The effect of a fat replacer on the physical, chemical, microbiological and sensory characteristics of blesbok (*Damaliscus pygargus phillipsi*) cabanossi

by
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Declaration

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Notes

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Abstract

Processed meat products usually have a high fat content and health conscious consumers tend to find this unacceptable. Traditional processed meat products, such as cabanossi, are furthermore produced with animal fat that contain high levels of saturated fatty acids (SFA). A diet high in SFA may pose health risks. There are vegetable oils that could provide a better fatty acid profile in meat products and could be used as fat replacers. Unfortunately, reformulation may affect the processed meat product's characteristics and this could easily decrease a product's market viability. This study was conducted to investigate the effect of a canola oil-based fat replacer in the form of a protein-based hydrocolloid gel (*FR*) at three concentrations, i.e. 10% (*FR1*), 20% (*FR2*) and 30% (*FR3*) with no pork back fat added, compared to the *Control* containing pork back fat on the physical, chemical, microbiological and sensory characteristics of blesbok (*Damaliscus pygargus phillipsi*) cabanossi.

The proximate analysis of all three *FR* treatments had higher ($P \leq 0.05$) moisture, protein and ash content than the *Control* and as expected, the fat content was lower ($P \leq 0.05$) in all three *FR* treatments. The lipid oxidation results were lower than expected with no difference ($P > 0.05$) between the *Control* and all three *FR* treatments; possibly due to the nitrates' antioxidant ability. The fatty acid composition did not differ ($P > 0.05$) at day 0 as well as after 60 days storage, however, the fatty acid composition for the treatments at day 0 and day 60 differed ($P \leq 0.05$). At day 0 and day 60, *FR2* and *FR3* had larger ($P \leq 0.05$) PUFA:SFA ratios (0.8-1.0) than the *Control* and *FR1*. Furthermore at day 0 and day 60, all three *FR* treatments had lower ($P \leq 0.05$) n-6:n-3 ratios (2.8-3.1) than the *Control*.

Descriptive sensory analysis was performed alongside an instrumental texture analysis to profile any changes in the cabanossi's characteristics (aroma, appearance, flavour and texture). The trained panel detected differences in all the characteristics between the *Control* and the three *FR* treatments, as well as differences between the three *FR* treatments. An unexpected bitter taste developed after 60 days storage, maintained at 4°C; however, there was no sign of rancidity development, as perceived by the sensory panel. In terms of physical characteristics at day 0 and day 60, the *Control* and *FR1* differed ($P \leq 0.05$) from *FR2* and *FR3* with the latter scoring the lowest ($P \leq 0.05$) in instrumental hardness. Microbiological quality must be in accordance with the country's legislation. Testing for *Staphylococcus aureus, Listeria monocytogenes, Salmonella* spp., coliforms and aerobic forming bacteria was conducted. At day 0 and after 60 days storage, maintained at 4°C; the *Control* and all three *FR* treatments were in accordance with various regulations and food safety guidelines from South Africa and several international specifications.

The fat content was successfully decreased by ~20% with an improved fatty acid profile and a limited lipid oxidation. The sensory and physical properties were easily discernible between the

Control and the three *FR* treatments. The animal fat used in the control had certain characteristics which the fat replacer was not able to mimic. In addition, during storage (60 days) a bitter taste developed in the three *FR* treatments which were undesirable. All the treatments were microbiologically safe and were in accordance with local, South Africa, and international regulations. The *FR* treatments were noticeably different to the *Control* however, a lower fat content, improved fatty acid profile and a 60 day shelf-life were achieved. The results have added more knowledge about fat replacers in processed meat products especially with regard to cabanossi.

Opsomming

Geprosesseerde vleisprodukte is bekend vir ‘n hoë vetinhoud en word daarom gereeld deur gesondheidsbewuste verbruikers as onaanvaarbaar beskou. Vleisprodukte soos cabanossi word tradisioneel van diervet gemaak en bevat dus ‘n hoë vlak versadigde vet (SFA). Aangesien ‘n dieet hoog in versadigde vet mag lei tot gesondheidsimplikasies kan sekere plantolies gebruik word as vetvervanger, om sodoende ‘n meer gewenste vetsuurprofiel te verseker. Herformulasie van so ‘n aard mag egter verandering in die eienskappe van die produk teweegbring en dus ook die haalbaarheid daarvan beïnvloed. Hierdie studie is uitgevoer om die effek van 'n vetvervanger met ’n kanola-olie basis in die vorm van 'n proteïen-gebaseerde hidrokolloïde jel (*FR*) te ondersoek op die fisiese, chemiese, mikrobiologiese en sensoriese eienskappe van blesbok (*Damaliscus pygargus Phillipsi*) cabanossi. Die eksperimentele monsters het bestaan uit by drie konsentrasies *FR*, nl. 10% (*FR1*), 20% (*FR2*) en 30% (*FR3*), en geen varkvet, terwyl die *Kontrole monster* varkvet en derhalwe geen *FR* bevat het nie.

