on average 2.8mg/100 g) than values reported more recently (2.3mg/100 g) by Cooper & Horbanczuk (2002) and was also higher than that reported for poultry (0.9g/100g) and beef (2.2mg/100g).

**Table 3.5** Mineral content (mg/100g) of the fan fillet meat from ostriches fed increasing dietary levels of CSOCM

	Experimental Diets					P-Value	RSD <sup>(1)</sup>
	Control	CSOCM 3%	CSOCM 6%	CSOCM 9%	CSOCM 12%		
	Samples, No.	16	18	17	21		
Ca	4.59	4.71	4.68	4.59	4.69	ns	0.20
P	243	245	244	238	247	ns	13.2
K	309	310	312	303	309	ns	19.8
Na	40.3	40.7	40.4	39.6	41.3	ns	2.08
S	199	201	200	198	203	ns	8.19
Mg	24.6	24.8	24.5	24.4	24.7	ns	0.75
Fe	2.75	2.90	2.77	2.70	2.91	ns	0.49
Zn	1.92	1.92	1.90	1.68	2.05	ns	0.54
Cu	0.16	0.16	0.15	0.16	0.16	ns	0.02

<sup>(1)</sup> Residual standard deviation

Analogously to rabbit meat (Dalle Zotte & Szendrő, 2011), ostrich meat is characterized by lower Na content (on average 41mg/100 g in the present study) than beef (63mg/100g) or chicken meat (77 mg/100g) (Sales & Hayes, 1996), and this is favourable for modern consumers in general, even more so for those who suffer from hypertension.

### 3.3.5 Fan fillet meat FA profile

Results illustrated in Table 3.6 show that the experimental diets did not affect the FA profile (% of total identified FAME) of ostrich fan fillet meat. The average total SFA content was 32.9% of the total FA, which is in accordance with literature (Sales et al., 1996; Sales et al., 1999; Cooper & Horbańczuk, 2002; Girolami et al., 2003; Hoffman et al., 2005; Hoffman, Brand et al., 2012), in which the SFA content was around 33-34% of total FAME. Only Horbańczuk et al. (1998) found slightly different results when comparing the FA composition of different ostrich subspecies (35.2% and 37.7% total FAME for red neck and blue neck ostrich, respectively).

Specifically, the average percentage value for C16:0 in this study was 18.6% of total FAME, similar to the value reported by Sales et al. (1996, 1999), whereas other authors have reported higher values (Horbańczuk et al., 1998; Cooper & Horbańczuk, 2002; Girolami et al., 2003; see also review by Hoffman, 2008). Average percentage value for C18:0 in this study was around 13% total

FAME, which corresponded to literature, except for studies by Girolami et al. (2003) and Sales et al. (1998), in which values of 9 and 16.1% total FAME were found, respectively. Regarding the MUFA amount, the average values found in literature varied between 34.9 and 39.1% total FAME, which were higher than that reported in this study (31.3% total FAME on average).

Further the PUFA content found here was lower (21% total FAME) than the values reported in literature for the same ostrich muscle (26% to 30% total FAME). Specifically, the average EPA and DHA contents observed in this study were 0.37 and 0.36% respectively, whereas those reported in literature varied from 0.28 to 1.78% and between 0.1 and 1.79%, respectively (Sales et al., 1996; Sales et al., 1999; Horbańczuk et al., 1998; Girolami et al., 2003). Interestingly, results of this study showed that the EPA content of meat from the Control group was slightly lower than the meat of groups in which CSOCM was used, even if not statistically significant (Table 3.6). Also the Control group DHA content had the lowest value, and an increasing trend from the Control to the 6% CSOCM group appeared evident, and although the 9 and 12% CSOCM groups did not follow the same trend, their DHA content was also higher than the 0% CSOCM group, even if no statistical significance supported this assumption.

The PUFA/SFA balance and the n-3 PUFA content are very important in human health for the role they play in protection against cardiovascular and inflammatory disease and ensuring the health of the brain (Diniz et al., 2004; Coad et al., 2011). For these reasons the suggested n-6/n-3 and PUFA/SFA ratios should be less than 4 and above 0.4, respectively (Wood et al., 2004). The average n-6/n-3 value obtained in this study was 7.4, which was higher than the recommendation mentioned above but still in line with what Girolami et al. (2003) reported in their study.

Monogastric animals like pigs, chickens, and fowl absorb dietary FA intact through the small intestine and incorporate them unchanged into tissue lipids. This makes it possible to modify the FA composition of meat and fat tissue in these animals through dietary treatment (Hoffman et al., 2005; Sales, 2006; Wood et al., 2008). The ostrich is also a monogastric animal, but unlike other species, its ability to digest fibrous fractions improves with age, reaching an efficiency of 66% for hemicellulose and 38% for cellulose at about 17 weeks of age (Swart, 1988; Swart et al., 1993a,b,c). This characteristic is a result of the specific adaptation of ostrich digestion: its hindgut is 8 meters long, constituting 57% of the total digestive tract (Swart, 1998). This increases digesta retention time to 48 hours and creates a favourable environment for both microbial fermentation and the production of volatile FA which account for more than 70% of the ME (Metabolisable Energy) requirements (Swart, 1988, Swart et al 1993a,b,c; Cooper et al., 2004). The lack of relationship between dietary FA composition and meat FA composition could therefore be explained by the fermentative action in the lower digestive tract. The lower PUFA content found in the meat compared to the quantity present in the diets may be explained by the hydrogenation of these FA through the microbial action that takes place in the hindgut.

**Table 3.6** Fatty acid profile of fan fillet meat from ostriches fed increasing dietary levels of CSOCM (% of total identified FAME)

	Experimental Diets					P-Value	RSD <sup>(1)</sup>
	Control	CSOCM 3%	CSOCM 6%	CSOCM 9%	CSOCM 12%		
Samples, No.	16	18	15	21	18		
C10:0	0.04	0.04	0.04	0.05	0.04	ns	0.02
C12:0*	0.06	0.06	0.08	0.06	0.06	ns	1.98
C14:0	0.90	0.89	0.95	0.83	1.05	ns	0.30
C15:0	0.12	0.11	0.12	0.13	0.12	ns	0.05
C16:0	18.5	18.7	18.2	18.7	18.9	ns	3.8
C17:0	0.19	0.17	0.16	0.17	0.16	ns	0.05
C18:0	13.0	12.9	13.8	13.1	12.0	ns	2.20
C20:0	0.02	0.02	0.02	0.04	0.02	ns	0.04
C22:0	0.04	0.01	0.01	0.02	0.01	ns	0.03
C23:0	0.01	0.01	0.00	0.00	0.02	ns	0.02
Total SFA	32.8	32.9	33.5	33.0	32.4	ns	2.80
C14:1*	0.11	0.13	0.16	0.11	0.15	ns	1.72
C15:1	0.03	0.02	0.02	0.02	0.02	ns	1.03
C16:1	5.70	5.87	6.14	5.36	6.12	ns	1.65
C17:1	0.30	0.38	0.36	0.44	0.33	ns	0.31
C18:1 <sub>n-9ct</sub>	22.7	22.8	22.0	22.5	21.6	ns	4.10
C18:1 <sub>n-11t</sub>	2.52	2.43	2.34	2.39	2.41	ns	0.46
C20:1 <sub>n-9</sub>	0.17	0.19	0.18	0.18	0.18	ns	0.05
C22:1 <sub>n-9</sub>	0.01	0.00	0.03	0.01	0.01	ns	0.03
Total MUFA	31.5	31.9	31.3	31.1	30.9	ns	5.40
C18:2 <sub>n-6ct</sub>	13.3	12.6	12.7	13.1	12.7	ns	1.90
C18:3 <sub>n-6</sub>	0.05	0.04	0.04	0.05	0.04	ns	0.02
C18:3 <sub>n-3</sub>	1.76	1.50	1.51	1.42	1.59	ns	0.68
C18:2c9t11	0.05	0.05	0.04	0.04	0.05	ns	0.03
C20:2*	0.09	0.08	0.08	0.11	0.12	ns	1.93
C20:3 <sub>n-6</sub>	0.45	0.44	0.42	0.40	0.43	ns	0.13
C20:4 <sub>n-6</sub>	5.42	4.51	4.68	4.64	5.15	ns	2.33
C20:3 <sub>n-3*</sub>	0.11	0.11	0.12	0.10	0.11	ns	2.92
C20:5 <sub>n-3</sub>	0.34	0.39	0.38	0.37	0.36	ns	0.23
C22:6 <sub>n-3*</sub>	0.26	0.28	0.54	0.37	0.34	ns	0.35
Total PUFA	21.9	20.1	20.7	20.8	21.1	ns	3.90
<i>n-6</i>	19.2	17.5	17.8	18.2	18.3	ns	3.80
<i>n-3</i>	2.57	2.41	2.72	2.41	2.58	ns	0.72
<i>n-6/n-3*</i>	7.74	7.37	6.73	7.84	7.11	ns	1.48

<sup>(1)</sup> Residual Standard Deviation; \* Data analyzed using logarithmic transformation

### 3.4 CONCLUSIONS

Chemical analysis revealed the gossypol content of the experimental diets to be very low, and therefore no adverse effects due to gossypol poisoning were noted. Partially replacing soybean oilcake meal with increasing levels of CSOCM had no adverse effects and improved live performances of growing ostriches at a 12% inclusion level. The dietary protein source, CSOCM, did not influence the proximate composition, mineral content, or FA profile of ostrich fan fillet meat. Considering the overall results, CSOCM seems to be a viable and less expensive alternative to soybean oilcake meal as a protein source in ostrich nutrition, considering gossypol levels.

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## CHAPTER 4

### THE EFFECT OF COTTONSEED OILCAKE MEAL ON THE PHYSICAL, CHEMICAL AND SENSORY CHARACTERISTICS OF OSTRICH MEAT

#### ABSTRACT

The object of this study was to determine whether the replacement of soy bean oilcake meal (SBOCM) with cottonseed oilcake meal (CSOCM) would be detected in ostrich (*Struthio camelus* var. *domesticus*) fan fillet (*Iliofibularis* muscle) on a sensory level. Two levels of CSOCM were investigated; 0% as a control and 9% CSOCM. No significant differences were found for the physical measurements (cooking loss percentage and shear force) as well as for the pH and proximate composition of the raw fan fillet. Cooked fan fillet in the control group had significantly higher fat content than the 9% CSOCM, but this was attributed to the cooking method and therefore not of biological significance. The control group presented a higher ( $P < 0.05$ ) MUFA content in the cooked fan fillet whereas the 9% CSOCM group showed a favourable increased ( $P < 0.05$ ) PUFA content when compared to the cooked control samples. As a result, the PUFA:SFA ratio in the 9% CSOCM group was also significantly higher. No differences ( $P > 0.05$ ) were found between the treatments for the n-6:n-3 (omega 6:omega 3) ratio even though Docosahexaenoic acid (DHA) (C20:5n-3) was significantly higher in the 9% CSOCM group. The 9% CSOCM group had a more intense beef aroma, had a higher level of initial and sustained juiciness as well as increased tenderness ( $P < 0.05$ ). Compared to SBOCM, inclusion of 9% CSOCM resulted in a favourable (in terms of consumer preference) cooked ostrich fan fillet.

*Keywords:* Cottonseed oilcake meal, Ostrich meat, Physicochemical analysis, Sensory analysis

## 4.1 INTRODUCTION

The demand for ostrich meat has increased drastically over the last couple of years. Not only due to catastrophic events like BSE (Bovine Spongiform encephalopathy) outbreaks in Europe or foot and mouth disease appearing in the UK but also due to a change in the consumer driven market for healthier red meat alternatives (Polawska et al., 2011). Currently there is also *Avian influenza* outbreaks that are placing a lot of pressure on the ostrich industry. On a live weight basis the skin contributes 7.0%, the feathers 1.9% and the carcass (lean, fat and bone) 58.6% of the live weight of the ostrich (Harris et al., 1994). The income generated from these ostrich products are 5%, 50% and 45% for feathers, leather and meat respectively (Hoffman et al., 2008; Hoffman, 2005) with a current drive towards the further development of the meat product (Balog & Almeida Paz, 2007). As a result, there is a demand for more information on ostrich meat as well as ways of improving and processing this novel meat product.

The attractiveness of a meat product will determine the consumers' decision on the quality of the product. Tenderness and flavour are two of the most important attributes for the consumer and both of these can be manipulated through feeding (Wood et al., 1999). Manipulation of tenderness through nutrition lies in the amount of fat present in the muscle, which is directly correlated to the amount and type of feed. Wood et al. (1999) explains how fat accumulation and deposition can affect the muscle composition and as a result modifying the tenderness. Accumulation of fat starts in the subcutaneous and intermuscular parts of the body and as this deposition increases it provides insulation for the muscles, protecting them from the effects of the low temperatures during cooling, for example cold shortening. Secondly it accumulates in the inner part of the muscle (intramuscular fat (IMF)), leading to a decrease in the force needed to shear the muscle during eating or cutting. This is achieved through a dilution of fibrous protein by the soft fat or due to fat cell expansion that forces the muscle fibres apart creating a more loose structure, easy for shearing.

Ostrich meat however is known worldwide for having low intramuscular fat values compared to other poultry species and beef (Palairet et al., 1998) making it very attractive for the health conscious consumer. Low lipid content however renders a meat product less juicy during mastication and also affects the tenderness of the product, but Balog & Almeida Paz (2007) states that tenderness is indeed one of the most appreciated characteristics of ostrich meat. They explain further that this characteristic is due to the low levels of saturated fat as well as the collagen to protein ratio in ostrich meat, making it easy to chew and digest.

Fatty acids (FA) and meat quality is a topic that is gaining much attention. Earlier works were more focused on the FA composition of the adipose tissue as this is where the majority of the body's FA are located, but currently the focus has shifted to the FA composition of the meat as consumers' requirements are for more information on what they eat. The aversion to visible fat at retail level

however, and the fact that meat is more significant as the food component has led to the focus aimed at mainly muscle fatty acid composition (Wood et al., 2008). Dietary guidelines are urging consumers to reduce the amount of total fat and saturated fatty acids (SFA) intake as a means to reduce the risks of coronary heart disease (Williams, 2000). Consuming products with increased polyunsaturated fatty acids (PUFA) levels is not always the answer though, as the lipoperoxidation products of these FA may have a negative effect on human health (Williams, 2000) as well as the oxidative stability of the meat product being consumed (Wood et al., 2008).

As results obtained in Chapter 3 showed CSOCM did not affect fatty acids (FA) composition in the raw ostrich *Iliofibularis* muscle, this study was conducted to see if differences could be detected on a sensory level on a cooked fan fillet (*Iliofibularis* muscle). This study therefore adds additional information on ostrich meat quality, especially on the chemical composition of cooked muscle.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 Birds, feed and management**

A total of 54 growing ostriches were used to study the effect of two levels of cottonseed oilcake meal (CSOCM) inclusion on the physical and sensory attributes of the *Iliofibularis* muscle. Birds of approximately 6 months ( $\pm 20$  days) of age were brought in to the experimental farm just outside of Oudsthoorn where they remained for the duration (7 months; May to September) of the trial. The experimental birds were subdivided into two treatment groups of 27 birds each. The treatment groups were then subdivided into three replicate pens containing nine birds each (approx. 200m<sup>2</sup>/bird). The Control group (CSOCM 0%) received a soy bean oilcake meal (SBOCM) based diet with zero cottonseed oilcake meal (CSOCM) and the treatment group received a 9% CSOCM inclusion diet, replacing the SBOCM. The Control and treatment groups consist of three replicates each with 9 birds per paddock. Two experimental diets were formulated and the chemical composition and fatty acid profile is presented in Table 4.2.

### **4.2.2 Slaughtering and meat sampling**

Slaughtering of the 54 thirteen month old ostriches took place at Ostriswell, a commercial abattoir in Swellendam, South Africa. Birds were kept in lairage (roofed pens) for two hours prior to slaughter. Electrical head stunning (90-110V, 400-600mA, 4-6s) was applied to induce unconsciousness. After stunning the ostriches were suspended by both legs and exsanguinated by a neck cut to the aortic vein followed by a thoracic stick (Hoffman, 2012). Bleeding was allowed for 10-15min followed by plucking, skinning, evisceration and a health inspection. Carcasses were chilled for 24 hours at 0-4°C after which the fan fillet (*Iliofibularis* muscle) was removed for physicochemical and sensory analysis. Fan fillets were individually vacuum packed and frozen at -20°C until further analysis was completed.

### 4.2.3 Physical measurements

The whole fan fillet (*Iliofibularis* muscle) was thawed under refrigerated (4°C) conditions over a 12 hour period. Two steaks of 1.5 – 2.5cm thickness were cut perpendicular to the muscle fibre direction from the centre of the fillet. Two pH measurements were taken from each steak using a Crison pH 25 handheld portable pH meter (Lasec (Pty) Ltd, South Africa) with an automatic temperature adjuster calibrated before each session with the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer. The two steaks were then used for the sensory evaluation, cooking loss and shear force measurements. The rest of the muscle was retained for the remaining chemical analysis. The remaining muscle was homogenised and divided into two vacuum bags, one for proximate analysis and the other for fatty acid analysis.

Cooking loss was determined by weighing the two 1.5 – 2.5cm thick steaks and then placing them into an oven bag (GLAD™) and onto an oven tray of a conventional oven (Defy, Model 835) connected to a computerised monitoring system responsible for regulation of the temperature (Viljoen et al., 2001). Thermocouple probes attached to a handheld digital temperature monitor (Hanna Instruments South Africa) was placed in one of the steaks and the roasting bags were closed by the use of a metal tie (AMSA, 1995). The oven was preheated to a regulated temperature of 160°C. When the internal temperature of the meat reached 68°C, the meat was removed from the oven and allowed to rest. During this time the residual heat causes the core temperature to rise to the desirable 75°C (Honikel, 1998). Once cooled down, the steaks were dabbed with a paper towel and then weighed back. Cooking loss was calculated as follows:

Warner-Bratzler shear (WBS) force measurements were performed on the cooked meat samples. With a core borer, six cylindrical core samples of 1.27cm diameter each were cut at random locations parallel with the muscle fibre on the cooked piece. Maximum shear force values were recorded by cutting the cylindrical core of cooked muscle perpendicular to the longitudinal orientation of the muscle fibres using a Warner–Bratzler Shear attachment (with a circular cross section of 1.27cm blade) at a crosshead speed of 200mm/min. An average WBS force value (N) was then calculated for each fan fillet. Care was taken to avoid cylindrical core samples that contained visible connective tissue that could influence WBS force results.