In die eerste studie (Hoofstuk 3) is proksimale analise, lipied oksidasie en die vetsuursamestelling bepaal. Die drie *FR* behandelings het hoër ($P \leq 0.05$) vog, proteïen en asinhoud as die *Kontrole* gehad en, soos verwag, was hul vetinhoud ook laer ($P \leq 0.05$). Die lipied oksidasie resultate was laer as wat verwag is met geen verskil ($P > 0.05$) tussen die *Kontrole* en die drie *FR* behandelings nie; moontlik as gevolg van die bygevoegde nitrate se vermoë om as anti-oksidant op te tree. Die vetsuursamestelling per behandeling het nie verskil ($P > 0.05$) op dag 0 en na 60 dae by 4°C nie, maar dit het wel op dag 0 en dag 60 tussen die *Kontrole* en die drie *FR* behandelings verskil ($P \leq 0.05$). Vetsuurverhoudings, veral die omega-6:omega-3 (n-6:n-3) verhouding, is ’n aanduiding van die voedingswaarde van dieetvet. Op dag 0 en dag 60 het *FR2* en *FR3* groter ($P \leq 0.05$) POVS:VVS verhoudings (0.8-1.0) as die *Kontrole* en *FR1* gehad. Verder, op dag 0 en dag 60 het al drie *FR* behandelings laer ($P \leq 0.05$) n-6:n-3 verhoudings (2.8-3.1) as die *Kontrole* gehad.

Die fisiese en sensoriese eienskappe is belangrik vir die sukses van ’n produk. In die tweede eksperimentele studie (Hoofstuk 4) is ’n beskrywende sensoriese analise saam met 'n instrumentele tekstuur-analise uitgevoer. Die opgeleide paneel het betekenisvolle verskille tussen die *Kontrole* en die drie *FR* behandelings opgetel, asook verskille tussen die drie *FR* behandelings. Daar het ’n onverwagse “Bitter smaak” na 60 dae se berging by 4°C ontwikkel, alhoewel die sensoriese paneel geen teken van galsterigheid waargeneem het nie. In terme van fisiese eienskappe op dag 0 en dag 60, het die *Kontrole* en *FR1* verskil ($P \leq 0.05$) van *FR2* en *FR3*, waar laasgenoemde die laagste ($P \leq 0.05$) vir instrumentele hardheid (Inst. Hardheid) gehad het.

Alhoewel die vet-vervangde cabanossi gesond moet wees en oor ’n wenslike sensoriese profiel beskik, moet die mikrobiologiese gehalte ook in ooreenstemming met Suid-Afrika se wetgewing

wees. In die derde studie (Hoofstuk 5) was ’n mikrobiologiese analise uitgevoer om te toets vir *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., Kolivorme en aërobiese vormende bakterieë. Op dag 0 en na 60 dae se berging by 4°C was die *Kontrole* en al drie *FR* behandelings in ooreenstemming met verskillende Suid-Afrikaanse regulasies en hul riglyne vir voedselveiligheid, asook verskeie internasionale spesifikasies. Daarom kan die produk geag word as veilig om te eet wanneer dit vir 60 dae by 4°C geberg word.

Vanuit hierdie resultate kan dit afgelei word dat die vet van blesbok (*Damaliscus pygargus Phillipsi*) cabanossi met 'n proteïen-hidrokolloïde jel, wat kanola-olie bevat, suksesvol vervang kan word. Dit het gunstige proksimale resultate, 'n verlaagde vetinhoud en is mikrobiologies veilig vir 'n bergingstydperk van 60 dae by 4°C.

Die studie het getoon dat die vetinhoud van geprosesseerde cabanossi suksesvol verlaag kan word met ~20%. Tesame hiermee het die vetvervanging ook gelei tot ‘n meer onversadigde vetsuur profiel met ‘n beperkte hoeveelheid lipied oksidasie wat plaasgevind het. Die verskille tussen die sensoriese en fisiese eienskappe van die kontrole en die FR behandelings was duidelik opletbaar. Die gebruik van diervet in die kontrole produk is verantwoordelik vir sekere eienskappe wat die vetvervanger nie kon naboots nie. Verder het ‘n ongewensde, bitter smaak ontwikkel in die FR behandelings gedurende die stoor tydperk. Alle behandelings was mikrobiologies veilig en in akkoord met die Suid Afrikaanse asook internasionale regulasies. Die resultate van die studie dra by tot ‘n beter begrip van die gebruik van vetvervangers in geprosesseerde vleisprodukte, veral met betrekking tot cabanossi.

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List of abbreviations

%	Percent
ºC	Degrees Celsius
a_w	Water activity
ANOVA	Analysis of variance
CFU	Colony forming units
CVD	Cardiovascular disease
EU	European Union
FR	Fat replacer
FR1	Fat replacer treatment 1
FR2	Fat replacer treatment 2
FR3	Fat replacer treatment 3
FUFOSE	Functional Food Science in Europe
g	Gram
HC	Health Canada
“Inst. Hardness”	Instrumental hardness
“Inst. Gumminess”	Instrumental gumminess
“Inst. Cohesiveness”	Instrumental cohesiveness
kg	Kilogram
LDL	Low density lipoproteins
MDA	Malondialdehyde
mg	Milligram
mL	Millilitre
nm	Nanometre
n-3	Omega 3
n-6	Omega 6
MUFA	Mono-unsaturated fatty acid
PAH	Polycyclic aromatic hydrocarbons
PF	Pork back fat
PUFA	Poly-unsaturated fatty acid
RTE	Ready-to-eat
SFA	Saturated fatty acid
SE	Standard error