#### 4.2.4 Proximate analysis

Proximate analysis of the meat samples were analysed according to the Association of Official Analytical Chemist's Standard Techniques (AOAC). A 2.5g homogenized meat (*Iliofibularis* muscle) sample was placed in a drying oven at 100 – 105°C for 24 hours (AOAC Official method 934.01) (AOAC, 2000a) in order to determine the moisture content after which the same samples was used to determine ash content by incinerating in an oven at 500°C for 6 hours (AOAC Official Method 942.05) (AOAC, 2000b). The chloroform/methanol (1:2 v/v) extraction method stipulated by Lee et al. (1996) was used to determine the total lipid (%) (intramuscular fat) of a 5g homogenised cooked meat sample. The fat free sample was placed in a drying oven to retain a moisture free sample. The % nitrogen was then determined on the fat and moisture free sample based on the Dumas combustion method 992.15 (AOAC, 2000c) using a Leco Nitrogen/Protein Analyser (FP-528, Leco Corporation). The Leco was calibrated with EDTA samples (Leco corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA, Part no. 502-092, Lot no. 1055) prior to every analyses session. The results were presented in % Nitrogen (N) which was then multiplied by a conversion factor (6.25) in order to determine the crude protein content of the meat samples. All proximate analyses are controlled by a National inter-laboratory scheme (AgriLASA: Agricultural Laboratory Association of South Africa). In order to assess the accuracy of the analyses, blind samples are analysed every other month.

#### 4.2.5 Fatty acid analysis

After thawing, 2g meat sample was extracted with a chloroform:methanol (1:2; v/v) solution according to a modified method of Folch, Lees, and Sloane-Stanley (1957). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (WiggenHauser, D-500 Homogenizer) was used to homogenise the sample with the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2h at 70°C using a methanol/sulphuric acid (19:1; v/v) solution as transmethylating agent. After cooling to room temperature, the resulting fatty acid methyl esters (FAMES) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

Analysis was on a Thermo Focus GC equipped with a flame ionized detector using a BPX70 capillary column (60m x 0.25mm internal diameter, 0.25µm film, SGE, Australia). Gas flow rates were 25ml/min for hydrogen and 2–4ml/min for the hydrogen carrier gas. Temperature programming was linear at 3.4°C/min, with an initial temperature of 60°C, a final temperature of 160°C, an injector temperature of 220°C and a detector temperature of 260°C. The FAMES were identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37



Component FAME Mix, 10mg/ml in CH<sub>2</sub>Cl<sub>2</sub>, Catalogue Number 47885-U. Supelco, North Harrison Road, Bellefonte, PA 16823-0048, USA).

#### **4.2.6 Descriptive Sensory analysis**

Sensory analysis was conducted on the two meat treatments with twelve replications per treatment. The samples were randomly selected for each of the twelve replications (n=12). The vacuum-packed and frozen fan fillets (whole) were thawed over 12 hours in a refrigerator (4°C) prior to each of the pre-determined sensory analysis sessions. After removal from the vacuum packaging, two steaks were cut from the centre of the fan fillet (*Iliofibularis* muscle) and placed inside a marked oven baking bag (GLAD™). No seasoning was added to any of the meat treatments throughout the sensory analysis process. The two steaks were treated as one entity. The roasting bag with the meat samples were then placed on a stainless steel grid, which was fitted on an oven roasting pan. Thermocouple probes attached to a handheld digital temperature monitor (Hanna Instruments South Africa) were placed inside the centre of each of the meat samples where after the roasting bags were closed by the use of a metal tie (AMSA, 1995). The prepared samples were then placed inside two conventional ovens (Defy, Model 835) connected to a computerised monitoring system responsible for regulation of the temperature (Viljoen et al., 2001). The ovens were pre-heated to 160°C (AMSA, 1995). The meat samples were removed from the oven when a core temperature of 75°C was indicated by the temperature probe (AMSA, 1995). After removal from the roasting bags the samples were cooled for 15 min where after they were cut into 1 cm x 1 cm cubes, individually wrapped in aluminium foil and placed into glass ramekins with a randomised three digit code. The coded ramekins, each containing two wrapped cubes, were then placed into a preheated oven at 100°C for 10 min after which they were removed and immediately served to the panel for evaluation. Descriptive sensory analysis was performed on the two meat treatments. The eleven judges were chosen based on previous experience regarding sensory analysis of meat.

The panellists were trained, according to the guidelines for sensory analysis of meat by the American Meat Science Association (AMSA, 1995) and the generic descriptive sensory analysis technique as described by Lawless and Heymann (2010). The panel undertook four training sessions and during each session the panellists received 1cm x 1cm cubes of meat from four reference standards, as well as the two meat treatments. The reference standards included: commercial free range chicken, beef rump, commercial ostrich steak as well as locally harvested blesbok (*Damaliscus pygargus phillipsi* - a free ranging wild ungulate) *Longissimus dorsi* muscle. The reference samples enabled the panellists to distinguish between chicken, beef and game aromas and flavours present in the different meat samples. The panel decided on 12 sensory attributes: game, beef, ostrich and cooked chicken aroma, initial and sustained juiciness as well as game, beef, ostrich and metallic flavour and tenderness and residue was also included. The definition for each of the attributes is described in Table 4.1. The sensory attributes were analysed using an unstructured line scale with zero (low intensity) on the left side and 100 (high intensity) on the right side (AMSA, 1995).

The test re-test method was used for the sensory analysis. The panellists received the two treatments in a complete randomised order, while seated in individual tasting booths, the samples were analysed by completing the questionnaire validated during the training sessions. The sensory analysis sessions took place inside a temperature-controlled (21°C) and light-controlled (artificial daylight) room (AMSA, 1995). In order to cleanse and refresh their palates between sample analyses, the panellists received distilled water (21°C), apple quarters and water biscuits (Carr, UK).

**Table 4.1** Definition and scale for each attribute used for the descriptive sensory analysis.

<b>Attribute</b>	<b>Definition</b>	<b>Scale</b>
Game aroma	Aroma associated with game meat	0 = Extremely bland 100 = Extremely intense
Beef aroma	Aroma associated with beef	0 = Extremely bland 100 = Extremely intense
Ostrich aroma	Aroma associated with ostrich	0 = Extremely bland 100 = Extremely intense
Cooked chicken aroma	Aroma associated with chicken	0 = Extremely bland 100 = Extremely intense
Initial juiciness	The amount of fluid exuded from the cut surface when pressed between the thumb and forefinger	0 = Extremely dry 100 = Extremely juicy
Sustained juiciness	The level of juiciness perceived after the first five chews using the molar teeth	0 = Extremely dry 100 = Extremely juicy
Game flavour	Flavour associated with game	0 = Extremely bland 100 = Extremely intense
Beef flavour	Flavour associated with beef	0 = Extremely bland 100 = Extremely intense
Ostrich flavour	Flavour associated with ostrich	0 = Extremely bland 100 = Extremely intense
Metallic flavour	Flavour associated with metal/liver	0 = Extremely bland 100 = Extremely intense
Tenderness	Impression of tenderness after first five chews using the molar teeth	0 = Extremely tough 100 = Extremely tender

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Residue	The amount of residue left inside the mouth	0 = None
	after the first 10 chews	100 = Abundant

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Aroma and flavour were analysed orthonasally and retronasally, respectively.

#### **4.2.7 Statistical analysis**

The model for the experimental design for the physical and chemical data is defined by the following equation:

Where  $y_{ij}$  defines the response on treatment  $i$  in replication  $j$ , the overall mean is identified by  $\mu$ . The effect due to feeding treatment  $I$  is defined by  $t_i$  and  $\epsilon_{ij}$  indicates the random error associated with response on treatment  $i$  in replication  $j$ .

For the sensory analyses, the experimental design was a randomised block design with two treatments (Control and 9% CSOCM) randomly replicated in 12 evaluation sessions. The trained panel consisted of 11 judges and the two treatments were analysed for the 12 attributes decided on during the two training sessions.

The following equation describes the model of the experimental design for the sensory data:

Where  $y_{ij}$  defines the response on treatment  $i$  in evaluation session  $j$ ,  $\mu$  indicates the overall mean, the effect due to evaluation session  $j$  is indicated by  $s_j$ ,  $t_i$  defines the effect due to feeding treatment  $i$  and  $\epsilon_{ij}$  is the random error associated with response on treatment  $i$  in evaluation session  $j$ .

Univariate analysis of variance was performed, according to the model for the experimental design, on all variables accessed (sensory and instrumental), using GLM (General Linear Models) Procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Sensory data was pre-processed by subjecting it to a test-retest analysis of variance (ANOVA), using SAS, to test for panel reliability. Judge\*Replication and Judge\*Sample interactions were used respectively as measures of temporal stability (precision) and internal consistency (homogeneity) of the panel. Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Any outliers based on the test for normality were discarded before final analysis of the complete data. Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests. In addition to the univariate ANOVAs, the data was also subjected to Multivariate methods like Principal component analysis (PCA) and Discriminant Analysis (DA) (XLStat, Version 2011, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and their attributes.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Diet composition

The chemical composition (g/100g as fed) of the two experimental diets (Control and 9% cottonseed oilcake meal (CSOCM) inclusion) are presented in Table 4.2.

**Table 4.2** Chemical composition (g/100g as fed) and fatty acid (FA) profile (% of total FA detected) of the Control and 9% cottonseed oilcake meal diets.

Component (g/100g as fed)	Control	9% CSOCM
Moisture	9.56	9.77
Crude fat	2.49	2.75
Crude protein	16.91	14.38
Ash	9.64	8.97
ADF	15.92	15.71
NDF	26.62	28.38
Free Gossypol (ppm)	ND*	10 - 20
SFA	21.1	22.3
MUFA	23.1	20.1
PUFA	45.5	42.5
PUFA:SFA	2.2	1.9
(n-6)/(n-3)	4.0	11.0

\* Not detected, detection limit of 20 ppm; chemical analysis performed in duplicate, SFA = Saturated fatty acids, MUFA = Mono unsaturated fatty acids, PUFA = Polyunsaturated fatty acids.

### 4.3.2 Physical measurements

The means and standard deviations ( $\pm$  SD) of the pH, cooking loss and Warner-Bratzler shear (WBS) force of the *Iliofibularis* muscle for the two treatments are presented in Table 4.3. No differences ( $P > 0.05$ ) were found between the two dietary treatments for any of the physical attributes. Rapid pH drop post mortem, is the result of muscle glycogen undergoing anaerobic glycolysis, resulting in the production of lactic acid (Lawrie, 1998). In general ostrich meat is classified as an intermediate type meat with regards to  $pH_u$  with values lying between normal  $< 5.8$  and extreme dark, firm, dry  $> 6.2$  (Balog & Almeida Paz, 2007). Although similar values ranging from 5.86 to 6.13 have been reported (Sales & Mellet, 1996; Sales, 1996 and Paleari, et al., 1998),

in this study however, pH values were found to be in the extreme dark, firm, dry range (average 6.3; Table 4.3). These values are slightly higher than the 6.04 and 6.13 presented by Sales (1996) and Sales & Mellet (1996), respectively. Lawrie (1998) explains that this phenomenon of high final pH as the result of depleted glycogen reserves due to ante mortem stress. Other post mortem factors also contribute greatly to the pH<sub>u</sub> for example slaughter method, stunning, bleeding, deboning, packaging and storage (Hoffman et al., 2008). According to Balog et al. (2006) as cited by Polawska et al. (2011), the *Iliofibularis* muscle is one of the muscles that do not follow the usual post mortem pH drop, but rather shows rapid decrease for the initial two hours where after it increases slightly before stabilizing (Sales & Mellet, 1996). This is in accordance with Hoffman et al. (2008) that presented the *Iliofibularis* muscle as having the highest pH<sub>u</sub>. Cooking loss percentage in this study was slightly lower than the 36% cooking loss presented by Sales (1996). The lower cooking loss may be attributed to an increased water holding capacity (WHC) of the muscles caused by the higher pH<sub>u</sub> (Lawrie, 1998). However, Hoffman & Fisher (2001) when comparing ostriches of different ages noted a mean cooking loss value of 31.91% for 14 month old birds, similar to the 9% CSOCM group. The value obtained for shear force (WBS) is higher than the value of 29.6 N found by Harris et al. (1994) but in agreement with the 43.5N presented by Sales (1996). Contradicting results with regards to WBS of the *Iliofibularis* muscle may be attributed to the fact that this particular muscle is largest (1.16kg) of all the leg muscles, leading to greater intramuscular variations (Sales, 1996). However wide the range of average tenderness values measured by the Warner Brazler (20.0N to 45.0N; Balog & Almeida Paz, 2007) for ostrich meat, it still remains much lower than what is seen in beef (45.0N to 60.0N) and similar to what is found in poultry (19.0N to 30.0N) (Lawrie, 1998). Shear force and cooking loss are highly correlated (Hoffman et al., 2008). This is explained by Tarrant (1998) through collagen (the principal fibrous protein in connective tissue) contraction. As collagen concentration in the meat increases, more contraction will occur at high temperatures (cooking), resulting in increased fluid exuded (cooking loss) from the muscle and as a result, tougher meat. According to Bailey & Simms (1977) this contraction and increased fluid exudation due to increased tension is the result of collagen contracting to a quarter of its length, at temperatures of ~65°C, and resembling a rubber-like substance.

**Table 4.3** Physical measurements of cooked ostrich meat (*Iliofibularis* muscle) (Mean ± Standard deviation)

Attribute	Control	9% CSOCM	LSD <sup>a</sup>
pH	6.2 ± 0.26	6.4 ± 0.15	0.18
Cooking Loss (%)	34.1 ± 4.21	31.6 ± 5.41	4.10
WBS <sup>b</sup> (N)	43.3 ± 7.91	39.2 ± 7.93	6.70

<sup>a</sup>Least significant difference; <sup>b</sup> Warner-Bratzler Shear force values

### 4.3.3 Proximate composition

In Table 4.4 the means and standard deviation ( $\pm$  SD) for the proximate composition of raw and cooked ostrich meat are depicted. Very little information is available on the chemical composition of cooked ostrich meat. In 1995, Sales et al. (1995) did however compare the nutritive value of raw and cooked ostrich *Iliofibularis* muscle and found moisture content in the raw sample (76.1g/100g) to be in accordance with that found in this investigation (76.5 and 76.1g/100g for Control and 9% CSOCM respectively; Table 4.4). Moisture content of cooked *Iliofibularis* muscle (68.4%) examined by Harris et al. (1994) was also in accordance with the present study's findings. Decrease in moisture content due to cooking however was much greater in the present study (76.5 to 67.4g/100g for Control and 76.1 to 69.3g/100g 9% CSOCM ostrich *Iliofibularis* muscle) than that noted by Sales et al. (1995); 76.1 to 71.5g/100g.

The amount of fat in the raw *Iliofibularis* muscle depicted in Table 4.4 is much higher than that reported in literature (0.4g/100g to 1.6g/100g) (Sales et al., 1995; Sales & Hayes, 1996; Sales, 1996; Paleari et al., 1998). In the aforementioned studies however, fat was extracted using the ether extraction method, whereas in the present study, chloroform methanol extraction (1:2 (v/v)) was used to determine the fat content of the cooked samples, which is the procedure (Lee et al. (1996)) when analysing meat samples with < 5% fat (eg. game and fish) whereas in Harris et al. (1994) a 2:1 ratio was used. The cooked control group showed a higher ( $P < 0.05$ ) fat content than the cooked 9% CSOCM group (Table 4.4). The higher moisture content found in the cooked 9% CSOCM sample may be the reason for this as the cooking process causes a loss of moisture and a subsequent increase in the concentration of non-volatile compounds within the meat (Browning et al., 1990). Therefore in the cooked 9% CSOCM sample, less moisture was lost during cooking resulting in a seemingly lower fat concentration within the samples. Lipid content (%) presented by Harris et al. (1994) was found to be much lower ( $2.74 \pm 0.33$ ) than that found in the present study ( $6.4 \pm 0.79$ ). In the present study a chloroform:methanol of 1:2 was used to determine the fat content of the cooked samples, which is the procedure (Folch, Lees, & Sloane-Stanley (1957)) when analysing meat samples with < 5% fat (eg. Game and fish) whereas in Harris et al. (1994) a 2:1 ratio was used.

No differences ( $P > 0.05$ ) were found for protein or ash content between the dietary treatments for cooked and raw *Iliofibularis* muscle. Values obtained in this study for protein content in raw samples (17.7 and 19.0g/100g) was slightly lower than that reported elsewhere (20.9g/100g to 22.2g/100g) whereas the ash content (1.2g/100g) was similar (1.03g/100g to 1.1g/100g) (Sales & Hayes, 1996; Sales, 1996 and Paleari et al., 1998). Protein and ash content of cooked ostrich *Iliofibularis* muscle in the present study (Table 4.4) was found to be similar to values reported by Harris et al. (1994).

**Table 4.4** Proximate composition (g/100g meat) of raw and cooked *Iliofibularis* muscle samples (Mean  $\pm$  Standard deviation)

Attribute	Control		9% CSOCM	
	Raw	Cooked	Raw	Cooked
Moisture	76.5 $\pm$ 3.34	67.4 $\pm$ 6.13	76.1 $\pm$ 0.76	69.3 $\pm$ 7.65
Fat	5.6 $\pm$ 1.44	6.8 <sup>a</sup> $\pm$ 0.94	4.9 $\pm$ 1.30	6.0 <sup>b</sup> $\pm$ 0.63
Protein	17.7 $\pm$ 3.29	25.8 $\pm$ 5.88	19.0 $\pm$ 1.49	26.0 $\pm$ 7.49
Ash	1.2 $\pm$ 0.28	3.4 $\pm$ 1.86	1.2 $\pm$ 0.07	3.2 $\pm$ 1.73

<sup>a,b</sup> Rows with different letters within cooking treatment differed significantly (P<0.05)

#### 4.3.4 Fatty acid composition

The means and standard deviations ( $\pm$  SD) for the fatty acid (FA) composition of the cooked ostrich *Iliofibularis* muscle samples for the two treatments are presented in Table 4.5. The composition of fatty acids is presented as percentages of the total fatty acids identified. All the fatty acids identified were analysed and are presented but only the more relevant FA will be discussed as there is limited studies conducted on the FA composition of cooked ostrich meat.