Chapter 1

Introduction

Meat has been a staple dietary requirement for centuries and will continue to be an important food group. Processed meat products have been designed to provide consumers with ready-to-eat (RTE) products that are convenient and financially accessible (Clonan *et al*., 2015). Various institutions all around the world have stipulated that for the prevention of lifestyle diseases such as obesity, diabetes and cardiovascular diseases (CVD), individuals need to reduce their daily intake of processed meat products with high fat content. Cabanossi has a high fat content (25-30%) which may have become undesirable to the consumer, especially when keeping in mind that the daily intake of fat for an individual's diet should be between 20-35%, i.e. according to the USA's dietary guidelines (Smolin & Grosvenor, 2003)**.** In certain countries, Denmark, a fat tax on the saturated fatty acid (SFA) content of foods that have been deemed unhealthy such as meat and meat products have been implemented to promote healthier living (Jensen *et al*., 2015). Although the fat tax policies have been reviewed and repealed, the issue surrounding high fat content meat products is still a growing concern. This will prompt manufacturers in investigating the use of substitute ingredients for animal fat with lower fat content, as well as fat alternatives.

South Africa has a growing game industry and as consumers move towards the consumption of leaner meats, the game industry will keep growing in popularity. Game meat has a lower fat content than pork meat (Aidoo & Haworth, 1995; Hoffman *et al.*, 2010). In addition, there is a tendency for pork fat to be replaced in processed meat products, completely and partially, with combinations of vegetable oil, hydrocolloids as well as plant, animal and dairy proteins (Hoffman & Mellet, 2003; Fernández-Ginés *et al*., 2005; Egbert & Payne, 2009; Xiong, 2009; Utrilla *et al*., 2014; Reddy *et al*., 2015). However, replacing the animal fat can be detrimental to a processed meat product as mimicking of animal fat can be very difficult. The concern when using fat replacers is the negative effect it could have on the original products properties, furthermore the use of an incorrect fat replacer can have a negative impact on the overall quality of the meat product (Brewer, 2012). There are numerous processed meat products with fat replacers, however, the importance of choosing a replacer correctly has been documented, e.g. the addition of an emulsified and non-emulsified vegetable oil to a processed meat product, chorizo, negatively affected the texture with the chorizo having a softer texture with the emulsified vegetable oil and a harder texture with the non-emulsified vegetable oil (Beriain *et al*., 2011).

Fat replacers have been used in a variety of processed meat products ranging from dry fermented to cooked emulsified sausages however, there is limited literature on the use of a fat

replacer on cabanossi, a cooked smoked semi-dry sausage. Generally, cabanossi is made from a combination of beef and pork but in order to reduce the fat content of the beef and pork, the meat can be substituted with game meat. However, with replacing fat, essential fatty acids need to be reintroduced; this can be done with the addition of vegetable oil (Arntfield, 2011). Canola oil is readily available and inexpensive, furthermore, it has a favourable fatty acid composition consisting of high levels of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) alongside good PUFA:SFA ratios and omega-6 and omega-3 ratios (Hu, 2003; Ganesan *et al*., 2014). Although adding a fat replacer with a favourable fatty acid composition sounds enticing, lipid oxidation can occur and steps need to be put in place in order to prevent off-flavours developing (Ganesan *et al*., 2014).

In addition, to preventing off-flavour formation descriptive sensory analysis in conjunction with an instrumental texture analysis can be performed to profile the attributes of the new fat replaced product. The purpose of these profiling tests is to see similarities as well as differences amongst treatments and how it impacts on the product's sensory profile. New products are developed around the world every day, however, altering products ingredients can have a major influence on the microbiological quality. The main pathogenic microorganisms in ready-to-eat (RTE) products are *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Escherichia coli* and *Salmonella* spp. (Yu *et al*., 2016). Due to microbes various growing conditions, manufacturers use a technique called hurdle technology or combination preservation techniques which incorporate several microbe limiting parameters (Malik & Sharma, 2010).

In view of the above, the fat content of processed meat products needs to be significantly lowered. Due to the effect a reduction of animal fat and addition of a fat replacer could potentially have on the characteristics of a processed meat product such as cabanossi, various analyses need to be conducted to profile the change in quality, if any. A canola oil-based fat replacer in the form of a protein-based hydrocolloid gel has the appearance and texture mimicking properties of animal fat. The aim of this study was to use this fat replacer and to build a chemical, physical, sensory and microbiological profile for game cabanossi. The chemical analysis will provide nutritional background for the product. The microbiological analysis was done to assess if the product has a shelf-life of 60 days with the physical and sensory analysis providing insight on the aroma, flavour, appearance and texture changes.

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Chapter 2

Literature Review

2.1 Introduction

Consumers around the world wish to purchase healthier alternatives to the popular meat products sold by retailers. Due to the many processed products available on the market, it is becoming easier to consume more fat than required per day. The daily intake of fat for an individual's diet should be between 20-35% according to the US Department of Health (Smolin & Grosvenor, 2003; Institute of Medicine, Food & Nutrition Board, 2002). The game industry in South Africa is growing and as a result the availability of lean meat in increasing (Hoffman *et al.,* 2010). This prompted this study, i.e. to produce a popular processed meat product such as *cabanossi* but to substantially reduce the fat content therein. The use of a fat replacer in combination with canola oil, could open up the possibility of developing a "functional food". The accepted definition for a functional food is as follows: "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. The functional food is consumed as part of a normal diet and is not regarded as a pill, a capsule or any form of dietary supplement." (Diplock *et al*., 1999; European Commission, 2010; Grasso *et al.*, 2014). The product produced would substantially reduce the saturated fat and cholesterol content which will help prevent heart disease. Processed meat products are known to have a high fat content, however; salami's have one of the highest fat content (30-50%) (Jiménez-Colmenero *et al.*, 2001).

Canola is an edible vegetable oil that belongs to the *Brassicaceae* family which forms part of the rapeseed group (Weiss *et al.*, 2010). It has a high concentration of mono-unsaturated fatty acids (MUFA), the most abundant being oleic acid. Canola oil is a readily available vegetable oil that can be obtained from most local retailers. Vegetable oils such as olive oil have been used in various processed meat products to lower or reduce the fat content (Ferandez-Gines *et al*., 2005), however, the addition of canola oil into a processed meat product is relatively unique and unexplored.

The demand for foods with a significantly reduced fat content is increasing and this has led to a trend where manufacturers are reformulating so-called "unhealthy" food items. This is an attempt to change the preconceptions associated with the product and make it enticing for health conscious consumers (Ferandez-Gines *et al*., 2005). The meat industry is one market that has started reducing the fat content and reformulating new low fat and reduced fat processed meat products (Vasconcellos, 2001). Research into the reduction or replacement of fat is expanding and will increase over the

coming years as health and nutrition become more important. Fat replacement is an exciting and challenging possibility for the meat industry. The combination of pork collagen and alginate has not been researched as a possible fat replacer in a cooked semi-dry processed meat product with the added health benefit of canola oil.

2.2 Fat replacers used in different processed meat products

Animal fat has been used in dry fermented sausages as it provides flavour and juiciness. Fat has contributed to the functionality and success of processed meat products. Animal fat adds flavour, texture and most notably the characteristic appearance, visible fat, of some processed meat products. For example, in traditionally made processed meat products, pork back fat is used even though it contains a high content of saturated fatty acids (SFA) and cholesterol, it provides unique characteristics which are difficult to mimic or replace (Krauss *et al.*, 2000; Del Nobile *et al*., 2009). According to Grasso *et al*. (2014), the link between diet and health has come to the modern consumers' attention; the implication being that they are looking to purchase products that provide additional benefits, making them healthy and nutritious.

An article in the South African Food Review (2014) stated that the South African Department of Health (DOH) have decided to propose changes to the food nutrition labels in South Africa, with an emphasis on the nutrients of the product and the link to chronic diseases such as obesity, diabetes and heart disease. One of the changes that have been highlighted was the clear labelling of the saturated fatty acids (SFA), cholesterol and trans fatty acid content. Smolin & Grosvenor (2003) found that cholesterol is produced by the liver which means that it is not as important in human's diets as was previously thought. Thus, the proposed change put forward by the South African Department of Health indicates the seriousness and concern that they are placing on fat content of products. Table 2.1 provides a list of several popular processed meat products, with their fat content, that consumers purchase. Jiménez-Colmenero (2000), Jiménez-Colmenero *et al*. (2001) and Keeton (1994) stated that due to consumer demand for low fat/reduced fat meat products, the meat industry has had to make changes and modify the composition of the processed meat products.

Table 2.1 Several meat products and their corresponding fat contents (Jiménez-Colmenero, 2000)

Meat Product	Fat content (%)
Frankfurters	20-30
Bologna	20-30
Fresh pork sausage	30-50
Nugget	20-25
Salami	30-50
Beef patty	20-30
Ham	<10

The reality is that the fat content of processed meat products need to be altered. The regulatory bodies in South Africa, as well as the consumers have applied pressure on manufacturers to produce low fat processed meat products. Many advances have been made in reducing the fat content in processed meat products, however, industry need to reformulate these products to make it acceptable to the consumer. Brewer (2012) noted that when replacing or reducing fat; the quality of the meat product needs to be retained so that it still remains acceptable to the consumer. Therefore, the alteration of the product should not have an impact on the sensory, nutritional and functional properties of the product.

There are many strategies that can be taken to reduce the fat content of processed meat products. Jiménez-Colmenero *et al.* (2001) suggested that there are two main approaches that can be taken when making low-fat processed meat products. The first is to use leaner meat in the product, however, this approach could be expensive. The second is by adding water, in this way there is little to no contribution towards the kilojoule (kJ) content. Fat adds to the overall kilojoule content of a product and if the fat is reduced or replaced with an ingredient such as water then the kilojoule content would decrease. Fat replacers typically used in the meat industry can be categorised as protein-based (collagen, whey protein isolate and sodium caseinate), lipid-based (soy lecithin acts as an emulsifier) and carbohydrate-based (gums, fibres, starches and cellulose) (Brewer, 2012).

Fat replacers have mainly been used in an attempt to reduce and even replace the animal fat in processed meat products. The reduction of fat has been approached in two manners; the addition of water which involves the partial substitution of fat; the other is the use of other ingredients such as emulsifiers and gelling agents (Olmedilla-Alonso *et al.*, 2013). The addition of water often results in a product with a softer texture which can be undesirable (Ruusunen *et al.*, 2003), therefore, alternative fat replacers need to be used that are able to replicate or mimic, and in some cases improve, the texture of a low fat or reduced fat processed meat product (Garcia-Garcia & Totosaus, 2008). Fat replacers are lipid-based, protein-based and carbohydrate-based, the large variety of fat replacers is necessary

due to different products requiring fat replacers that have a certain functionality (Lucca & Tepper, 1994; Akoh, 1998; Brewer, 2012). Studies have been conducted testing different fat replacers of processed meat products as well as building chemical, physical and sensory profiles for the reformed products. Hoffman & Mellett (2003) used modified starch to replace pork fat in ostrich burgers. Non-digestible fibres such as potato starch, carrageenan and locust bean gums (LBG) were added to frankfurter sausages where the LBG increased cooking yield however, when the potato starch was combined with either the LBG or carrageenan moisture retention increased (Garcia-Garcia & Totosaus, 2008).