The meat of the Control group presented a higher (P<0.05) MUFA content than that of the 9% CSOCM group (34.7% and 28.2%), whereas the latter showed a favourable increased (P<0.05) PUFA content compared to the control meat (25.1% and 20.5% respectively). The PUFA to SFA ratio in the 9% CSOCM group was as a result, also significantly higher. Sales et al. (1995) noted slightly lower SFA and MUFA (32% and 32.9% respectively) in cooked *Iliofibularis* muscle than that noted in this investigation (avg. 43.85% and 31.45% respectively). However, the PUFA for the present study was much lower (22.8%) compared to the 36.31% noted by Sales et al. (1995). According to Harris et al. (1994), the FA composition of cooked ostrich muscle shows MUFA to be most prevalent, followed by SFA and lastly PUFA. In this study however, the SFA were most prevalent (Table 4.5).

In comparison to other meats, ostrich meat fatty acid composition presents lower MUFA levels and higher PUFA levels. The fatty acid found most abundantly in ostrich meat is Oleic acid (C18:1), followed by Palmitic acid (C16:0) and then Linoleic acid (C18:2n-6) (Palafox et al., 1998; Sales & Horbanczuk, 1998; Hoffman & Fisher, 2001). In this study the same profile was observed, deeming the general composition of the fatty acid in agreement with the literature. The two major PUFA are Linoleic (C18:2n-6) and Linolenic acid (C18:3n-3) of which the former is more readily absorbed into muscle tissue (Wood et al., 2008). The reason for this is that Linolenic acid (C18:3n-3) forms a greater part of high fibre or forage diets, much like the diet composition for ostriches and these diet constituents have a much longer transit time leading to more biohydrogenation of these fatty acids leaving very little available for uptake into muscle or adipose tissue. Percentages for these PUFA detected in the *Iliofibularis* muscle samples depicts this phenomenon with the more readily

absorbed PUFA (Linoleic acid (C18:2n-6)) presenting a much greater proportion (19%) of the total PUFA than the less readily absorbed Linolenic acid (C18:3n-3) presenting only 0.3% of total PUFA (Table 4.5).

No differences ( $P > 0.05$ ) were found between the treatments for the n-6:n-3 (omega 6 to omega 3 ratio). The ratio in this study is very high and not favourable, but as meat is generally associated with low levels of omega 3 fatty acids this is not uncommon (Lawrie, 1998). Imbalanced n-6:n-3 is mainly the result of insufficient omega 3 fatty acid intake in the human diet, especially in the more developed countries and this can lead to serious coronary heart disease (Williams, 2000). Other diseases also linked to omega 3 deficiencies are hypertension, inflammatory and immune disorders as well as depression and neurological dysfunction. According to Sales (1996) however, this ratio can be manipulated through feeding diets with increased levels of omega 3. Of the major omega 3 (n-3) fatty acids (C22:6n-3; DHA and C20:5n-3; EPA) present, docosahexaenoic acid (DHA) (C20:5n-3) was seen to be significantly higher in the 9% CSOCM group. The significance of the FA composition of cooked ostrich meat on a nutritional basis will be more relevant however once human dietary studies are performed (Harris et al., 1994).



**Table 4.5** Fatty acid (FA) composition (% of total FA detected) of cooked ostrich meat (*Iliofibularis* muscle) (Mean  $\pm$  standard deviation).

	Control	9% CSOCM	LSD*
<b>Saturated Fatty Acids (SFA)</b>			
C14:0	0.5 $\pm$ 0.19	0.4 $\pm$ 0.16	0.15
C15:0	0.2 $\pm$ 0.03	0.2 $\pm$ 0.05	0.04
C16:0	26.0 $\pm$ 2.61	24.2 $\pm$ 2.09	2.00
C18:0	15.2 <sup>b</sup> $\pm$ 1.99	18.7 <sup>a</sup> $\pm$ 2.17	1.76
C20:0	0.3 $\pm$ 0.06	0.3 $\pm$ 0.05	0.04
C21:0	0.0 $\pm$ 0.01	0.0 $\pm$ 0.01	0.01
C22:0	0.7 $\pm$ 0.16	0.7 $\pm$ 0.17	0.14
C24:0	0.2 $\pm$ 0.04	0.2 $\pm$ 0.04	0.03
<b>Total SFA</b>	43.0 $\pm$ 3.02	44.7 $\pm$ 2.06	2.19
<b>Mono unsaturated Fatty Acids (MUFA)</b>			
C14:1	0.1 $\pm$ 0.03	0.1 $\pm$ 0.01	0.02
C16:1	6.0 <sup>a</sup> $\pm$ 1.45	3.6 <sup>b</sup> $\pm$ 1.43	1.22
C18:1n9c	28.0 <sup>a</sup> $\pm$ 3.13	23.7 <sup>b</sup> $\pm$ 3.12	2.64
C18:1n9t	0.3 $\pm$ 0.03	0.3 $\pm$ 0.15	0.09
C20:1	0.1 $\pm$ 0.01	0.1 $\pm$ 0.02	0.01
C22:1n9	0.3 <sup>a</sup> $\pm$ 0.07	0.3 <sup>b</sup> $\pm$ 0.10	0.07
C24:1	0.3 <sup>b</sup> $\pm$ 0.07	0.4 <sup>a</sup> $\pm$ 0.05	0.05
<b>Total MUFA</b>	34.7 <sup>a</sup> $\pm$ 3.82	28.2 <sup>b</sup> $\pm$ 4.23	3.41
<b>Polyunsaturated Fatty Acids (PUFA)</b>			
C18:2n6c	18.9 <sup>b</sup> $\pm$ 2.02	23.0 <sup>a</sup> $\pm$ 3.61	2.48
C18:2n6t	0.1 $\pm$ 0.02	0.1 $\pm$ 0.02	0.02
C18:3n3	0.3 $\pm$ 0.05	0.3 $\pm$ 0.04	0.04
C20:2	0.3 $\pm$ 0.08	0.5 $\pm$ 0.21	0.13
C20:3n3	0.0 $\pm$ 0.14	0.0 $\pm$ 0.12	0.01
C20:4n6	0.0 $\pm$ 0.01	0.0 $\pm$ 0.01	0.01
C20:5n3	0.2 <sup>b</sup> $\pm$ 0.06	0.3 <sup>a</sup> $\pm$ 0.04	0.04
C22:2	0.1 <sup>b</sup> $\pm$ 0.03	0.2 <sup>a</sup> $\pm$ 0.06	0.04
C22:5n3	0.0 $\pm$ 0.00	0.1 $\pm$ 0.28	0.17
C22:6n3	0.5 $\pm$ 0.56	0.8 $\pm$ 0.49	0.45
<b>Total PUFA</b>	20.5 <sup>b</sup> $\pm$ 2.24	25.1 <sup>a</sup> $\pm$ 3.39	2.44
PUFA:SFA	0.5 <sup>b</sup> $\pm$ 0.07	0.6 <sup>a</sup> $\pm$ 0.08	0.06
n6:n3	20.0 $\pm$ 6.21	17.7 $\pm$ 6.84	5.53

<sup>a,b</sup> Rows with different letters differed significantly ( $P < 0.05$ ); \* Least significant differences

#### 4.3.5 Sensory attributes

Tenderness, flavour and aroma, three meat quality aspects of importance to the consumer, are subjective characteristics of meat, generally evaluated by sensory panellists and then correlated to other traits such as shear force, pH, colour and chemical composition which can be measured more accurately (Polawska et al., 2011). The sensory means and standard deviations ( $\pm$  SD) of the attributes established during training (Table 4.1) are presented in Table 4.6. No differences ( $P < 0.05$ ) were found for any of the flavour attributes between the two treatment groups. A stronger ( $P < 0.05$ ) beef aroma, indirectly less game aroma, was found for the 9% CSOCM group. This finding may be favourable as some consumers are not accustomed to a game flavour, which may inhibit them from purchasing game or ostrich meat products. In general panellists will compare the taste of ostrich meat to that of beef. Harris et al. (1994) found only slight non-significant differences between ostrich steaks and Choice beef top loin steaks in terms of like and texture like. However, Balog & Almeida Paz (2007) found contradicting responses noting that the consumer preferred ostrich, a preference based mainly on its lower fat content. In this investigation the panellists noted that the 9% CSOCM treatment had a stronger beef aroma but did not find any difference between the treatments for beef flavour. Also, the beef aroma score was closer to the mid-scale used whereas the beef flavour was slightly lower indicating that the panel did not associate the ostrich strongly with beef.

The type of muscle affects the flavour (Polawska et al., 2011), but not the aroma (Hoffman et al., 2008; Girolami et al., 2003) of the muscle. According to Polawska et al. (2011) muscles that are situated more internally (closer to the bone), eg. *Iliofemoralis* muscle have a stronger flavour, whereas the external muscles, eg. *Gastrocnemius pars externa* muscle would have a less prominent flavour. Harris et al. (1994) found a similar result with the *Obturatorius medialis* muscle (more internally situated) having a more intense flavour than the *Iliofibularis* muscle. The *Iliofibularis* muscle is positioned more externally than internally and would therefore be expected not to have a very strong ostrich flavour (flavour associated with ostrich meat). Ostrich is also frequently noted to have a metallic flavour due to the higher myoglobin and thus iron content (Yancey et al., 2006) however in this investigation there was no difference between treatments for this attribute and the panel used the lower part of the scale indicating that this was not a prominent flavour component.

Cooked *Iliofibularis* muscle samples from the 9% CSOCM group achieved higher ( $P < 0.05$ ) scores for tenderness than the control group and also significantly higher scores for sustained juiciness. In some cases a panellist may perceive a juicier sample as being more tender, which may explain the higher values obtained for both these attributes. In reality however, sustained juiciness is more closely correlated with fat content that causes saliva production to be increased and consequently an impression of juicier meat. Tenderness is one of the major quality contributors that determine the consumers' purchasing/re-purchasing intent and product acceptability. It is also a subjective

attribute that is affected by many variables, cooking methods and temperatures being of the more important variables (Polawska et al., 2011). According to Wood et al. (2008), there exists an inverse relationship between nutritional value and eating quality of meat products as the total lipid content of muscle (Intramuscular fat/marbling) plays an important role when evaluating the tenderness and more importantly the juiciness of cooked meat. Ostrich meat is known for its low lipid content, however according to Balog & Almeida Paz (2007), tenderness even in the presence of low lipid content, is one of the favourable characteristics of ostrich meat. Harris et al. (1994) also found tenderness values for a sensory analysis of ostrich meat to be comparable to that of Choice beef top loin steaks.

Although residue was found to be significantly higher for the control group, the lower end of the scale was used. This higher score for the control treatment may be attributed to the lower tenderness and juiciness scores noted in this treatment group (Table 4.6) as tougher (less tender) meat is associated with leaving a residue in the mouth after chewing due to fluids being released more rapidly during mastication leaving less residue in the mouth (Tshabalala et al., 2003).

**Table 4.6** Comparison of sensory results for cooked ostrich meat (*Iliofibularis* muscle) obtained from birds receiving a control diet or a diet containing 9% cottonseed oilcake meal.

Attribute	Control	9% CSOCM	LSD
Game Aroma	9.2 ± 4.13	9.1 ± 5.23	0.70
Beef Aroma	40.3 <sup>b</sup> ± 8.20	41.8 <sup>a</sup> ± 7.47	0.66
Ostrich Aroma	42.5 ± 7.20	43.1 ± 8.53	1.25
Cooked Chicken Aroma	8.3 <sup>a</sup> ± 5.34	6.8 <sup>b</sup> ± 4.80	0.66
Initial Juiciness	32.8 ± 9.51	35.0 ± 9.44	3.15
Beef Flavour	36.7 ± 6.75	37.2 ± 6.06	1.26
Metallic Flavour	6.0 ± 4.53	6.6 ± 4.73	1.17
Game Flavour	8.7 ± 3.85	8.4 ± 3.58	0.59
Ostrich Flavour	36.8 ± 5.47	36.5 ± 6.06	0.63
Sustained Juiciness	49.2 <sup>b</sup> ± 12.60	52.0 <sup>a</sup> ± 11.79	2.18
Tenderness	63.7 <sup>b</sup> ± 13.96	66.7 <sup>a</sup> ± 12.64	2.65
Residue	2.8 <sup>a</sup> ± 3.32	2.0 <sup>b</sup> ± 2.60	0.77

<sup>a,b</sup> Rows with different letters differ significantly ( $P < 0.05$ ); Data presented as Mean ± standard deviation

#### 4.3.6 Relationship between chemical and sensory attributes

In Fig. 4.1(a) a principal component analysis (PCA) bi-plot of the physical, proximate and sensory data (Fig. 4.1(a)) as well as the fatty acid profile and the sensory data (Fig. 4.1(b)) are presented. The PCA is concerned with explaining the (co)variance structure of the set of variables (meat attributes) through a few linear combinations of these variables (Johnson & Wichern, 2007). The

PCA bi-plot demonstrates the presence of possible correlations amongst the attributes whereas the correlation coefficients ( $r$ ) between attributes indicate the magnitude of these relationships (Table 4.7).

In Fig. 4.1(a) the combination of the two components (F1 and F2) explained 47.68% of the total variance of which F1 explained 32.65% and F2 explained 15.02% of the total variance. As F1 explained a larger proportion of the total variance, the layout of the plot will be investigated vertically as opposed to horizontally. In Fig. 4.1 (b) the combination of the two components; F1 and F2 explained 39.79% of the total variance of which F1 explained 26.47% of the total variance and F2 explained 13.31% of the total variance and will therefore also be investigated vertically.

From Fig. 4.1(a) it can be seen that 9% CSOCM fan fillet associates well with the attributes on the right side of F1, on the PCA bi-plot (juiciness attributes, tenderness, beef aroma and metallic flavour) and these results are substantiated by the higher mean scores for these attributes as can be seen in Table 4.6. It is evident that there was a very strong correlation between sustained juiciness and sensory tenderness ( $r = 0.743$ ;  $P < 0.0001$ ; Tabel 4.7) Davis et al. (1979) also found a positive correlation between these attributes, as panellists experience a juicier sample with tenderness scores increasing accordingly. Hoffman et al. (2008) noted a strong correlation between shear force and residue, a finding in agreement with this study ( $r = 0.387$ ;  $P < 0.0001$ ). Sensory tenderness and shear force are presented in the PCA plot to be negatively correlated ( $r = -0.371$ ;  $P < 0.0001$ ). This result is an indication of the panellists' ability to perceive toughness (Table 4.1). Sensory tenderness is correlated to the amount of moisture or juiciness the panellist perceive during the first initial bites (Hoffman et al., 2008) of the meat sample (initial juiciness) and this correlation was shown to be significant in this study ( $r = 0.579$ ;  $P = 0.003$ ). The relationship of these attributes to the 9% CSOCM samples is further supported by the higher ( $P < 0.05$ ) mean scores presented in Table 4.6. Tenderness and residue show a strong negative correlation ( $r = -0.618$ ;  $P = 0.001$ ). This is further substantiated by the results presented in Table 4.6 as Control fan fillets presented a higher mean score for residue with lower ( $P < 0.05$ ) tenderness in comparison to 9% CSOCM which had higher tenderness with lower residue scores ( $P < 0.05$ ). The 9% CSOCM associated strongly to beef aroma and was significantly higher than the Control group (Table 4.6). In Figure 4.1(b) beef aroma presents a strong correlation ( $r = 0.442$ ;  $P = 0.031$ , Fig. 4,7) with the saturated fatty acid (SFA) C18:0 (Stearic acid) and the 9% CSOCM presents a higher mean ( $P < 0.05$ ) for this same SFA presented in Table 4.5. Raes, et al. (2003) found values for Stearic acid (C18:0) to represent close to 50% of total SFA in the *Longissimus lamborum* of Belgium blue beef cattle and it is due to this finding that it may be assumed that Stearic acid may have contributed to the formation of a characteristic beef flavour in the 9% CSOCM treatment groups cooked fan fillet samples.