A strategy that can be used to increase the functionality and health benefits of the product is to provide an improved fatty acid profile. Vegetable oils high in n-3 PUFA have been successfully incorporated with oil-in-water emulsions (Valencia *et al*., 2008). Processed meat products containing fat replacers that incorporate vegetable oils are able to provide a "healthier" fatty acid profile and have the potential to be a functional food. Rodriguez-Carpena *et al.* (2012) noted that replacing 50% of the pork fat with either avocado, sunflower and olive oil lead to an improved MUFA and PUFA content. Vegetable oils have been used in the partial substitution of animal fat; the vegetable fats are high in mono-unsaturated fatty acids (MUFA) and omega-3 poly-unsaturated fatty acids (n-3 PUFA) compared to that of animal fat which is high in saturated fatty acids (SFA) (Olmedilla-Alonso *et al.*, 2013). Youssef & Barbut (2011) used canola oil (which contains high levels of oleic acid) in the partial substitution of pork fat in meat emulsions. Utrilla *et al.* (2014) noted that the pork fat was partially replaced (15, 25, 35, 45 & 55%) with an emulsified olive oil organogel (containing soy protein and water).

Although there are a variety of fat replacers the functionality of each one differs which ultimately determines which processed meat product will benefit with the specific fat replacer addition. Table 2.2 showed a short summary of three typical fat replacer categories and what processed sausages have been reformulated using a specific fat replacer, however, it is evident from Table 2.2 that many of the lipid-based fat replacers are used in combination with another fat replacer. Vegetable oils high in mono-unsaturated and poly-unsaturated fatty acids (olive oil, canola oil etc.) are liquid at room temperature. Pelser *et al.* (2007) noted that the liquid nature of flaxseed oil lowered the hardness compared to a solid fat such as animal fat. The liquid character of vegetable oils require the use of other fat replacers (protein and/ or carbohydrate-based) to improve binding (Table 2.2). Fat replacers continue to develop and improve in functionality, literature has no information on the use of a protein and alginate gel containing canola oil therefore, researching the effect on a processed meat product will add new knowledge to the scientific database.

Table 2.2 Fat replacers with different properties and the different sausages containing them

Type of sausage	Fat replacer(s) used	Reference
Protein-based		
Bologna	Soy protein concentrate	Shand (2000)
Chorizo	Soy protein isolate	Muguerza *et al*. (2003)
Sucuk	Kashar cheese	Ercoşkum (2014)
Bologna	Pork skin & cellulose	De Oliveira Faria *et al.* (2015)
Fat-based		
Frankfurters	Olive oil & sodium lactate	Bloukas, Paneras & Fournitzis (1997)
Chorizo	Oilve oil & soy protein isolate	Muguerza *et al.* (2001)
Salami	Olive oil & sodium caseinate	Severini *et al.* 2003)
Frankfurter	Palm, cottonseed & olive oil with sugar-beet fibre	Vural *et al.* (2004)
Dutch style cervelat (summer sausage)	Flaxseed oil Canola oil Flaxseed & encapsulated flaxseed Encapsulated fish oil	Pelser *et al*. (2007)
Chorizo	Olive oil, alginate & Inulin	Beriain *et al.* (2011)
Salchichon	Olive oil & soy protein concentrate	Utrilla *et al*. (2014)
Carbohydrate & hydrocolloid-based		
Frankfurter	Carrageenan	Bloukas, Paneras & Papadima (1997)
Chorizo	Inulin	Mendoza *et al.* (2001)
Bologna	Carrageenan Potato starch Barley flour	Shand (2000)
Fermented cooked	Fructooligosaccharides	Dos Santos *et al.* (2012)
Chorizo	Konjac gel	Ruiz-Capillas *et al*. (2012)
Merguez	Konjac gel & olive oil	Triki *et al.*(2013)
Frankfurter	Inulin & pectin	Mendez-Zamora *et al.* (2015)

2.2.1 Sensory relating to different fat replacers and how they affected processed meat products

Cabanossi has a particular appearance, as well as certain sensory attributes that make the product acceptable from a quality point of view. The type of fat used in the production of cabanossi has a great impact on the success or failure of the processed meat product. Traditionally animal fat, in particular pork back fat, is used in the product and provides the characteristic appearance, texture and

flavour associated with cabanossi. Therefore, the addition of a fat replacer in a cabanossi could be a challenge. Other processed meat products' fats have, however, been replaced successfully. Salcedo-Sandoval *et al.* (2013) partially substituted pork back fat with a vegetable oil (olive oil) and combining the oil with konjac gel (polysaccharide produced from *Amorphophallus konjac*). The results showed that even though the hardness had increased, the sensory quality was not affected. Garcia-Garcia & Totosaus (2008) added carrageenan and locust bean gums to low fat sausages which improved the texture and water retention with only minor effects on the colour of the product. Unfortunately, no sensory tests were conducted, therefore; there are no results on the sensory quality of the products. Buscailhon *et al.* (1994) added olive oil to a reduced animal fat chorizo which significantly ($P < 0.05$) effected the MUFA content of the product due to the abundance of oleic acid. During storage mono-unsaturated fatty acids are more chemically stable due to fewer double bonds.

Muguerza *et al*. (2001) produced Pamplona chorizo partially substituted with 25% olive oil which the consumers found acceptable. Muguerza *et al*. (2001) with the substitution of approximately 20-30% pork back fat with olive oil in the chorizo found that the linoleic acid (n-6) content decreased during the curing process due to the high degree of unsaturation. According to Beriain *et al.* (2011) chorizos containing emulsified olive oil using alginate had increased MUFA's and decreased SFA's and PUFA's. The shelf-life was stable over a period of time (day 0, 10, 17, 2 and 31) with an added health benefit provided by the high MUFA content and it was concluded that the combination could be used to produce a reduced fat Pamplona-style chorizo. In a study by Rubio *et al*. (2008) it was noted that when panellists evaluated the shelf-life stability of salchichón, a dry fermented Spanish sausage, which was enriched with mono-unsaturated and poly-unsaturated fatty acids, they found no rancid notes. Techniques have been developed to aid the inhibition of lipid oxidation. Estrada-Muñoz *et al*. (1998) found that liquid smoke (conc. 1.5%) was able to retard lipid oxidation in precooked beef patties that were stored for 90 days at -15 ˚C. Jiménez-Colmenero (2007) noted that the use of emulsification with proteins and gel forming hydrocolloids could reduce flavour degradation caused by lipid oxidation of the vegetable oils. Game salami made from gemsbok, zebra and kudu were regarded as being of good quality whilst those made from springbok meat were found to be less acceptable from a quality point of view (Van Schalkwyk *et al*., 2011). The characteristics that stood out in this study were the low game flavour scores which may have been due to the high animal fat included in the processed meat products. The replacement of animal fat will have impact on the sensory and textural properties of the product.

2.2.2 Fat replacers effect on the Instrumental texture of different fat replacers

Texture profiling of fat replaced sausages is important as it contributes significantly towards the sensory results. Processed meat products contain high levels of animal fat which contributes to the overall texture and juiciness of the product. Reducing the fat content in these products could lead to physical changes. Bloukas *et al.* (1997) and Muguerza *et al.* (2001) found that sausages in which pre-emulsified olive oil was used as a substitute for animal fat were harder than those containing only pork back fat. Muguerza *et al.* (2001) processed a dry fermented sausage, chorizo, where by 20% of the pork back fat was replaced with olive oil and sodium caseinate. Sodium caseinate is typically used to emulsify the olive oil with the meat and was found to increase the hardness of the sausage. Severini *et al*. (2003) produced salami containing different levels of pork back fat that were partially substituted with olive oil and the study focused on three sensory characteristics; aroma, firmness and colour that were evaluated at the end of ripening and after 30 days of storage. The 5% olive oil salami had small differences between the end of ripening and storage. At the end of ripening and storage the 7.5% olive oil salami started to develop pungent odours, which could indicate that due to the high degree of unsaturation the product at this concentration can be susceptible to lipid oxidation and may not be suitable for storage. Olivares *et al.* (2010) found no change in hardness or springiness between the different fat levels (pork back fat) of a fermented sausage (salami). In a study on chorizo, the drying procedure of chorizo containing the emulsified olive oil resulted in harder products compared to the control, however; the springiness did not differ between the two treatments (Beriain, *et al*., 2011). Youssef & Barbut (2011) noted that in the partial substitution of pork fat (10% & 17.5%) in meat emulsions with canola oil and pre-emulsified canola oil (sodium caseinate, soy and whey protein isolate). It was found that the chewiness, gumminess and cohesiveness all increased. In terms of texture, it was clear from previous literature that the texture of the fat replaced processed meat products are altered and negatively impact on the texture. Some increase the hardness where others soften the product; this is due to the decreased lubricity and structure that animal fat provides in processed meat products (Barbut, 2011; De Hoog *et al.*, 2011).

2.3 Canola oil production and characteristics

Canola is grown all around the world and is one of the most abundant vegetable oils. The initial preparation of the plant is important to maximise oil yield. The production from start to end is as follows (Unger, 2011): The canola plant is harvested once mature and the moisture content adjusted within a range of 7-7.5% depending on configuration. The canola plants temperature is important, if it is too low the seed fractures along the cells which decreases the oil yield when pressed. The extraction process begins with the seeds traveling through two rollers which split the cellular

structure, a process known as flaking. The canola flakes are heated to ~90°C for 30-40 min to deactivate myrosinase and reduce the moisture content to 4.5-6.0%. Due to canola oil's high oil content and fragile flakes a screw press is used and 60-70% of the oil is extracted. Also, the oil cake is transferred to a solvent for extraction and desolventizing of most of the residual oil. The solvent runs through distillation columns where the solvent and oil are separated. Degumming is the process where the oil from both the pressing and solvent extraction is combined and the phospholipids present are removed. The crude oil is then refined and deodorised as free fatty acids can influence the odour, flavour and shelf-life of the oil.