**Table 4.7** Correlations and P values for sensory, physical and chemical attributes of cooked *Iliofibularis* muscle.

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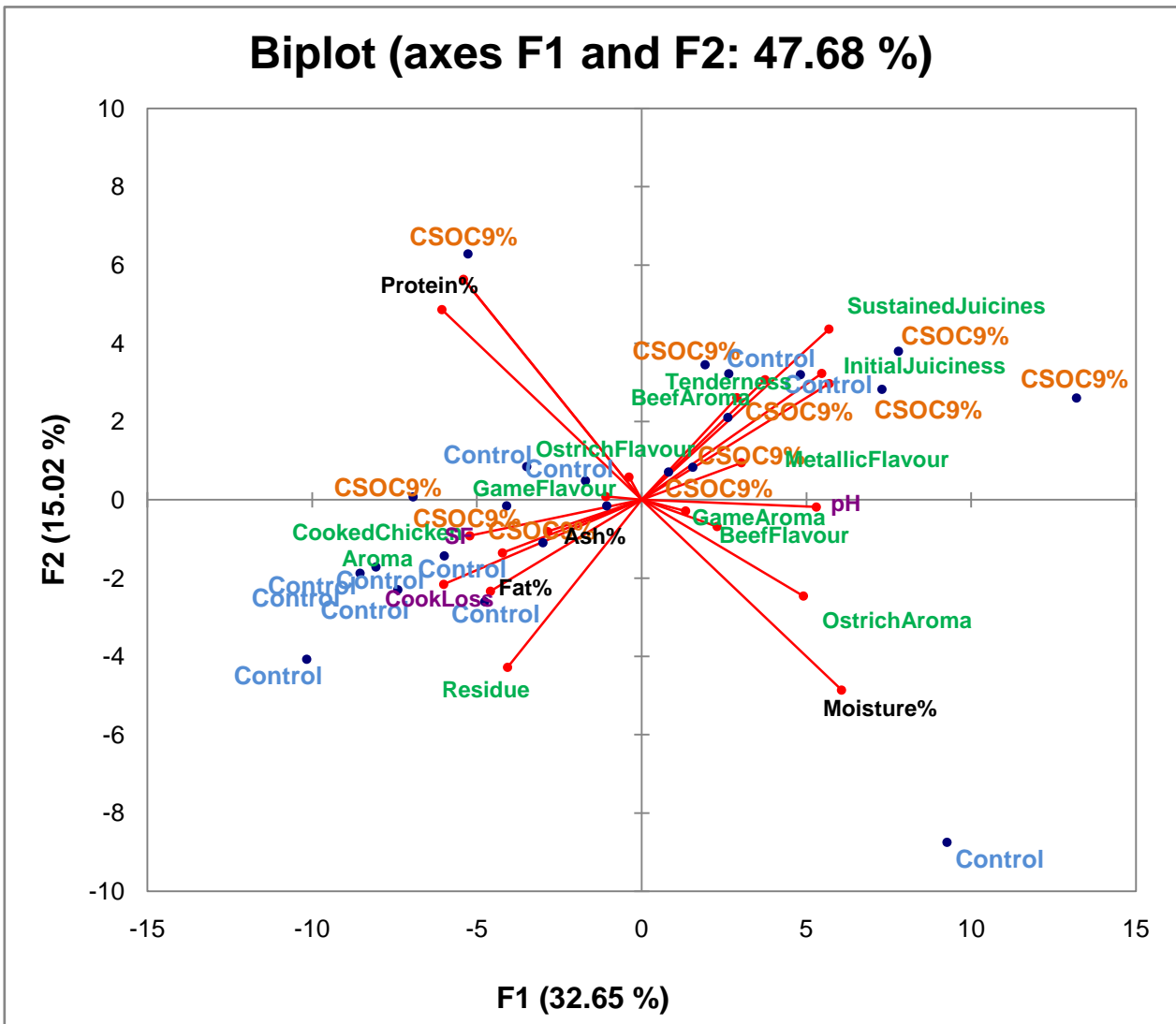
	Beef aroma	Initial juiciness	Sustained juiciness	Tenderness	Residue	C18:0	SFA	C24:1	C18:2n-6c	C20:3n-3	C22:2	PUFA	Shear force
<b>Beef aroma</b>	1	0.298; 0.157	0.348; 0.096	0.335; 0.110	-0.231; 0.278	0.442; 0.031	0.415; <0.0001	0.472; <0.0001	0.422; <0.0001	0.412; <0.0001	0.081; <0.0001	0.485; <0.0001	-0.124; <0.0001
<b>Initial juiciness</b>	0.298; 0.157	1	0.758; <0.0001	0.579; 0.003	-0.472; 0.020	0.140; 0.514	0.203; <0.0001	0.152; <0.0001	0.203; <0.0001	0.176; <0.0001	-0.282; <0.0001	0.228; <0.0001	-0.502; <0.0001
<b>Sustained juiciness</b>	0.348; 0.096	0.758; <0.0001	1	0.743; 0.0001	-0.593; 0.002	0.270; 0.202	0.413; <0.0001	0.161; <0.0001	0.188; <0.0001	0.156; <0.0001	0.038; <0.0001	0.227; <0.0001	-0.499; <0.0001
<b>Tenderness</b>	0.335; 0.110	0.579; 0.003	0.743; <0.0001	1	-0.618; 0.001	0.115; 0.591	0.277; <0.0001	0.065; <0.0001	0.127; <0.0001	0.035; <0.0001	0.039; <0.0001	0.148; <0.0001	-0.371; <0.0001
<b>Residue</b>	-0.231; 0.278	-0.472; 0.020	-0.593; 0.002	-0.618; 0.001	1	-0.396; 0.056	-0.437; <0.0001	-0.424; <0.0001	-0.147; <0.0001	-0.010; <0.0001	-0.158; <0.0001	-0.474; <0.0001	0.387; <0.0001
<b>C18:0</b>	0.442; 0.031	0.140; 0.514	0.270; 0.202	0.115; 0.591	-0.396; 0.056	1	0.800; <0.0001	0.832; <0.0001	0.115; <0.0001	0.415; <0.0001	0.565; <0.0001	0.860; <0.0001	0.280; <0.0001
<b>C24:1</b>	0.415; <0.0001	0.203; <0.0001	0.413; <0.0001	0.277; <0.0001	-0.437; <0.0001	0.800; <0.0001	1	0.645; 0.001	0.335; <0.0001	0.484; <0.0001	0.389; <0.0001	0.711; <0.0001	0.439; <0.0001
<b>C18:2n-6c</b>	0.472; <0.0001	0.152; <0.0001	0.161; <0.0001	0.065; <0.0001	-0.424; <0.0001	0.832; <0.0001	0.645; 0.001	1	0.100; <0.0001	0.296; <0.0001	0.277; <0.0001	0.984; <0.0001	-0.277; <0.0001
<b>C20:3n-3</b>	0.422; <0.0001	0.203; <0.0001	0.188; <0.0001	0.127; <0.0001	-0.147; <0.0001	0.115; <0.0001	0.335; <0.0001	0.100; <0.0001	1	0.542; 0.006	-0.240; 0.258	0.154; 0.472	0.102; 0.636
<b>C22:2</b>	0.412; <0.0001	0.176; <0.0001	0.156; <0.0001	0.035; <0.0001	-0.010; <0.0001	0.415; <0.0001	0.484; <0.0001	0.296; <0.0001	0.542; 0.006	1	0.018; 0.934	0.385; 0.063	0.138; 0.521
<b>SFA</b>	0.081; <0.0001	-0.282; <0.0001	0.038; <0.0001	0.039; <0.0001	-0.158; <0.0001	0.565; <0.0001	0.389; <0.0001	0.277; <0.0001	-0.240; 0.258	0.018; 0.934	1	0.235; 0.270	-0.103; 0.630
<b>PUFA</b>	0.485; <0.0001	0.228; <0.0001	0.227; <0.0001	0.148; <0.0001	-0.474; <0.0001	0.860; <0.0001	0.711; <0.0001	0.984; <0.0001	0.154; 0.472	0.385; 0.063	0.235; 0.270	1	-0.306; 0.145
<b>Shear force</b>	-0.124; <0.0001	-0.502; <0.0001	-0.499; <0.0001	-0.371; <0.0001	0.387; <0.0001	0.280; <0.0001	0.439; <0.0001	-0.277; <0.0001	0.102; 0.636	0.138; 0.521	-0.103; 0.630	-0.306; 0.145	1

Values presented as correlation (r); P value

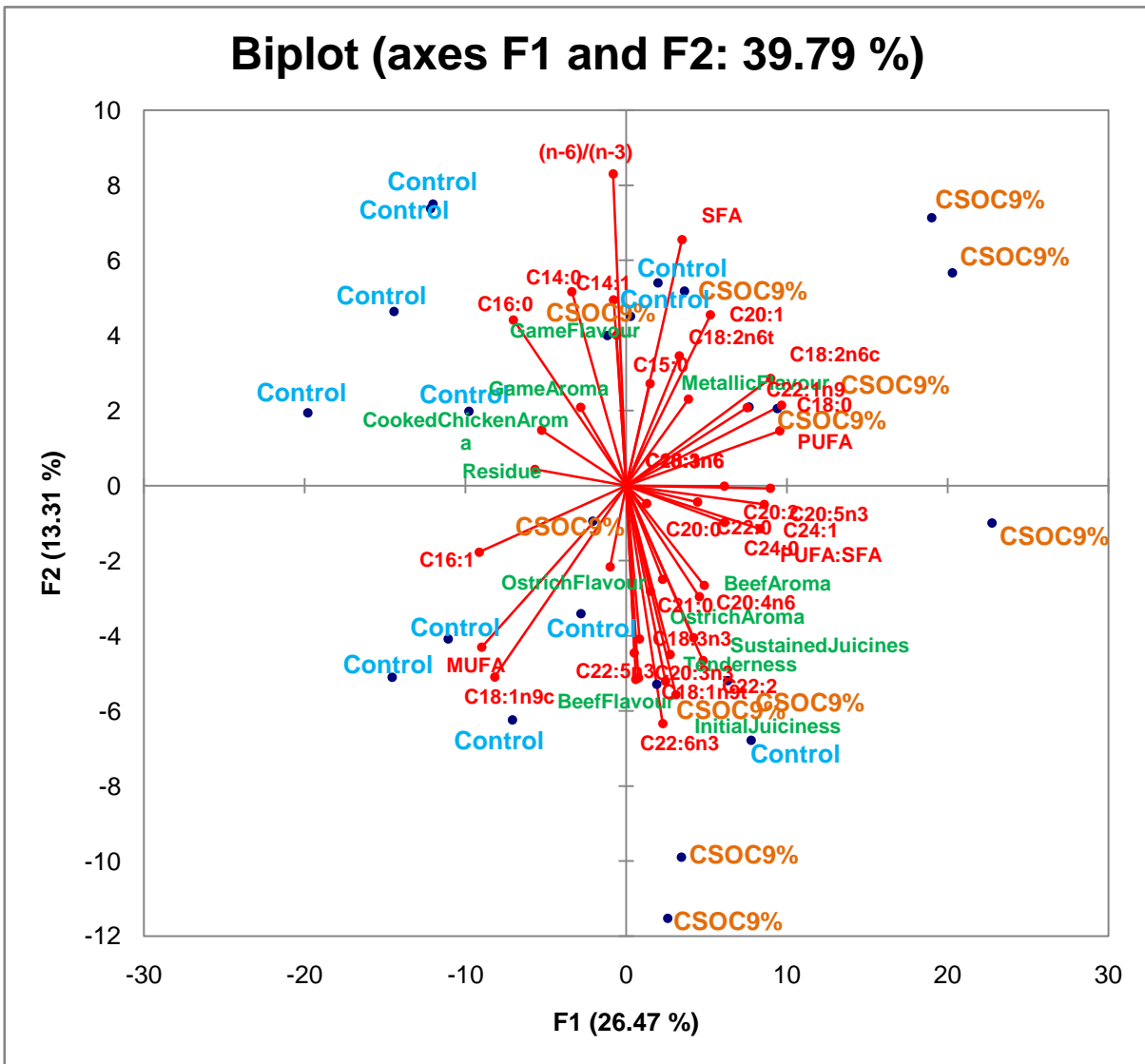
Attributes of the Control fan fillets associated more with the left side of F1 (cooked chicken aroma, residue, shear force, cooking loss and fat %). The correlation between residue and Control fan fillets is supported by the higher ( $P < 0.05$ ) mean scores presented in Table 4.6. The relationship between cooked chicken aroma and the Control group is also substantiated by the higher ( $P < 0.05$ ) score presented in Table 4.6 when compared to the 9% CSOCM group. Fat content was not found to be affected by the treatments although the PCA-biplot indicates a relationship with the Control group. Interestingly though is that there was no relationship between fat content and any of the flavour attributes. According to Melton (1990), intramuscular fat content is one of the most important factors with regards to flavour formation, but as it is well known that ostrich meat is very low in intramuscular fat the lack of intensity in flavour may be explained by this characteristic – all the flavour attributes in Table 4.6 had scores from the lower section of the scale used.

Over the years, the relationship between fatty acid profile and flavour formation in meat has been reported and emphasised by several authors (Wood et al., 1999, Lawrie, 1998, Wood et al., 2004) and the variation in fatty acid composition has immense effects on meat quality (Wood et al., 2008). Fatty acids have different melting points causing the fat component of meat to be different in terms of firmness or softness and where these fat components gather, the amount of solidified or liquid fat will determine the appearance and/or colour of the fat. More importantly however is the ability of some fatty acids to oxidise rapidly. Unsaturated fatty acids, especially those with more than one double bond are more susceptible to oxidising, affecting the shelf life of the meat products. But it is also this ability to oxidise that determines and influences the flavour formation during cooking (Wood et al., 2004).

Ostrich meat is generally associated with flavours similar to game species, metallic flavours or game flavour and aroma. In literature it has been reported that game flavour and aroma is associated mostly with polyunsaturated fatty acids (PUFA) (Swanson & Penfield, 1991). In this study however, no correlations were found between PUFA and any of the expected game or metallic flavours, but a moderate correlation with beef aroma ( $r = 0.485$ ;  $P < 0.0001$ ) and the 9% CSOCM presented higher ( $P < 0.05$ ) mean scores for both PUFA and beef aroma as presented in Table 4.5 and Table 4.6, respectively. There was also some individual fatty acids that presented a moderate correlation with beef aroma indicated in Fig. 4.1(b) and Table 4.7; Stearic acid (C18:0) ( $r = 0.442$ ;  $P = 0.031$ ), Nervonic acid (C24:1) ( $r = 0.415$ ;  $P < 0.0001$ ), Linoleic acid (C18:2n-6c) ( $r = 0.472$ ;  $P < 0.0001$ ), Eicosatrienoic Acid (C20:3n-3) ( $r = 0.442$ ;  $P < 0.0001$ ) and Docosadienoic acid (C22:2) ( $r = 0.412$ ;  $P < 0.0001$ ). No other significant correlations were found for any of the attributes with any other individual fatty acids. As mentioned before, it is the amount and composition of fatty acids that are responsible for the flavour development during cooking. The favourable characteristic of ostrich meat being low in intramuscular fat (Table 4.3) could therefore be the reason for a lack of intensity in flavour (Table 4.6) development during cooking.



**Figure 4.1(a).** Principle component analysis bi-plot of the sensory attributes, physical attributes and chemical composition of ostrich meat (*Iliofibularis* muscle) receiving diets containing 0 or 9% cottonseed oilcake meal of diets.



**Figure 4.1(b).** Principle component analysis bi-plot of the sensory attributes and fatty acid composition of ostrich meat (*Iliofibularis* muscle) samples receiving diets containing 0 or 9% cottonseed oilcake meal of diets.



#### 4.4 CONCLUSION

The inclusion of cottonseed oilcake meal (and replacement of soybean oilcake meal) at a 9% level did not affect the physical or chemical composition of cooked or raw ostrich *Illofibularis* muscle. A favourable FA profile was observed in the 9% CSOCM group with increased PUFA levels as well as docosahexaenoic acid (DHA; C20:5n-3) quantities. The 9% CSOCM presented a less intense game aroma, closer to the aroma associated with cooked beef, which can be a favourable result from feeding CSOCM. This attribute can be used to promote an exotic product, with favourable nutritional characteristics without the game flavour which can sometimes discourage consumers from purchasing the products, as they are not accustomed to or dislike this “wild” flavour. Tenderness and sustained juiciness was also scored higher in the 9% CSOCM group, rendering these samples, in terms of consumer importance, as the favourable product. Cottonseed oilcake meal did therefore prove to have a favourable result on the sensory profile of ostrich (*Illofibularis* muscle) fillet steaks, but as stated by Wood et al. (2008), there remains an inverse relationship between nutritional value and eating quality. Very little information is available with regards to cooked ostrich fatty acid composition. Further research on this subject is necessary as ostrich meat is primarily consumed as a cooked product and information on the nutritional value is necessary. The future of ostrich production is based on the idea of providing ostrich meat as a healthy alternative for the consumption of traditional meats (Balog and Almeida Paz, 2007) and traditional meats are used in the production of a great variety of value added products. Ostrich meat and its products therefore need to be utilized and experimented with in order to substantiate its place in the meat industry.

#### 4.5 REFERENCES

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## CHAPTER 5

### REPLACING PORK FAT WITH OLIVE OIL IN OSTRICH CABANOSSİ AND THE EFFECT THEREOF ON CHEMICAL AND SENSORY ATTRIBUTES

#### ABSTRACT

The effect of olive oil as replacement for pork fat on the chemical composition and sensory characteristics in ostrich cabanossi was investigated. Fan fillet (*Iliofibularis* muscle) from 13 month old birds receiving no cottonseed oilcake meal but soybean oilcake meal (Control) or 9% cottonseed oilcake meal (9% CSOCM) replacing the soybean oilcake meal, was used. In addition to the ostrich meat, warthog meat was used to replace commercial pork meat in the recipe. Olive oil was included at three levels (0%, 1% and 2%). Six treatments were under investigation: Control 0% olive oil, Control 1% olive oil, Control 2% olive oil, 9% CSOCM 0% olive oil, 9% CSOCM 1% olive oil and 9% CSOCM 2% olive oil. The Control and 9% CSOCM ostrich meat presented no differences ( $P < 0.05$ ) with regards to chemical composition or fatty acid profile. After smoking and drying the fat content in the cabanossi containing 0%, 1% and 2% olive oil averaged 7.2%, 7.45% and 8.65% respectively. Processed meat products containing less than 10% fat are classified as a low-fat meat product. Olive oil is a mono unsaturated vegetable oil containing mainly Oleic acid (C18:1n9c), and low quantities of saturated fatty acids and polyunsaturated fatty acids. Total mono unsaturated fatty acids in the cabanossi increased from 47.0% to 73.0%, whilst total saturated fatty acids and total polyunsaturated fatty acids decreased from 40.6% to 19.9% and 11.6% to 6.6% respectively as olive oil increased from 0% to 2%. Cabanossi produced from the Control ostrich meat presented a lower fat and higher crude protein content ( $P < 0.05$ ). The inclusion of olive oil at 2% resulted in cabanossi with increased tenderness, juiciness and cured red meat colour, all factors that appeal greatly to the consumer. Overall flavour was not adversely affected by the inclusion of olive oil.

**Keywords:** Processed ostrich meat product, Vegetable oil, Consumer acceptability

## 5.1 INTRODUCTION

As a result of European Union (EU) regulations, banning the export of South African ostrich meat due to an ongoing outbreak of *Avian influenza*. This viral infection has been identified as the H5N2 strain and is predominantly found among waterfowl, shorebirds and gulls (Cooper et al., 2004). According to the World Health Organisation (WHO), the World Organisation for Animal Health (OIE) and the South African Institute for Communicable Diseases this strain poses no risk to humans, as humans do not have receptors for the virus in their respiratory tract (Cooper et al., 2004).

As a result a surplus of ostrich meat is available in South Africa at a price unaffordable for the local consumer. Increasing the variety of processed or value added ostrich meat products is necessary. Ostrich meat is classified as a healthy red meat due to its leanness and favourable fatty acid profile. Another characteristic of ostrich meat is its high ultimate pH (pH<sub>U</sub>) which is favourable in processed meat products as it increases the water holding capacity (WHC) (Fisher et al., 2000) but comes as a disadvantage in terms of shelf life, flavour and its ability to absorb curing agents (Sales & Mellet, 1996). Several value added ostrich products have already been manufactured but these are mainly based on established technologies used on other red meat types and generally just applied as is on ostrich meat (Hoffman, 2005). Cabanossi is a dry sausage that is only slightly spiced and then smoked and dried for several hours. Generally it consists of a mixture of beef and pork meat but can be produced using a variety of meats for example chicken and duck with alternatively turkey meat, even venison in some areas. The general recipe calls for a composition of 38% lean meat, 32% pork meat (60:40 meat to fat ratio) plus an added 23% of bacon (50:50 meat to fat ratio) which results in a product consisting of almost 25% pork fat.