Canola oil is readily available in South Africa and especially in the Western Cape where it is grown. Canola is an edible vegetable oil that belongs to the *Brassicaceae* family, forming part of the rapeseed group. There are a large variety of vegetable oils containing high levels of α-linolenic acid are maize, soy, canola, linseed, grape seed, walnut and others which could improve the fatty acid profile of the processed meat products (Weiss *et al.*, 2010). Canola oils popularity stems from the n-6 (linoleic acid) and n-3 (α-linolenic acid) fatty acid ratio of 2:1, as well as a high content of vitamin E (Akhtar, 2014). Rapeseed oil was bred to contain lower amounts of erucic acid; this breed is now called canola and is one the largest consumed vegetable oils in the world (Beriain *et al*., 2011). Studies about mono-unsaturated fatty acids (MUFA) are steadily increasing due to their association with improving blood lipid profiles, the mediation of blood pressure, improved insulin sensitivity and regulated glucose levels (Keys *et al.*, 1986; Krauss *et al*., 2000; American Heart Association Nutrition Committee *et al*., 2006) with dietary guidelines recommending an increased consumption of MUFA's replacing the saturated fatty acids (SFA) in the diet. Table 2.3 illustrates the fatty acid composition of canola oil and indicates the oils popularity due to its low SFA's and high MUFA's, PUFA's and n-3 PUFA's (α-linolenic acid) content (Hu, 2003). The direct comparison with olive oil supports the reason why it has become a popular oil as it contains 7.4% saturated fatty acid (SFA), 28.1% poly-unsaturated fatty acid (PUFA) and 63.3% mono-unsaturated fatty acids (MUFA) (USDA, 16/07/2014).

T**able 2.3** Summarized comparison of the fatty acid contents between canola and olive oil

	kJ	**Total fat (g)**	**SFA (g)**	**MUFA (g)**	**PUFA (g)**	**n-6 PUFA (g)**	**n-3 PUFA (g)**
Canola	3 699	100	7.4	63.3	28.1	19.0	9.1
Olive	3 699	100	13.8	73.0	10.5	9.8	0.7

*Source: USDA National Nutrient Database for Standard Reference. United States Department of Agriculture Website (http://www.nal.usda.gov/fnic/foodcomp/search/). 16/07/2014.

An increased number of consumers are more aware of their daily dietary fat intake (Eckel *et al*., 2009). Thus, giving rise to the consumers purchasing "healthier" oils which contain larger amounts of MUFA's. Canola oil's most common/abundant MUFA is oleic acid (OLA); making it a good substitution for other oils and fats which contain high levels of SFA's and trans fatty acids (TFA) (Smolin & Grosvenor, 2003; Tarrago-Trani *et al*., 2006).

In 2006, The United States Food and Drug Administration (FDA) authorised a health claim that stated the daily consumption of approximately 19 g of oils high in MUFA's could reduce the risk of cardiovascular disease (FDA, 2009). Wood *et al*. (2003) highlighted that with vegetable oils the n-6:n-3 ratio is important to keep balanced, as they have conflicting physiological functions in the body, the recommended ratio for optimum balance is 4:1. Johnson (2007) noted that canola oil use has been escalating and if it were to replace other oils in cooking and products it could lead to consumers complying with the dietary fatty acid intake recommendations.

According to the Dietary Guidelines for Americans (2005) and Harris (2007) the dietary intake of PUFA's especially n-3 fatty acids are under consumed and lower than the recommended level set by the American Heart Association (2006). Kaushik *et al*. (2014) went on to say that due to the recommendations the demand for n-3 fatty acids has increased in the functional food market. Field *et al*. (2007) has insisted that there is a stronger correlation between the fat qualities in a product than the amount of fat in a product on weight gain. Other studies have found and reported that the intake of MUFA's is not associated with weight gain or the increase in waist circumference (Koh-Banerjee *et al*., 2003; Field *et al*., 2007).

The addition of quality fat into an individual's diet is needed; this can be accomplished by adding canola oil into processed meat products. The level of addition is low but if the product is intended to be nutritionally significant the addition must be approximately 50-100 $mg.kg^{-1}$ (Decker & Park, 2010). As previously mentioned, if vegetable oils (canola oil) containing high levels of omega-3 fatty acids were incorporated into emulsified or gelled systems, they would have an advantage in decreasing oxidation without effecting the bioavailability (Decker & Park, 2010).

2.4 Blesbok (*Damaliscus pygargus phillipsi*) meat characteristics

Traditional processed meat products use pork meat and back fat. Negative connotations about saturated fatty acids and cholesterol contents in processed meat products have forced food manufactures to find alternative meat sources with a better nutritional profile (Whitney & Rolfes, 2002). Animal species availability in a geographical area also influences what processed meat products food manufactures are able to produce for the consumer. In terms of the environmental impact in South Africa, the culling of surplus game animals is essential for wild-life management as most farmers do not have natural predators to control the number (Lewis *et al*., 1997). In South Africa

this is a meat resource that is frequently overlooked, particularly in the production of processed game meat products. There are various types of game meat found in South Africa however, blesbok meat was chosen as it has a low fat content and is readily available (Smit, 2004; Hoffman 2007). The other possible game that could have been chosen was springbok (*Antidorcas marsupialis*), however, the fat content of springbok (2.2%) was higher than the blesbok (0.9-1.2%) (Smit, 2004; Hoffman, 2007). Furthermore, the leaner meat of the raw material (blesbok meat) would assist in reducing the products fat content. Asalyng (2009) noted that one of the biggest challenges for the meat industry going forward would be the need to meet the consumer's demand for healthy reduced fat processed meat products that have an optimal fatty acid composition. Neethling *et al*. (2014) noted that blesbok meat has a favourable fatty acid composition which could help improve the fat quality present in the processed meat product. Hoffman *et al.* (2008) added that due to the blesbok living in a natural environment it could be expected that the blesbok meat should have a healthy fatty acid profile. Furthermore, the meat from these animals need to be utilised and processed meat products such as cabanossi has great potential as it uses low cost cuts and has a long shelf-life. Previous literature indicates that the possible use of game in place of pork meat could aid in lowering the fat content.