Pork fat, specifically the back fat is generally used as an ingredient in processed meat products and has high contents of saturated fatty acids (SFA) and cholesterol (German & Dillard, 2004; Mugerza et al., 2003). Over the last decade however, consumer preferences have changed drastically and there is an emphasis on nutrition and health, specifically with regards to saturated fat and cholesterol content of meat products (Resurreccion, 2003). In most developed countries obesity and cardiovascular disease has become a topic of grave concern (Williams, 2000) and it has been proposed that intake of total fat and SFA should decrease to less than 10% of dietary energy in order to reduce the risk of contracting cardiovascular diseases. This resulted in the promotion of consuming or changing the diet composition to increased polyunsaturated fatty acid (PUFA) content, specifically the long chain omega-3 PUFA docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) for their beneficial physiological responses. The presence of these PUFA in the typical western diet is very low due to the small amount of fish and fish oils consumed. Williams (2000)

further explains however, that even if it is possible to achieve favourable levels of these Omega-3 PUFA (EPA and DHA) by consuming fish and fish oils, the general consumer perceive these types of products as unpalatable. With regards to processed meat products however, the interest is not so much in increasing PUFA but with increasing the mono unsaturated fatty acid (MUFA) content as it has also been associated with decreasing coronary heart disease (Bloukas & Paneras, 1993), as well as having a protective effect against low density lipoproteins (LDL) oxidation and against oxidative stress in humans (Ansorena & Astiasaran, 2004). The other objective for focusing on increasing MUFA in processed meat products is because it is not as susceptible to oxidation as PUFA which could lead to unfavourable sensory properties. A strategy to enhance the nutritional value of meat products by increasing MUFA content and adding natural anti-oxidants such as tocopherols as well as reducing cholesterol intake is to replace animal fat with certain vegetable oils (Rodríguez-Carpena, Morcuende & Estévez, 2012).

A study to determine the functionality and nutritional effect of avocado, sunflower and olive oil as a replacement for pork backfat in the production of hamburger patties (Rodríguez-Carpena, Morcuende & Estevez, 2011) showed that the most favourable of these vegetable oils were avocado and olive oil. Olive oil has shown to have positive effects with regards to nutritional value and oxidative stability as well as demonstrating protection against several cancer types (Escrich et al., 2007). It is one of the most mono unsaturated vegetable oils containing 56.3 to 86.5% MUFA, 8 to 25% SFA and 3.6 to 21.5% PUFA (Bloukas and Paneras, 1993). A variety of value added meat products have already been manufactured with olive oil as a replacement for animal fat (Rodríguez-Carpena, Morcuende & Estévez, 2012; Ansorena & Astiasaran, 2004; Pappa, Bloukas & Arvaritoyannis, 2000; Bloukas & Paneras, 1993) and has proven to be very successful with regards to nutritional value as well as sensory quality.

Ostrich meat however is already known for its favourable level of MUFA and PUFA as well as low intramuscular fat (IMF) content (Sales, 1994) and therefore the addition of olive oil instead of a saturated animal fat in the production of value added products seems very promising. As it is vital to ensure commercial success and dietary benefits, the consumer's perception of any low fat or reduced fat products is important (Resurreccion, 2003). This study investigated the use of olive oil (at three inclusion levels of 0%, 1% and 2%) as a replacement for pork fat and warthog meat at a 50:50 ratio with ostrich meat to replace the pork meat component in the production of a cabanossi sausage. The effect of replacing pork fat with olive oil has not yet been investigated in the production of cabanossi. This study is therefore aimed at investigating the effect of olive oil on the chemical composition as well as the sensory attributes of the ostrich cabanossi meat product.



## 5.2 MATERIALS AND METHODS

### 5.2.1 Processing of *cabanossi*

Ostrich meat was obtained from the 13 month old birds slaughtered during the cottonseed oilcake meal (CSOCM) trial discussed in Chapter 4 from the Control and 9% CSOCM treatment groups. Slaughtering of ostriches took place at Ostriswell (Swellendam, South Africa) a commercial abattoir in Swellendam, South Africa. Birds were kept in lairage (roofed pens) for two hours prior to slaughter. After electrical head stunning (90-110V, 400-600mA, 4-6s), the ostriches were suspended by both legs and exsanguinated by a neck cut to the aortic vein followed by a thoracic stick (Hoffman, 2012). Bleeding (10-15min) was followed by plucking, skinning, evisceration and a health inspection. Carcasses were chilled for 24 hours at 0-4°C after which the fan fillet (*Iliofibularis* muscle) was excised for chemical analysis. Meat samples were vacuum packed and frozen at -20°C until further analysis at Stellenbosch University.

Six *cabanossi* treatments were under investigation: Olive oil was included at three levels (0%, 1% and 2%); Control 0% olive oil, Control 1% olive oil, Control 2% olive oil, 9% CSOCM 0% olive oil, 9% CSOCM 1% olive oil and 9% CSOCM 2% olive oil.

All six treatments were produced from the fan fillet (*Iliofibularis* muscle) of ostriches fed the Control and 9% CSOCM batch. The fan fillets were vacuum packed and frozen before being transported to Stellenbosch, where it was stored at -20°C until processing. A single batch of cold-pressed extra-virgin olive oil (Frontoia variety) from Tokara Olive Farm (Tokara Olive Shed, Helshoogte Pass, Stellenbosch, South Africa) was used. All the remaining ingredients were provided by a single provider, Deli Spices (25 Bertie Avenue, Epping 2, Cape Town, South Africa). From the Control group (0 % inclusion of CSOCM), 30 fan fillets were used and for the 9% CSOCM treatment group 31 fan fillets were used. The *cabanossi* recipe for each batch contained 50% of ostrich meat and 50% of warthog meat with one *cabanossi* spice pack from Deli spice. For the 1% olive oil inclusion, 50 ml was added to a 5 kg batch and for the 2% olive oil, 100ml was added to a 5kg batch. No additional water or ice was added.

Both the ostrich fan fillets and the warthog meat (off cuts as well as belly) was first minced through a 12mm diameter disc where after the two meat components were mixed together. The *cabanossi* spice was then added and mixed by hand until thoroughly mixed. The meat and spice mixture was then minced again through a 5mm diameter disc to ensure adequate mixing of the ingredients. Finally the olive oil was added to the mixture. The *cabanossi* mixture was then placed into a hand sausage filler (Tulsa model, DMD Foodtec Code T-0102 5-89) and filled into natural sheep casings (25 – 30mm).

The cabanossi were placed into a Reich Airmaster® UKF 2000 BE (Reich Klima-Räuchertechnik, Urbach, Germany) with a SmartSmoker and TradiSmoker LS 500 HP electronic that was controlled automatically by a Microprocessor (Unicontrol 2000). The program run is depicted in Table 5.1. After completion of the process, the cabanossi were removed and placed into vacuum bags and stored at -20°C until further analysis was completed.

**Table 5.1** Production program for ostrich cabanossi made in a Reich Airmaster® UKF 2000 BE

Activity	Temperature <sup>a</sup> (°C)	RH <sup>b</sup> (%)	Time (hrs)
Reddening	40	80	2.00
Drying	30	30	2.00
Cold smoking	30	20	0.30
Smoke destruction	30	30	0.10
Drying	30	30	2.00
Cold smoking	30	20	0.20
Smoke destruction	30	20	0.10
Drying	30	30	8.00

<sup>a</sup> Temperature in °C, <sup>b</sup> Relative humidity in percentage

### **5.2.2 Proximate analysis**

After thawing at 4°C for 24 hours, cabanossi samples of the six treatments (of a randomly selected cabanossi within each treatment) were homogenised and analysed in duplicate for total percentage of moisture, ash, fat and crude protein content.

Proximate analysis of the cabanossi samples were analysed according to the Association of Official Analytical Chemist's Standard Techniques (AOAC). A 2.5g homogenized cabanossi sample was placed in a drying oven at 100 – 105°C for 24 hours (AOAC Official method 934.01) (AOAC, 2000a) in order to determine the moisture content after which the same samples was used to determine ash content by incineration in an oven at 500°C for 6 hours (AOAC Official Method 942.05) (AOAC, 2000b). The chloroform/methanol (1:2v/v) extraction method stipulated by Lee et al. (1996) was used to determine the total lipid (%) (intramuscular fat) of a 5g homogenised cabanossi sample. The fat free sample was placed in a drying oven to retain a moisture free sample. The % nitrogen (N) was then determined

on the fat and moisture free sample based on the Dumas combustion method 992.15 (AOAC, 2000c) using a Leco Nitrogen/Protein Analyser (FP-528, Leco Corporation). The Leco was calibrated with EDTA samples (Leco corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA, Part no. 502-092, Lot no. 1055) prior to every analyses session. The results were presented in % N which was then multiplied by a conversion factor (6.25) in order to determine the crude protein content of the cabanossi samples. All proximate analyses are controlled by a National inter-laboratory scheme (AgriLASA: Agricultural Laboratory Association of South Africa). In order to assess the accuracy of the analyses, blind samples are analysed every other month.

**Table 5.2** Means ( $\pm$  Standard deviation) of proximate composition (%) of warthog and ostrich (control & 9% cottonseed oilcake meal (CSOCM)) meat used in the production of ostrich cabanossi with increasing levels of olive oil.

	Warthog*	Control	9% CSOCM	LSD
Moisture	70.6	75.6 $\pm$ 0.83	76.1 $\pm$ 1.31	1.54
Crude Protein	22.0	20.4 $\pm$ 0.61	19.5 $\pm$ 1.75	1.87
Fat	5.8	3.8 $\pm$ 0.37	4.6 $\pm$ 0.67	0.76
Ash	1.2	1.1 $\pm$ 0.03	1.1 $\pm$ 0.04	0.05

\* A single batch was minced and then added to the ostrich meat and olive oil treatment batches.

### 5.2.3 Fatty acid analysis

After thawing, 2g cabanossi sample was extracted with a chloroform:methanol (1:2 v/v) solution according to a modified method of Folch, Lees, and Sloane-Stanley (1957). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (WiggenHauser, D-500 Homogenizer) was used to homogenise the sample with the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2h at 70°C using a methanol/sulphuric acid (19:1 v/v) solution as transmethylating agent. After cooling to room temperature, the resulting fatty acid methyl esters (FAMES) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. Analysis was done on a Thermo Focus GC equipped with a flame ionized detector using a BPX70 capillary column (60m x 0.25mm internal diameter, 0.25µm film, SGE, Australia). Gas flow rates were 25ml/min for hydrogen and 2-4ml/min for the hydrogen carrier gas. Temperature programming was linear at 3.4°C/min, with an initial temperature of 60°C, a final temperature of 160°C, an injector

temperature of 220°C and a detector temperature of 260°C. The FAMEs were identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH<sub>2</sub>Cl<sub>2</sub>, Catalogue Number 47885-U. Supelco, North Harrison Road, Bellefonte, PA 16823-0048, USA).

**Table 5.3** Fatty acid profile (% of total fatty acids) of raw materials used in production of ostrich cabanossi.

	Raw materials			
	Olive oil	Warthog	Control	9% CSOCM
<i>Saturated fatty acids</i>				
C14:0	0.0	1.2	0.4 ± 0.16	0.6 ± 0.24
C15:0	0.0	0.3	0.2 ± 0.04	0.2 ± 0.04
C16:0	19.1	33.1	22.3 ± 8.39	27.1 ± 2.06
C18:0	1.1	15.9	13.8 ± 0.77	16.1 ± 2.33
C20:0	0.0	0.4	0.3 ± 0.04	0.3 ± 0.09
C21:0	0.0	0.1	0.1 ± 0.01	0.1 ± 0.12
C22:0	0.0	0.2	0.6 ± 0.08	0.6 ± 0.13
C24:0	0.0	0.0	0.1 ± 0.02	0.1 ± 0.07
Total SFA	20.2	51.2	37.7 ± 8.46	45.1 ± 2.58
<i>Mono unsaturated fatty acids</i>				
C14:1	0.0	0.1	0.1 ± 0.02	0.1 ± 0.03
C16:1	0.2	3.6	6.6 ± 1.59	4.3 ± 0.96
C18:1n-9c	78.1	41.4	26.8 ± 8.16	25.2 ± 0.89
C18:1n-9t	0.9	0.1	0.2 ± 0.03	0.5 ± 0.43
C20:1	0.1	0.1	0.1 ± 0.01	0.1 ± 0.02
C22:1n-9	0.0	1.6	0.1 ± 0.02	0.3 ± 0.28
C24:1	0.0	0.1	0.3 ± 0.06	0.3 ± 0.08
Total MUFA	79.35	46.9	33.8 ± 8.63	30.3 ± 1.31
<i>Polyunsaturated fatty acids</i>				
C18:2n-6c	0.0	0.3	17.9 ± 1.47	18.6 ± 1.70
C18:2n-6t	0.1	0.1	0.0 ± 0.01	0.1 ± 0.02
C18:3n-6	0.0	0.0	0.0 ± 0.00	0.0 ± 0.00
C18:3n-3	0.2	0.3	0.3 ± 0.05	0.3 ± 0.03
C20:2	0.1	0.2	0.3 ± 0.04	0.4 ± 0.07
C20:3n-6	0.0	0.0	3.3 ± 0.49	1.0 ± 1.55
C20:3n-3	0.0	0.0	0.1 ± 0.00	0.1 ± 0.06
C20:4n-6	0.0	0.1	0.0 ± 0.00	0.0 ± 0.01
C20:5n-3	0.0	0.0	0.0 ± 0.01	0.0 ± 0.01
C22:2	0.0	0.1	0.1 ± 0.02	0.2 ± 0.32
C22:5n-3	0.0	0.0	0.0 ± 0.00	0.3 ± 0.39
C22:6n-3	0.0	0.1	0.3 ± 0.04	0.3 ± 0.17
Total PUFA	0.4	1.2	22.3 ± 1.32	21.2 ± 1.29
PUFA:SFA	0.02	0.0	0.6 ± 0.20	0.5 ± 0.05
n-6:n-3	2.83	0.7	31.4 ± 4.01	22.1 ± 6.73

Rows with different letters differed significantly ( $P < 0.05$ ), \* Least significant difference

#### **5.2.4 Descriptive sensory analysis**

The purpose of the sensory analysis was to determine the effect of olive oil inclusion on the sensory quality characteristics of ostrich cabanossi. All cabanossi packages (stored at -20°C) were thawed for 24h at 4°C before sensory analysis. After opening, two pieces of 2cm thickness were sliced and packed into ramekin bowls and covered with a plastic lid 2h prior to their pre-assigned sensory analysis sessions.

A descriptive sensory analysis was performed all six cabanossi treatments. The panel was chosen based on their experience in sensory analysis and on their availability. Panellists were trained, in accordance with the generic descriptive analysis techniques, as described by Lawless & Heymann (1998). A panel of ten members were trained in two interactive sessions to familiarise the panellists with the treatments and to identify the sensory characteristics to be evaluated. A questionnaire was compiled during the first training session. The questionnaire was refined and tested during the second training session. An unstructured line scale ranging from zero (low intensity) on the left side and 100 (high intensity) on the right side (AMSA, 1995) was used to analyse the sensory characteristics. Table 5.3 depicts the characteristics and definitions used. The sensory tests were performed in individual booths in a temperature (21°C) and light controlled (equivalent to daylight) room. Two samples of each of the six treatments were served to the panellists in a randomised order in six sessions. Distilled water, apple and crackers were given to the panellists with each sensory session. Each sample was coded with randomly selected three digit numbers and served at a room temperature of 21°C.

**Table 5.4** Definition and scale for each attribute used for the descriptive sensory analysis of ostrich cabanossi.

<b>Descriptor</b>	<b>Definition</b>	<b>Scale</b>
Smoky aroma	Aroma associated with smoked meats	0 = Extremely bland 100 = Extremely intense
Olive oil aroma	Aroma associated with olive oil	0 = Extremely bland 100 = Extremely intense
Fatty meat aroma	Aroma associated with meat products containing large amounts of fat	0 = Extremely bland 100 = Extremely intense
Visible fat	Amount of fat visibly present on visual inspection	0 = No fat present 100 = Large amount of fat present
Cured red meat colour	Colour associated with cured meat products	0 = Light red colour 100 = Intense dark red colour
Surface appearance	Presence of oily substance on surface	0 = Dry surface appearance 100 = Extremely oily appearance
Cured pork flavour	Flavour associated with cured pork products	0 = Extremely bland 100 = Extremely intense
Game flavour	Flavour associated with game meat	0 = Extremely bland 100 = Extremely intense
Fishy flavour	Flavour associated with fish products	0 = Extremely bland 100 = Extremely intense
Smoky flavour	Flavour associated with smoked meat products	0 = Extremely bland 100 = Extremely intense
Saltiness	Impression of amount of salt present	0 = Extremely bland 100 = Extremely salty
Peppery flavour	Flavour associated with pepper	0 = Extremely bland 100 = Extremely intense
Olive oil flavour	Flavour associated with olive oil	0 = Extremely bland 100 = Extremely intense
Tenderness	Impression of tenderness after first five chews using the molar teeth	0 = Extremely tough 100 = Extremely tender
Juiciness	The level of juiciness perceived after the first five chews using the molar teeth	0 = Extremely dry 100 = Extremely juicy
Firmness	The degree of force required to bite the sample	0 = Extremely soft 100 = Extremely firm

### **5.2.5 Statistical analysis**

The experimental design was a randomised block with each of the six treatment combinations randomly replicated in six evaluation sessions. The treatment design was a

2x3 factorial with two feeding treatments (Control, 9%CSOCM) and three levels of olive oil (0%, 1%, 2%).

The model for the experimental design for the proximate and fatty acid data is defined by the following equation:

Where  $y_{ijk}$  defines the response obtained for the  $k$ 'th observation in the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment. The overall mean is defined by  $\mu$ , the effect due to feeding treatment  $i$  is presented by  $t_i$ ,  $o_j$  presents the effect due to olive oil level  $j$ . The effect due to the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment is defined by  $to_{ij}$  and  $\epsilon_{ij}$  defines the random error associated with response on the  $k$ 'th observation in the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment.

The model for the experimental design for the sensory data is defined by the following equation:

Where  $y_{ijk}$  presents the response obtained for the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment in the  $k$ 'th evaluation session,  $\mu$  depicts the overall mean, the effect due to evaluation session  $k$  is presented by  $s_k$ . The effect due to feeding treatment  $i$  is defined by  $t_i$ , where  $o_j$  presents the effect due to olive oil level  $j$ . The effect due to the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment is depicted by  $to_{ij}$  and  $\epsilon_{ij}$  depicts the random error associated with response on the  $i$ 'th level of the feeding treatment and the  $j$ 'th level of the olive oil treatment in the  $k$ 'th evaluation session.

Univariate analysis of variance was performed, according to the model for the experimental design, on all sensory and chemical variables accessed using the GLM (General Linear Models) Procedure of SAS (Version 9.2; SAS Institute Inc, Cary, USA). Sensory data was pre-processed by subjecting it to a test-retest analysis of variance (ANOVA), using SAS, to test for panel reliability. Judge\*Replication and Judge\*Sample interactions were used respectively as measures of temporal stability (precision) and internal consistency (homogeneity) of the panel. Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

In addition to the univariate ANOVAs, the data was also subjected to Multivariate methods such as principal component analysis (PCA) and discriminate analysis (DA) (XLStat, Version



2011, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and their attributes.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Proximate analysis

The proximate composition of the ostrich cabanossi manufactured with increasing levels of olive oil is presented in Table 5.5. The average moisture content (%) of the ostrich cabanossi samples was initially 70.9% and reduced to 50.3% after processing. No significant differences were found for the moisture content between treatments. This was expected as all batches were prepared according to the same recipe and showed a similar ( $P>0.05$ ) weight loss percentage of approximately 40%. For a semi dry sausage the moisture content is in accordance with other typical dried sausages (Yun-Sang Choi et al., 2010; Muguerza et al., 2002; Bloukas et al., 1997a; Bloukas & Paneras, 1993).

Crude protein was highest ( $P<0.05$ ) in the 0 and 1% olive oil inclusion in the Control group (36.2 and 36.3% respectively) and lowest in the 9% CSOCM group with 2% olive oil (33.6%). Fat percentage was higher ( $P<0.05$ ) for the 9% CSOCM group with 2% olive oil inclusion than the 0 and 1% olive oil treatments in the Control group. No major differences were expected as the ostrich meat (Control and 9% CSOCM) used in the production of the cabanossi (Table 5.2) did not differ ( $P>0.05$ ) in proximate composition. Differences observed are therefore mainly contributed to the olive oil inclusion at 1 and 2% or can also be due to insufficient mixing of ingredients. Warthog meat used in this study presented a slightly higher fat content (5.8%) than the Control and 9% CSOCM ostrich meat (3.8 and 4.6% respectively) (Table 5.2) which also contributes to the fat content differences ( $P<0.05$ ) found in the dried and smoked cabanossi samples. According to Muguerza et al. (2003) dry sausages can contain as much as 32% fat which can rise to almost 40% due to the initial drying process (Table 5.1). In the present study fat content in the Control treatment increased from 3.9% to 6.9% and in the 9% CSOCM treatment from 5.1% to 8.6% (Average values calculated). Bloukas & Paneras (1993) found that the greater the moisture loss when producing low fat frankfurters, the higher the concentration of the other constituents such as protein and fat. It was also found that when the fat content of frankfurters were reduced from 27% to 10% by using lean meat the moisture content increased from about 50-55% to about 70% (Bloukas et al., 1997a). The lower fat content for the Control treatment (Table 5.5) did however not present the increased moisture content as presented by Bloukas et al. (1997a). This finding may be attributed to the fact that the fat content of the ostrich cabanossi is in general much lower than processed sausages cited here. Ash percentage was higher ( $P<0.05$ ) for the 2% olive oil treatment in the 9% CSOCM group than the 1% olive oil

treatment in this same group (6.9 and 5.1%, respectively). This may also be explained by insufficient mixing of cabanossi ingredients resulting in some sausages containing a greater content of spices. Ash content of other processed sausages was found to be in the range of 2.5% to 3.2% (Fernandez et al., 2003; Bloukas et al., 1997a; Bloukas & Paneras, 1993) which is slightly lower than what was found in this study, but may be explained by the amount of additives in the form of spices and preservatives added to the respective sausage recipes.

The chemical composition of traditionally processed South African cabanossi has not been well documented and could therefore not be compared to the ostrich cabanossi produced in the current study. Dry sausages generally contain quite high levels of fat though (Ansorena & Astiasaran, 2004). Low fat frankfurters produced in several investigations (Pappa et al., 2000; Bloukas et al., 1997a; Bloukas & Paneras, 1993) was highly acceptable with 9% fat and 13% protein. According to Pappa et al. (2000), a fat reduced product will contain between 15 and 18% fat and a low fat product will be lower than 10% fat content. In this investigation, ostrich cabanossi resulted in a processed meat product containing 6.5 – 9.6% fat but with a much higher crude protein content of 33.6 – 36.3% (Table 5.5) rendering it a low fat processed meat product but with very high contents of protein.

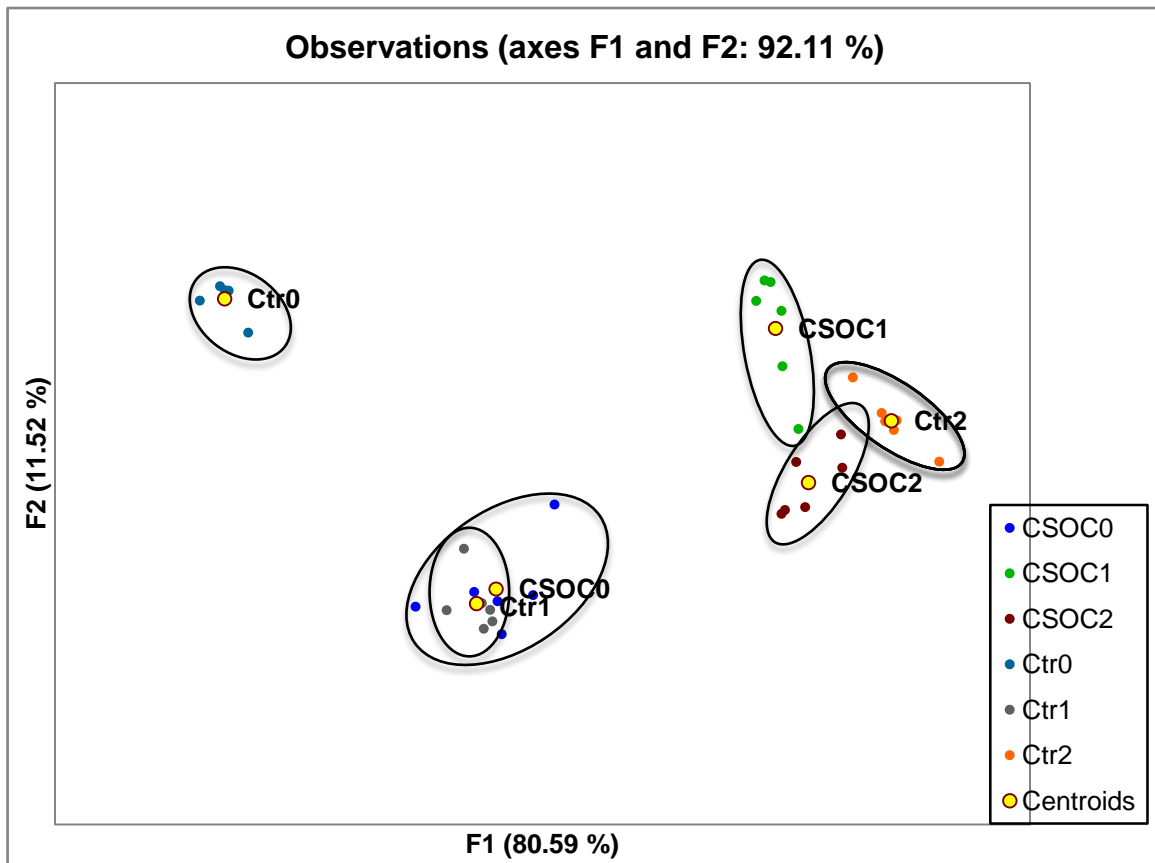
**Table 5.5** Means ( $\pm$  Standard deviation) of the proximate composition (%) of smoked and dried ostrich cabanossi with increasing levels of olive oil.

	Treatments						LSD
	Control			9% CSOCM <sup>1</sup>			
	0%	1%	2%	0%	1%	2%	
Moisture	50.9 <sup>a</sup> $\pm$ 2.33	50.5 <sup>a</sup> $\pm$ 1.60	50.5 <sup>a</sup> $\pm$ 1.66	50.0 <sup>a</sup> $\pm$ 1.58	50.0 <sup>a</sup> $\pm$ 1.58	50.0 <sup>a</sup> $\pm$ 0.72	1.94
Crude Protein	36.2 <sup>a</sup> $\pm$ 2.24	36.3 <sup>a</sup> $\pm$ 2.27	35.5 <sup>ab</sup> $\pm$ 1.10	35.4 <sup>ab</sup> $\pm$ 2.73	34.6 <sup>ab</sup> $\pm$ 0.81	33.6 <sup>b</sup> $\pm$ 2.03	2.34
Fat	6.6 <sup>b</sup> $\pm$ 1.96	6.5 <sup>b</sup> $\pm$ 1.68	7.7 <sup>ab</sup> $\pm$ 1.09	7.8 <sup>ab</sup> $\pm$ 2.65	8.4 <sup>ab</sup> $\pm$ 2.05	9.6 <sup>a</sup> $\pm$ 3.12	2.58
Ash	5.4 <sup>ab</sup> $\pm$ 0.39	6.2 <sup>ab</sup> $\pm$ 1.90	5.4 <sup>ab</sup> $\pm$ 0.16	5.5 <sup>ab</sup> $\pm$ 1.50	5.1 <sup>b</sup> $\pm$ 0.34	6.9 <sup>a</sup> $\pm$ 2.22	1.60

<sup>ab</sup>Rows with different letters differ significantly ( $P < 0.05$ ); <sup>1</sup> Cottonseed oilcake meal; \*Least significant difference

### 5.3.2 Fatty acid analysis

Table 5.6 presents the fatty acid composition of ostrich cabanossi formulated with three levels of olive oil. Figure 5.1 shows the difference in the fatty acid profiles of the six ostrich meat and olive oil treatments by means of the Discriminant Analysis (DA).



**Figure 5.1** Discriminant Analysis (DA) plot of the fatty acid data for the six treatments of ostrich and olive oil cabanossi. Ctr0 = Control with 0% olive oil, Ctr1 = Control with 1% olive oil and Ctr2 = Control with 2% olive oil, CSOC0 = 9% CSOCM with 0% olive oil, CSOC1 = 9% CSOCM with 1% olive oil and CSOC2 = 9% CSOCM with 2% olive oil.

The combination of the two components, F1 and F2 of the DA, explained 92.11% of the total variance of which F1 explained 80.59% of the total variance and F2 explained 11.52% of the total variance. The DA was used to analyse the differences between groups of data and to visualize the observations to see the relationship of one group in terms of another.

From the DA plot the differences in fatty acid profile between Ctr0 and Ctr1 and Ctr2 is visible as the centroid for Ctr0 is in an outlier position. Furthermore, CSOC0 is also removed from CSOC1 and CSOC2 indicating the difference in fatty acid profile between these treatments. The grouping of CSOC1, CSOC2 and Ctr2 indicates a close resemblance in fatty acid profile as is the grouping of CSOC0 and Ctr1.

Due to the low intramuscular fat content of ostrich meat it was expected that the inclusion of olive oil would drastically alter the fatty acid profile of the low fat ostrich cabanossi. Palmitic acid (C16:0) accounts for 19.1% of the total 20.2% of saturated fatty acids in the olive oil and

is also present in relatively high quantities in the warthog as well as the ostrich meat used here (Table 5.3). It was expected therefore that Palmitic acid (C16:0) would increase as the percentage olive oil increased, which was not found in this study (Table 5.6). A study on replacing beef fat with olive oil in producing Soudjouk, a popular dried sausage in turkey (Kayaard & Gök, 2003) also reported Palmitic acid (C16:0) to decrease as the olive oil percentage increased. Total SFA in the 2% olive oil inclusion treatments was significantly higher in the Control and 9% CSOCM treatments (17.5% and 22.3% respectively). The decrease of SFA in both treatment groups were therefore in the range of 19.9-21.4%. Low fat frankfurters produced with including grape seed, corn, canola, soybean or olive oil also presented decreases in total SFA in the range of 25-35% (Yun-Sang Choi et al., 2010).

The fatty acid most prevalent in olive oil is Oleic acid (C18:1n9c) at 78.1% of the total fatty acids present (Table 5.6). Similar values for Oleic acid in olive oil in several studies including olive oil as an animal fat replacement was noted (Rodriguez-Carpena, Morcuende & Estevez, 2011; Yun-Sang Choi et al., 2010; Ansorena & Astiasaran, 2004; Pappa et al., 2000). As expected there was a significant difference between the treatments with the 2% olive oil inclusion presenting the highest content of Oleic acid within the Control and 9% CSOCM groups (72.8% and 70.9%, respectively) confirming an increase in Oleic acid as olive oil inclusion increases in the ostrich cabanossi. These findings are in accordance with similar studies with olive oil replacing animal fat in processed meat products (Rodriguez-Carpena, Morcuende & Estevez, 2011; Yun-Sang Choi et al., 2010; Kayaard & Gök, 2003). In a study where avocado, sunflower and olive oil was used as a replacement for pork fat (Rodriguez-Carpena, Morcuende & Estevez, 2011), the vegetable oils were included at a 5% level, whereas in this study the highest inclusion level was 2%. The percentage of Oleic acid present in the burger patties containing olive oil was 76.83%, even lower than in the present study. The cabanossi recipe used in this study however did not include any additional water or ice whereas the burger patties contained 180g distilled water per kilogram of patty which could have had a diluting effect on the various constituents. Total mono unsaturated fatty acids (MUFA) presented similar results to the individual fatty acid Oleic acid, which is to be expected as it represents 50 to 60% of the total MUFA in all treatments. Total MUFA was significantly higher in the 2% olive oil treatment group for the Control (74.1%) as well as 9% CSOCM (71.9%) (Table 5.6). Within the Control treatment it increased from 48.9% to 74.1% and in the 9% CSOCM treatment from 45.1% to 71.9%. This is in accordance with Yun-Sang Choi (2010) that noted an increase in total MUFA from 37.0% to 64.6% when comparing grape seed, corn, canola, soybean and olive oil as fat replacements, with olive oil inclusion resulting in the greatest increase of total MUFA.

Of the polyunsaturated fatty acids (PUFA), Linoleic acid (C18:2n-6c) presented interesting results. In the olive oil there is no Linoleic acid (Table 5.6) but in the Control and 9% CSOCM ostrich meat used to produce the ostrich cabanossi Linoleic acid (C18:2n-6c) is present at 17.9% and 18.6% respectively, making up almost the total amount of PUFA (Table 5.3). Warthog meat used in the cabanossi also presents very low levels of Linoleic acid (0.3%; Table 5.3). In Table 5.6 it can be seen that the amount of Linoleic acid decreases as the amount of olive oil increases, with the Control 0% olive oil presenting the highest amount of Linoleic acid (8.4%) and the Control 2% olive oil and 9% CSOCM 2% olive oil presenting the lowest (2.7% and 2.6% respectively). Ostrich meat has a very low level of intramuscular fat (Sales & Horbanczuk, 1998). The increase of olive oil in the meat product therefore has a diluting effect on the fatty acids present in the ostrich and warthog meat, which results in a meat product gradually taking on the fatty acid profile of the olive oil, explaining the unexpected decrease in Linoleic acid in the finished product.

Total PUFA decreased most significantly in the 9% CSOCM with 2% olive oil and presented the highest concentration in the Control with 0% olive oil. This is to be expected as olive oil is not very rich in PUFA and is also in agreement with other studies (Rodriguez-Carpena, Morcuende & Estevez, 2011; Yun-Sang Choi et al., 2010; Ansorena & Astiasaran, 2004; Pappa et al., 2000). No significant differences were found for the ratio between polyunsaturated fatty acids and saturated fatty acids (PUFA:SFA). In terms of human health, the balance between PUFA and SFA and the content of n-3 PUFA are very important for their role in protection against cardiovascular and inflammatory diseases as well as brain health (Williams, 2000). For these reasons, the suggested n-6:n-3 and PUFA:SFA ratios should be less than 4 and above 0.4, respectively (Wood et al., 2004). In this study an acceptable ratio of PUFA to SFA was obtained in all treatment groups as presented in Table 5.6. With regards to n-6:n-3 ratio a value which greatly exceeded the recommended value was obtained. The reason for this can be ascribed to the fact that ostrich meat and other meats are generally a poor source of omega 3 fatty acids (Lawrie, 1998), but ostrich meat, as presented in Table 5.6 is quite rich in omega 6 fatty acids (42.4% of total fatty acids) and even though the olive oil presents a favourable n-6:n-3 ratio, the omega 3 fatty acids (C18:3n-3, C20:3n-3, C20:5n-3 (Eicosapentaenoic acid), C22:5n-3 (Docosapentaenoic acid) and C22:6n-3 (Docosahexaenoic acid) are present in too low concentrations to reduce the n-6:n-3 ratio effectively.

**Table 5.6** Means ( $\pm$  Standard deviation) of the fatty acid (FA) profile (% of total FA detected) of smoked and dried ostrich (meat from birds receiving 0 or 9% cottonseed oilcake meal (CSOCM)) cabanossi with increasing levels of olive oil.

Olive oil %	Olive oil %	Control			9% CSOC			LSD*
		0	1	2	0	1	2	
<i>Saturated Fatty Acids (SFA)</i>								
C14:0	0.0	0.6 <sup>ab</sup> $\pm$ 0.35	0.3 <sup>bc</sup> $\pm$ 0.05	0.3 <sup>c</sup> $\pm$ 0.10	0.7 <sup>a</sup> $\pm$ 0.63	0.4 <sup>abc</sup> $\pm$ 0.21	0.2 <sup>c</sup> $\pm$ 0.02	0.36
C15:0	0.0	0.1 <sup>a</sup> $\pm$ 0.07	0.1 <sup>b</sup> $\pm$ 0.01	0.1 <sup>b</sup> $\pm$ 0.02	0.1 <sup>a</sup> $\pm$ 0.11	0.1 <sup>ab</sup> $\pm$ 0.04	0.0 <sup>b</sup> $\pm$ 0.00	0.07
C16:0	19.1	29.1 <sup>ab</sup> $\pm$ 10.12	17.9 <sup>bc</sup> $\pm$ 12.36	13.2 <sup>c</sup> $\pm$ 8.84	32.5 <sup>a</sup> $\pm$ 12.19	20.7 <sup>abc</sup> $\pm$ 10.05	18.6 <sup>bc</sup> $\pm$ 11.42	12.86
C18:0	1.1	8.6 <sup>a</sup> $\pm$ 4.79	4.1 <sup>ab</sup> $\pm$ 0.94	3.9 <sup>ab</sup> $\pm$ 2.16	8.6 <sup>a</sup> $\pm$ 7.73	5.8 <sup>ab</sup> $\pm$ 3.17	3.4 <sup>b</sup> $\pm$ 0.40	4.78
C20:0	0.0	0.0 <sup>a</sup> $\pm$ 0.08	0.0 <sup>b</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.02	0.1 <sup>ab</sup> $\pm$ 0.06	0.1 <sup>b</sup> $\pm$ 0.04	0.0 <sup>b</sup> $\pm$ 0.01	0.05
C21:0	0.0	0.0 <sup>a</sup> $\pm$ 0.03	0.0 <sup>b</sup> $\pm$ 0.00	0.0 <sup>b</sup> $\pm$ 0.00	0.0 <sup>b</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.00	0.01
C22:0	0.0	0.2 <sup>a</sup> $\pm$ 0.12	0.1 <sup>b</sup> $\pm$ 0.02	0.1 <sup>b</sup> $\pm$ 0.02	0.1 <sup>ab</sup> $\pm$ 0.09	0.1 <sup>b</sup> $\pm$ 0.07	0.0 <sup>b</sup> $\pm$ 0.02	0.08
Total SFA	20.2	38.9 <sup>ab</sup> $\pm$ 11.00	22.4 <sup>c</sup> $\pm$ 11.98	17.5 <sup>c</sup> $\pm$ 9.50	42.2 <sup>a</sup> $\pm$ 17.6	27.1 <sup>bc</sup> $\pm$ 10.14	22.3 <sup>c</sup> $\pm$ 11.07	14.36
<i>Mono unsaturated Fatty Acids (MUFA)</i>								
C14:1	0.0	0.1 <sup>a</sup> $\pm$ 0.02	0.0 <sup>b</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.01	0.1 <sup>a</sup> $\pm$ 0.03	0.0 <sup>ab</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.01	0.02
C16:1	0.2	3.0 <sup>a</sup> $\pm$ 1.83	1.3 <sup>b</sup> $\pm$ 0.34	1.1 <sup>b</sup> $\pm$ 0.20	1.3 <sup>b</sup> $\pm$ 0.10	1.4 <sup>b</sup> $\pm$ 0.76	0.8 <sup>b</sup> $\pm$ 0.11	1.09
C18:1n9c	78.1	44.5 <sup>bc</sup> $\pm$ 13.69	67.7 <sup>a</sup> $\pm$ 10.53	72.8 <sup>a</sup> $\pm$ 13.22	43.4 <sup>c</sup> $\pm$ 18.86	60.9 <sup>ab</sup> $\pm$ 15.09	70.9 <sup>a</sup> $\pm$ 11.50	16.60
C18:1n9t	0.9	0.3 <sup>a</sup> $\pm$ 0.25	0.1 <sup>a</sup> $\pm$ 0.14	0.6 <sup>a</sup> $\pm$ 1.35	0.9 <sup>a</sup> $\pm$ 1.96	0.2 <sup>a</sup> $\pm$ 0.29	0.1 <sup>a</sup> $\pm$ 0.09	1.17
C20:1	0.1	0.9 <sup>a</sup> $\pm$ 2.10	0.0 <sup>a</sup> $\pm$ 0.01	0.0 <sup>a</sup> $\pm$ 0.03	0.1 <sup>a</sup> $\pm$ 0.04	0.1 <sup>a</sup> $\pm$ 0.03	0.0 <sup>a</sup> $\pm$ 0.02	1.01
C22:1n9	0.0	0.3 <sup>a</sup> $\pm$ 0.29	0.2 <sup>b</sup> $\pm$ 0.10	0.1 <sup>b</sup> $\pm$ 0.06	0.2 <sup>ab</sup> $\pm$ 0.09	0.2 <sup>ab</sup> $\pm$ 0.10	0.1 <sup>b</sup> $\pm$ 0.08	0.17
C24:1	0.0	0.1 <sup>a</sup> $\pm$ 0.06	0.0 <sup>b</sup> $\pm$ 0.01	0.0 <sup>b</sup> $\pm$ 0.01	0.1 <sup>ab</sup> $\pm$ 0.04	0.0 <sup>b</sup> $\pm$ 0.04	0.0 <sup>b</sup> $\pm$ 0.01	0.04
Total MUFA	79.35	48.9 <sup>bc</sup> $\pm$ 11.67	69.3 <sup>a</sup> $\pm$ 10.86	74.1 <sup>a</sup> $\pm$ 13.30	45.1 <sup>c</sup> $\pm$ 18.75	62.6 <sup>ab</sup> $\pm$ 14.66	71.9 <sup>a</sup> $\pm$ 11.62	16.2
<i>Poly unsaturated Fatty Acids (PUFA)</i>								
C18:2n-6c	0.0	8.4 <sup>a</sup> $\pm$ 5.29	4.1 <sup>ab</sup> $\pm$ 1.38	2.7 <sup>b</sup> $\pm$ 2.22	5.4 <sup>ab</sup> $\pm$ 4.93	4.1 <sup>ab</sup> $\pm$ 4.80	2.6 <sup>b</sup> $\pm$ 1.46	4.42
C18:2n-6t	0.1	0.1 $\pm$ 0.05	0.0 $\pm$ 0.01	0.0 $\pm$ 0.05	0.1 $\pm$ 0.16	0.0 $\pm$ 0.03	0.0 $\pm$ 0.02	0.09
C18:3n-6	0.2	2.4 $\pm$ 2.86	3.5 $\pm$ 0.62	4.6 $\pm$ 4.86	5.7 $\pm$ 4.77	5.3 $\pm$ 2.50	2.6 $\pm$ 0.69	3.78
C18:3n-3	0.1	0.2 <sup>a</sup> $\pm$ 0.12	0.1 <sup>b</sup> $\pm$ 0.03	0.1 <sup>b</sup> $\pm$ 0.03	0.1 <sup>ab</sup> $\pm$ 0.10	0.1 <sup>ab</sup> $\pm$ 0.07	0.1 <sup>b</sup> $\pm$ 0.01	0.08
C20:2	0.0	0.1 <sup>a</sup> $\pm$ 0.09	0.1 <sup>b</sup> $\pm$ 0.02	0.1 <sup>b</sup> $\pm$ 0.03	0.1 <sup>ab</sup> $\pm$ 0.08	0.1 <sup>ab</sup> $\pm$ 0.06	0.0 <sup>b</sup> $\pm$ 0.01	0.07
C20:3n-3	0.0	0.0 $\pm$ 0.02	0.0 $\pm$ 0.01	0.0 $\pm$ 0.01	0.0 $\pm$ 0.02	0.0 $\pm$ 0.01	0.0 $\pm$ 0.01	0.02
C20:4n-6	0.0	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.01
C20:5n-3	0.0	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.01	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.01
C22:2	0.0	0.0 $\pm$ 0.03	0.0 $\pm$ 0.01	0.0 $\pm$ 0.01	0.0 $\pm$ 0.02	0.0 $\pm$ 0.02	0.0 $\pm$ 0.00	0.02
C22:5n-3	0.0	0.0 $\pm$ 0.04	0.0 $\pm$ 0.04	0.0 $\pm$ 0.05	0.0 $\pm$ 0.04	0.1 $\pm$ 0.11	0.0 $\pm$ 0.04	0.07
C22:6n-3	0.0	0.2 <sup>a</sup> $\pm$ 0.21	0.1 <sup>ab</sup> $\pm$ 0.07	0.1 <sup>b</sup> $\pm$ 0.06	0.1 <sup>ab</sup> $\pm$ 0.05	0.1 <sup>ab</sup> $\pm$ 0.05	0.0 <sup>b</sup> $\pm$ 0.03	0.12
Total PUFA	0.4	11.6 <sup>a</sup> $\pm$ 3.46	7.9 <sup>ab</sup> $\pm$ 1.47	7.6 <sup>ab</sup> $\pm$ 3.78	11.6 <sup>a</sup> $\pm$ 4.18	9.8 <sup>ab</sup> $\pm$ 6.02	5.5 <sup>b</sup> $\pm$ 1.76	4.44
PUFA:SFA	0.02	0.3 $\pm$ 0.19	0.5 $\pm$ 0.22	0.5 $\pm$ 0.16	0.3 $\pm$ 0.17	0.4 $\pm$ 0.17	0.3 $\pm$ 0.20	0.22
n-6:n-3	2.83	29.7 $\pm$ 16.49	36.7 $\pm$ 14.49	33.3 $\pm$ 26.74	39.7 $\pm$ 18.31	31.2 $\pm$ 11.24	28.4 $\pm$ 12.86	20.56

Rows with different letters differed significantly (P&lt;0.05), \* Least significant difference

### 5.3.3 Sensory attributes

The means ( $\pm$  Standard deviation) for the sensory attributes of the ostrich cabanossi with three levels of olive oil added is presented in Table 5.7; definitions of the attributes and the scale used was presented in Table 5.4.

Although olive oil inclusion results in a meat product with a favourable fatty acid profile, adverse effects on sensorial characteristics are frequently noted, as the important functions of fat in dry sausages, such as flavour, texture, appearance and colour are limited by replacing (partially or in full) the fat component with vegetable oils (Bovolenta et al., 2008; Muguerza et al., 2001). Frankfurters made with olive oil was found to be chosen or refused on basis of their characteristic taste and soft texture (Luruena-Martinez, Vivar-Quintana & Revilla, 2004).

With regards to cooked meat, flavour and aroma remains two of the most important characteristics with regards to intent to purchase. Both these attributes are subjective characteristics generally judged by trained panellists and then further correlated to physical measurements of meat quality such as pH, colour and shear force (Polawska et al., 2011). For processed meat products however, visible appearance followed by flavour and texture, which will vary considerably between products and the origin of the product, will determine the general likeability of a product (Resurreccion, 2003). Muguerza et al. (2001) notes that the inclusion of olive oil in a processed meat product such as frankfurters, did not result in any changes with regards to textural properties. Olive oil as fat replacement did however affect the colour properties of low fat frankfurters, resulting in sausages with a darker red colour (Bloukas et al., 1997b). This is in agreement with the results presented in Table 5.7, where 9% CSOCM with 2% olive oil presented the highest cured red meat colour (73.4) in comparison to the Control with 0% and 1% olive oil (70.8 and 70.9, respectively).

Smoky aroma was significantly higher for the Control and 9% CSOCM with 0% olive oil (68.1). The lowest score for smoky aroma was presented by the 9% CSOCM with 2% olive oil (66.3). In Table 5.5 it is shown that the 9% CSOCM with 2% olive oil contained the highest amount of fat (9.6%). As fat content greatly affects aroma and flavour formation within any meat product (Melton, 1990), the higher amount of fat and presence of olive oil may have suppressed the smoky flavour in the 9% CSOCM with 2% olive oil treatment to a large extent. This also explains the more prominent smoky aroma in the Control and 9% CSOCM treatments with 0% olive oil that contained the least amount of fat in the finished product (Table 5.5). The low fat content in the Control with 0% olive oil could also be the cause of a firmer ( $P < 0.05$ ) cabanossi from this treatment as Muguerza et al. (2001) found that a reduction in fat content in frankfurter sausages resulted in firmer, less juicy sausages.

The incorporation of olive oil in its liquid form has been reported to produce a sausage that is softer (Bloukas et al., 1997a), which is in agreement with what was found here for the Control with 2% olive oil presenting a significantly lower firmness (32.8) compared to the Control with 0% olive oil (36.3). A reduction in fat content when producing processed meats has also been shown to increase the saltiness of the product (Pappa et al., 2000). This effect was also observed in the present study with the Control containing 0% and 1% olive oil having the lowest fat content (6.6% and 6.5% presented in Table 5.5) having significantly higher scores for saltiness (28.9).

Juiciness and tenderness were both significantly higher in the Control and 9% CSOCM with 2% olive oil. According to Hoffman et al. (2008), sensory tenderness is correlated to the amount of moisture or juiciness the panellist perceive during the first initial bites of the meat sample. Wood et al. (2008) noted that total lipid content plays an important role when evaluating the tenderness and more importantly the juiciness of meat products; a phenomenon evident here as there were no significant differences found for moisture content between treatments (Table 5.5) but distinct, significant differences with regards to the tenderness and juiciness as determined by a trained panel. Therefore the increase in tenderness and juiciness can be attributed to the higher fat content in the cabanossi processed from Control and 9% CSOCM ostrich meat with 2% olive oil.

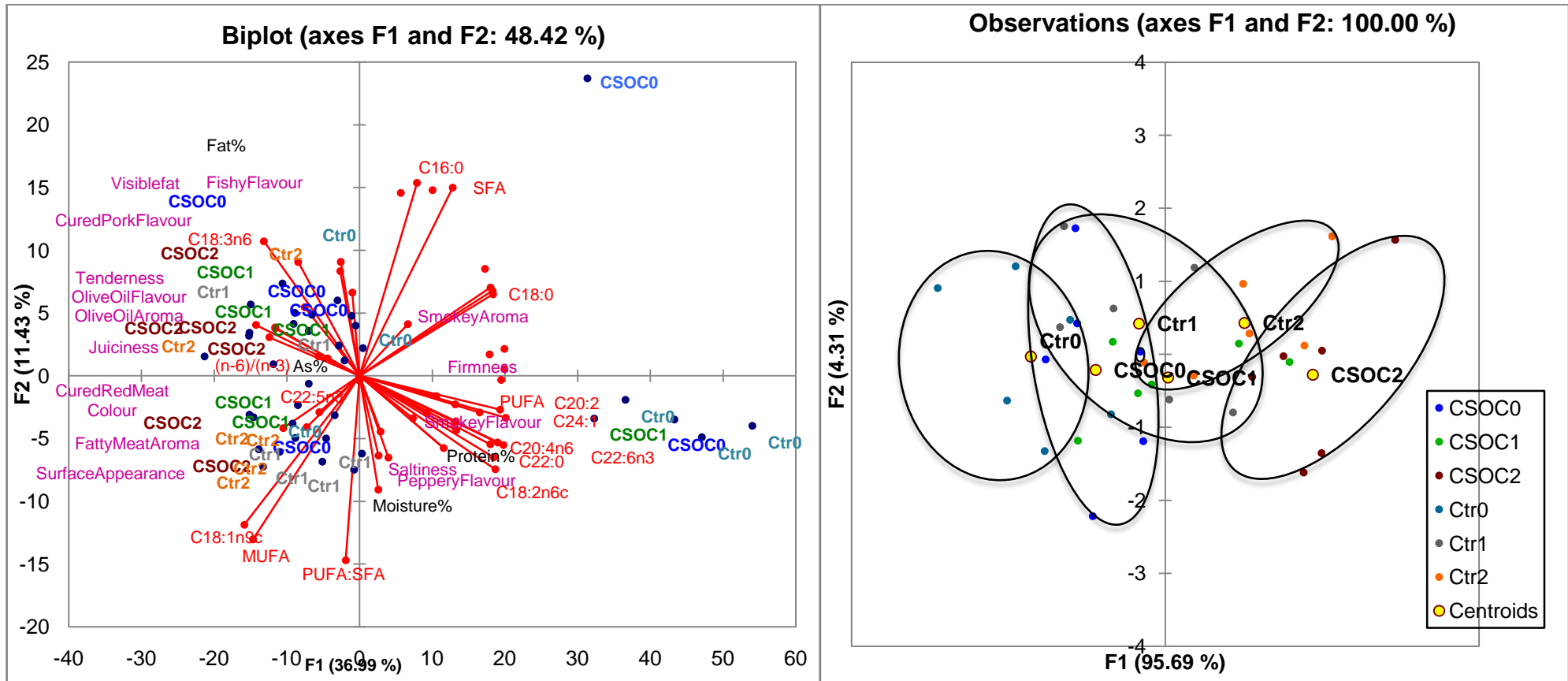
As expected, 9% CSOCM with 2% olive oil presented the highest ( $P < 0.05$ ) scores for olive oil aroma, olive oil flavour and fatty meat aroma (5.3, 10.5 and 14.8 respectively) (Table 5.7). This is substantiated by the evidence in Table 5.6 presenting 9% CSOCM with 2% olive oil with the highest content of Oleic acid (C18:1n9c), the main fatty acid present in olive oil. The olive oil inclusion was also found to be visibly present, as 9% CSOCM with 2% olive oil presented the highest scores for surface appearance, which according to the definition used (Table 5.3), is the presence of an oily substance on the outer surface of the cabanossi.



**Table 5.7** Means ( $\pm$  Standard deviation) of the sensory attributes of smoked and dried ostrich (meat from birds receiving 0 or 9% cottonseed oilcake meal (CSOCM)) cabanossi with increasing levels of olive oil.

Attributes	Treatments						LSD*
	Control			9% CSOCM			
	0%	1%	2%	0%	1%	2%	
Smoky aroma	68.1 <sup>a</sup> $\pm$ 5.14	67.2 <sup>ab</sup> $\pm$ 5.18	67.4 <sup>ab</sup> $\pm$ 5.24	68.1 <sup>a</sup> $\pm$ 4.72	67.2 <sup>ab</sup> $\pm$ 5.12	66.3 <sup>b</sup> $\pm$ 6.08	1.53
Olive oil aroma	2.9 <sup>c</sup> $\pm$ 4.35	4.2 <sup>b</sup> $\pm$ 4.94	4.1 <sup>b</sup> $\pm$ 4.62	3.9 <sup>bc</sup> $\pm$ 4.56	4.0 <sup>b</sup> $\pm$ 5.00	5.3 <sup>a</sup> $\pm$ 5.19	1.06
Fatty meat aroma	11.5 <sup>c</sup> $\pm$ 7.89	12.6 <sup>bc</sup> $\pm$ 7.03	13.5 <sup>ab</sup> $\pm$ 8.24	11.7 <sup>bc</sup> $\pm$ 7.70	11.2 <sup>c</sup> $\pm$ 7.16	14.8 <sup>a</sup> $\pm$ 7.07	1.90
Visible fat	8.8 $\pm$ 8.18	8.9 $\pm$ 5.05	10.1 $\pm$ 6.71	10.1 $\pm$ 6.56	9.1 $\pm$ 7.00	10.6 $\pm$ 5.61	2.12
Cured red meat colour	70.8 <sup>b</sup> $\pm$ 8.21	70.9 <sup>b</sup> $\pm$ 7.94	72.7 <sup>ab</sup> $\pm$ 6.67	71.4 <sup>ab</sup> $\pm$ 8.36	71.4 <sup>ab</sup> $\pm$ 8.68	73.4 <sup>a</sup> $\pm$ 6.54	2.13
Surface appearance	36.5 <sup>c</sup> $\pm$ 9.58	41.4 <sup>b</sup> $\pm$ 10.89	45.2 <sup>a</sup> $\pm$ 8.82	38.5 <sup>bc</sup> $\pm$ 10.54	40.8 <sup>b</sup> $\pm$ 10.37	46.3 <sup>a</sup> $\pm$ 10.06	3.38
Cured pork flavour	72.7 $\pm$ 6.98	72.7 $\pm$ 6.42	73.3 $\pm$ 6.54	73.9 $\pm$ 6.57	73.6 $\pm$ 6.42	72.7 $\pm$ 7.29	1.50
Game flavour	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.39	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.06
Fishy flavour	1.1 $\pm$ 3.74	0.9 $\pm$ 2.45	1.1 $\pm$ 2.92	0.9 $\pm$ 2.69	1.3 $\pm$ 3.08	1.2 $\pm$ 2.89	1.03
Smoky flavour	58.9 <sup>a</sup> $\pm$ 6.33	58.8 <sup>ab</sup> $\pm$ 6.44	57.4 <sup>ab</sup> $\pm$ 6.55	57.3 <sup>b</sup> $\pm$ 6.26	57.8 <sup>ab</sup> $\pm$ 5.8	57.9 <sup>ab</sup> $\pm$ 6.48	1.60
Saltiness	28.9 <sup>a</sup> $\pm$ 4.78	28.9 <sup>a</sup> $\pm$ 3.09	28.5 <sup>ab</sup> $\pm$ 5.44	27.8 <sup>b</sup> $\pm$ 5.04	27.7 <sup>b</sup> $\pm$ 5.20	28.1 <sup>ab</sup> $\pm$ 5.24	0.91
Peppery flavour	56.9 $\pm$ 16.08	57.6 $\pm$ 13.21	56.2 $\pm$ 14.82	55.9 $\pm$ 15.78	56.9 $\pm$ 15.81	56.1 $\pm$ 16.09	2.46
Olive oil flavour	6.1 <sup>c</sup> $\pm$ 8.17	7.3 <sup>bc</sup> $\pm$ 7.91	10.2 <sup>a</sup> $\pm$ 8.54	7.2 <sup>c</sup> $\pm$ 8.86	7.9 <sup>b</sup> $\pm$ 8.59	10.5 <sup>a</sup> $\pm$ 8.10	1.75
Tenderness	71.3 <sup>b</sup> $\pm$ 7.37	72.9 <sup>ab</sup> $\pm$ 7.52	75.2 <sup>a</sup> $\pm$ 6.17	71.8 <sup>b</sup> $\pm$ 7.56	71.2 <sup>b</sup> $\pm$ 7.32	74.5 <sup>a</sup> $\pm$ 6.62	2.40
Juiciness	59.5 <sup>b</sup> $\pm$ 10.20	60.8 <sup>ab</sup> $\pm$ 9.86	63.4 <sup>a</sup> $\pm$ 9.72	60.8 <sup>ab</sup> $\pm$ 9.82	60.9 <sup>ab</sup> $\pm$ 9.68	63.6 <sup>a</sup> $\pm$ 9.34	2.82
Firmness	36.3 <sup>a</sup> $\pm$ 9.19	34.8 <sup>abc</sup> $\pm$ 8.66	32.8 <sup>c</sup> $\pm$ 9.66	35.6 <sup>ab</sup> $\pm$ 8.98	35.6 <sup>ab</sup> $\pm$ 7.73	33.7 <sup>bc</sup> $\pm$ 9.75	2.32

Rows with different letters differed significantly ( $P < 0.05$ ); \* Least significant difference



**Figure 5.2 (a)** Principle component analysis bi-plot of the sensory attributes, proximate composition and fatty acids (for which there were differences ( $P < 0.05$ ) noted) of the six respective replications; **(b)** Discriminant analysis plot of the sensory attributes, proximate composition and fatty acid profile.

**Table 5.8** Correlations (r) and P values of relevant attributes for the six cabanossi treatments

	Olive oil aroma	Fatty meat aroma	Surface appearance	Saltiness	Olive oil flavour	Tenderness	Juiciness	C18:1n-9c <sup>a</sup>	MUFA <sup>b</sup>	Fat%
<b>Olive oil aroma</b>	1	0.392; 0.018	0.513; 0.001	0.109; 0.527	0.693; <0.0001	0.519; 0.001	0.613; <0.0001	0.397; <0.0001	0.359; <0.0001	0.348; <0.0001
<b>Fatty meat aroma</b>	0.392; 0.018	1	0.543; 0.001	-0.050; 0.773	0.482; 0.003	0.330; 0.049	0.460; 0.005	0.350; <0.0001	0.346; <0.0001	0.135; <0.0001
<b>Surface appearance</b>	0.513; 0.001	0.543; 0.001	1	0.005; 0.976	0.682; <0.0001	0.366; 0.028	0.447; 0.006	0.552; <0.0001	0.533; <0.0001	0.009; <0.0001
<b>Saltiness</b>	0.109; 0.527	-0.050; 0.773	0.005; 0.976	1	0.121; 0.481	0.133; 0.440	0.030; 0.860	0.092; <0.0001	0.110; <0.0001	-0.341; <0.0001
<b>Olive oil flavour</b>	0.693; <0.0001	0.482; 0.003	0.682; <0.0001	0.121; 0.481	1	0.661; <0.0001	0.753; <0.0001	0.354; <0.0001	0.319; <0.0001	0.252; <0.0001
<b>Tenderness</b>	0.519; 0.001	0.330; 0.049	0.366; 0.028	0.133; 0.440	0.661; <0.0001	1	0.817; <0.0001	0.431; <0.0001	0.394; <0.0001	0.507; <0.0001
<b>Juiciness</b>	0.613; <0.0001	0.460; 0.005	0.447; 0.006	0.030; 0.860	0.753; <0.0001	0.817; <0.0001	1	0.317; <0.0001	0.288; <0.0001	0.337; <0.0001
<b>C18:1n-9c</b>	0.397; <0.0001	0.350; <0.0001	0.552; <0.0001	0.092; <0.0001	0.354; <0.0001	0.431; <0.0001	0.317; <0.0001	1	0.995; <0.0001	0.220; <0.0001
<b>MUFA</b>	0.359; <0.0001	0.346; <0.0001	0.533; <0.0001	0.110; <0.0001	0.319; <0.0001	0.394; <0.0001	0.288; <0.0001	0.995; <0.0001	1	0.172; <0.0001
<b>Fat%</b>	0.348; <0.0001	0.135; <0.0001	0.009; <0.0001	-0.341; <0.0001	0.252; <0.0001	0.507; <0.0001	0.337; <0.0001	0.220; <0.0001	0.172; <0.0001	1

Values presented as correlation (r); P value, <sup>a</sup> Oleic acid, <sup>b</sup> Mono unsaturated fatty acids

### **5.3.4 Relationship between attributes and chemical composition**

A principal component analysis (PCA) bi-plot of the sensory, proximate and significant fatty acids is displayed in Fig. 5.2(a). The combination of the two components; Factor (F) 1 and F2 explained 48.42% of the total variance of which F1 explained 36.99% of the total variance and F2 explained 11.43% of the total variance. The PCA is concerned with explaining the (co)variance structure of the set of variables (meat attributes) through a few linear combinations of these variables. It demonstrates the presence of possible correlations amongst the attributes (Fig. 5.2a) whereas the correlation coefficients ( $r$ ) between attributes indicate the magnitude of these relationships (Table 5.8)

In Figure 5.2(b), the discriminant analysis (DA) presents the differences between the six treatments for sensory attributes, proximate composition and fatty acid profile. The combination of the two components of the DA; F1 and F2 explained 100.0% of the total variance of which F1 explained 95.69% of the total variance and F2 explained 4.31% of the total variance. The DA is used to analyse the differences between groups of data and to visualize the observations to see the relationship of one group in terms of another. As F1 explained a larger amount of the total variance in the PCA bi-plot, the layout of the plot will be investigated vertically as opposed to horizontally. The abbreviations on the PCA and DA plot were made for practical reasons and are defined as follows: Ctr0 = Control ostrich with 0% olive oil, Ctr1 = Control ostrich with 1% olive oil, Ctr2 = Control ostrich with 1% olive oil, CSOC0 = 9% CSOCM with 0% olive oil, CSOC1 = 9% CSOCM with 1% olive oil, CSOC2 = 9% CSOCM with 2% olive oil.

The treatments presented on the DA plot (Figure 5.2(b)) does not present such a clear indication of how the treatments differed, as they are all situated in the middle of the plot with no centroids presented as outliers. The treatments with 1% and 2% olive oil inclusion does however lie on the opposite side of F1 from the 0% olive oil treatments, indicating a significant difference as F1 explained 95.69% of the total variance.

As explained in the previous section, CSOC2 presented significantly higher scores for tenderness, juiciness, olive oil aroma and flavour as well as cured red meat colour and surface appearance which are reiterated by the PCA bi-plot (Figure 5.2 (a)). A strong correlation between olive oil flavour and olive oil aroma ( $r = 0.693$ ;  $P < 0.0001$ , Table 5.8) is visible. This further substantiates the results presented in Table 5.7, where the Control with 0% olive oil had the lowest olive oil aroma as well as olive oil flavour. Fat % presents correlations ( $P < 0.0001$ , Table 5.8) with several sensory attributes. A negative correlation with saltiness ( $r = -0.341$ ) and positive correlations with olive oil aroma ( $r = 0.348$ ), tenderness ( $r = 0.507$ ) and juiciness ( $r = 0.337$ ). All of these strong correlations support the

results with regards to the effect of fat content on saltiness (decreased fat content increases the saltiness of a meat product) and an increase in juiciness and tenderness as fat content increases (Table 5.6 and Table 5.7). Fatty meat aroma and surface appearance (presence of oily substance on surface) presents a strong correlation ( $r = 0.543$ ;  $P = 0.001$ , Table 5.8). The association of CSOC2 treatment with these attributes was further supported by the significantly higher mean scores (Table 5.7) of 14.8 and 46.3 received for fatty meat aroma and surface appearance respectively. The drying and smoking process, see Table 5.1, causes the olive oil to move towards the surface of the sausage creating this oily appearance on the surface and also enhances the fatty meat aroma.

A visible indication of the effect of Oleic acid (C18:1n9c) being the main fatty acid responsible for the total MUFA content in the ostrich cabanossi produced with olive oil is presented in the PCA bi-plot ( $r = 0.995$ ;  $P < 0.0001$ , Table 5.8). This specific fatty acid increased as olive oil inclusion increased (Table 5.6). As expected, SFA and PUFA are situated on opposite sides to the treatments containing olive oil as it was shown in Table 5.6 that concentrations of total SFA and PUFA decreased slightly as olive oil % increased.

#### **5.4 CONCLUSION**

The use of olive oil as a replacement for pork fat at an inclusion level of 1% and 2% in the production of ostrich cabanossi resulted in a value added ostrich meat product that satisfies the need of the modern day health conscious consumer.

Even though the olive oil resulted in an increase of percentage fat (oil) within the product, the range of 6.5 – 9.6% over all treatments still classifies it as a low-fat meat product. More importantly however, is the effect of olive oil on the fatty acid profile. Ostrich meat is already well known and favoured for its low levels of intramuscular fat (IMF), and high levels of MUFA and PUFA. It is this low IMF content however that causes ostrich meat to easily adopt the fatty acid profile of different ingredients added during the production of value added ostrich meat, due to a diluting effect. Therefore, the addition of olive oil, a highly MUFA vegetable oil with almost no PUFA, resulted in cabanossi with increasing levels of MUFA and decreasing amounts of PUFA and SFA as olive oil inclusion increased. An increase in MUFA within a processed meat product is favourable as higher levels of MUFA has proven to decrease incidences of coronary heart disease (Bloukas and Paneras, 1993) as well as having a protective effect against low density lipoproteins (LDL) oxidation and oxidative stress in humans (Ansorena and Astiasaran, 2004). From a technical perspective however, an increase in MUFA rather than PUFA is beneficial with regards to risks of rancidity due to lipid oxidation as PUFA, especially the longer chain PUFA, are more susceptible to oxidation which reduces the shelf life of a meat product. The inclusion of olive oil at 2% resulted in

cabanossi with increased tenderness and juiciness, two factors deemed as most important from a consumer's perspective. An ostrich cabanossi where olive oil replaces pork fat therefore seems to be a viable option to increase the variety of value added ostrich meat products available to the consumer.

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## CHAPTER 6

### GENERAL CONCLUSION

Cottonseed oilcake meal (CSOCM) was investigated as an alternative, less expensive protein source in rearing ostriches to a slaughter age of 13 months. Cottonseed oilcake meal was included at 0%, 3%, 6%, 9% and 12% of the total diet. Even though Aganga et al. (2003) stated that cottonseed or cottonseed meal should not be allowed in ostrich feed rations due to the presence of gossypol, no studies have been conducted to determine the viability of cottonseed products in ostrich nutrition. Production performance as well as the effect on meat quality of the *Iliofibularis* muscle (fan fillet) was also investigated. Partially replacing soybean oilcake meal with increasing levels of CSOCM was observed to improve live performances of growing ostriches from a 9% CSOCM dietary inclusion level. Ostrich meat is favoured by consumers as a result of its leanness and favourable fatty acid profile and results presented here showed that cottonseed oilcake meal (up to 12% in the diet) as a dietary protein source did not influence the proximate composition, mineral content, or fatty acid profile of ostrich fan fillet. Considering the overall results, CSOCM seems to be a viable and cheaper protein source in ostrich nutrition.

A descriptive sensory analysis was performed to determine whether the dietary manipulation of the fatty acid profile of *Iliofibularis* muscle as a result of feeding CSOCM could be detected. Cooked fan fillet from ostriches receiving 9% CSOCM presented a more intense beef aroma with no differences in flavour attributes when compared to the Control (0% CSOCM) treatment. The lack of differences in flavour between treatments may be explained by the low intramuscular fat content of ostrich meat (6.8% and 6.0% the Control and 9% CSOCM respectively) and lack of differences found for the fatty acid profile between treatments. Over the years, the relationship between fatty acid profile and flavour formation in meat has been reported and emphasized by several authors (Wood et al., 1999, Lawrie, 1998, Wood et al., 2004) and the variation in fatty acid composition has immense effects on meat quality (Wood et al., 2008). However, tenderness and juiciness was favourably influenced by the inclusion of CSOCM in the diet. Very little information is available with regards to the fatty acid composition in cooked ostrich meat. Further research on this subject is necessary as ostrich meat is primarily consumed as a cooked product and information on the nutritional value thereof is of importance to dieticians.

The current ban on the export of South African ostrich meat to the European countries due to an on-going outbreak of *Avian influenza* creates a surplus of ostrich meat available in South Africa. Consequently a demand arises to develop a greater range of processed and

value added ostrich meat products. Cabanossi, a semi dry sausage was produced using the fan fillet (*Iliofibularis* muscle) from the Control and 9% CSOCM treatment. Olive oil was added as a replacement for animal fat at three inclusion levels (0%, 1% and 2%) to determine whether olive oil can be used as a replacement for animal fat in the production of a “healthy” value added ostrich meat product. Olive oil has a high content of mono unsaturated fatty acids (MUFA) and ostrich meat presents higher quantities of polyunsaturated fatty acids (PUFA). Due to the low intramuscular fat content of ostrich meat a diluting effect of the fatty acids present in ostrich meat is observed, causing the processed ostrich meat product to adopt the fatty acid profile of the olive oil (or fat source) as well as other ingredients in the recipe. The result was an ostrich cabanossi with increasing levels of MUFA and decreasing amounts of PUFA and SFA as olive oil inclusion increased. From a technical perspective however, an increase in MUFA rather than PUFA is beneficial with regards to risks of rancidity due to lipid oxidation. The inclusion of olive oil at 2% resulted in cabanossi with increased tenderness and juiciness, two factors deemed as most important from a consumer’s perspective. Even though the olive oil resulted in an increase of percentage fat (oil) within the product, the range of 6.5 – 9.6% fat over all treatments still classifies it as a low-fat meat product.

It can be concluded that the inclusion of cottonseed oilcake meal as a source of protein in the diet of growing ostriches is a viable option to replace soybean oilcake meal partially or in full. The addition of CSOCM at 9% or 12% of the total diet resulted in acceptable production performance as well as meat quality. Cabanossi produced from the *Iliofibularis* muscle (fan fillet) of these birds fed CSOCM and added olive oil as a replacement for animal fat in the processing of ostrich cabbanossi is a valuable addition to the value added ostrich meat products currently being manufactured.

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## ANNEXURE 1

**INGREDIENTS AND CHEMICAL COMPOSITION OF OSTRICH FINISHER DIETS IN WHICH SOYBEAN OILCAKE MEAL (SOCM) WAS GRADUALLY REPLACED BY COTTONSEED OILCAKE MEAL (CSOCM)**

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	(0% CSOCM)	(3% CSOCM)	(6% CSOCM)	(9% CSOCM)	(12% CSOCM)
<b>Ingredients</b>					
Alfalfa meal (%)	45.38	41.66	37.93	34.21	30.48
Yellow maize (%)	37.83	37.83	37.83	37.83	37.83
CSOCM (%)	0	3.00	6.00	9.00	12.00
Wheaten bran (%)	0	2.96	5.91	8.87	11.83
Soybean oilcake meal	10.00	7.50	5.00	2.50	0
Molasses powder (%)	2.50	2.50	2.50	2.50	2.50
Limestone, ground (%)	1.63	1.69	1.74	1.80	1.85
MCP (%)	1.05	1.24	1.42	1.61	1.79
Fine salt	1.00	1.00	1.00	1.00	1.00
Vitamin&mineral Premix	0.50	0.50	0.50	0.50	0.50
DL-methionine 40%	0.10	0.10	0.09	0.08	0.08
L-lysine 95%	0	0.03	0.06	0.10	0.13
<b>Chemical Composition (calculated)</b>					
<i>Metabolisable Energy</i>	11.32	11.32	11.32	11.32	11.32
<i>Crude protein (%)</i>	15.45	15.44	15.42	15.41	15.40
<i>Crude fat (%)</i>	2.65	2.74	2.84	2.93	3.02
<i>Linoleic Acid (%)</i>	1.09	1.18	1.28	1.38	1.47
<i>Crude fibre (%)</i>	12.47	12.08	11.70	11.31	10.92
<i>Calcium (%)</i>	1.50	1.50	1.50	1.50	1.50
<i>Phosphorus total (%)</i>	0.49	0.57	0.65	0.73	0.81
<i>Phosphorus available (%)</i>	0.38	0.41	0.44	0.47	0.50
<i>Arginine (%)</i>	0.77	0.83	0.88	0.94	1.00
<i>Isoleucine (%)</i>	0.61	0.59	0.57	0.55	0.53
<i>Lysine (%)</i>	0.70	0.70	0.69	0.69	0.68
<i>Methionine+Cystine (%)</i>	0.50	0.50	0.50	0.50	0.50