2.5 Functional food and nutraceuticals defined

Functional foods have been mentioned previously, however, in this section the term is expanded and explained in more detail. Functional foods consist of various types of products which can and do possess different components which benefit an individual's diet. These components (vitamins, minerals, peptides, fibres, proteins, omega-3 poly-unsaturated fats, antioxidants and enzymes) are wide spread and can be found in a variety of raw materials (Deschênes, 2007). However, to understand what a functional food or nutraceutical is it must be defined. The terms definitions alter around the world Yeung *et al.* (2006) noted that there is no commonly accepted definition for functional foods and nutraceuticals which makes it increasingly difficult to regulate. Nutraceuticals was a term created and defined by the Foundation for Innovation in Medicine to distinguish between functional foods and "medical foods." (Hardy, 2000). Aruoma (2010) noted the Foundation for Innovation Medicine defined nutraceutical as: "Any substance that may be considered a food or part of a food and provides medical or health benefit including the prevention and treatment of disease." In addition, Sheeshka & Lacroix (2008) stated that Health Canada (HC) defined a nutraceuticals as: "A product isolated or purified from foods that is generally sold in medicinal forms not usually associated with foods. A nutraceutical is demonstrated to have physiological benefit or provide protection against chronic disease." Nutraceuticals as per the definitions are nutrients used in functional foods to add or improve the health benefit of a product.

Whereas, functional foods are defined as: “Similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions i.e. they contain bioactive compounds.” (Sheeshka & Lacroix, 2008).

Furthermore, Hawkes (2004) noted that the Japanese definition of a functional food was: “Foods which are expected to have a specified effect on health due to the relevant constituents, or food from which allergens have been removed.” Moreover, the FUFOSE (Functional Food Science in Europe) stated that: “Functional foods are those that satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either an improved state of health and well-being, or reduction of risk of disease.” (Howlett, 2008). Although, there are several different definitions from around the world, each definition highlighted how functional foods have the capability of improving the health of an individual by delivering nutritional benefits beyond what is recommended. Food with a functional purpose can have a positive impact on the health on individuals. The addition of nutraceuticals to foods could increase the functionality and increase the effectiveness in combating and reducing diseases. In an attempt to reduce the fat content in a game cabanossi a hydrogel was used containing canola oil. Previously mentioned, canola oil is high in omega-3 fatty acids which are involved in cell membranes, cellular signalling, gene expression and decrease the risks of CVD (McClemnets *et al.*, 2009). Functional foods need to contain a beneficial health or well-being function and it needs to be accepted by the scientific community with evidence of biological, biochemical and/or epidemiological data (Nowicka & Naruszewicz, 2004).

2.6 Meat consumption and economic impact

According to an article in South African Food Review (2014) the consumption of meat in South Africa has increased from R70 957 million in 2008 to R103 372 million in 2013. This increase in popularity indicates the importance meat has in a person’s diet. Fresh meat is expensive and not always accessible or easy to obtain therefore, shelf-stable processed meat products are growing in popularity as they are able to last for months and are economically viable. Processed meat products, as in raw meat products, are good sources of protein but can be high in fat which has become undesirable for the consumer. In a study conducted by Hoffman *et al*. (2005) it was found that consumers take the fat content of meat into account before purchasing the product. Moloney (2002) noted that the consumer’s decision for purchasing a product was based on the perception of healthiness and sensory quality.

Animal fat naturally contains a percentage of high saturated fatty acid (SFA) which has had many countries recommending a reduction in the consumption of animal fat. The latter author goes

on suggesting that the conditions for a new processed meat product need to remain similar to those of the original product. For example where the fat is firm, white, fresh and has a high melting point. Substituting the source of meat from pork or beef to game species could result in a lower fat content. Game meat contains high levels of protein and is low in both fat and connective tissue (Hoffman, 2000). Viljoen (1999) added that compared to beef, game meat species have a lower saturated fat content and are higher in poly-unsaturated fat.

Decker & Park (2010) suggested the selection of meat, dietary manipulation and the alteration of fatty acid composition as possibilities to minimise the health risks associated to a high saturated fat and cholesterol diet. Grasso *et al. (*2014) suggested the addition of bioactive ingredients during the processing of the meat product, as the type of ingredient and amount added can be controlled in order to comply with regulation and keep the product affordable. These various additions are becoming more necessary as food regulations are forcing companies to be aware of the consumer's health. Due to the evidence of chronic diseases associated with a high fat diet (Leis, 1991), the World Health Organization WHO (2003) have released recommendations for the consumption of fat: it stated that fat should provide between 15-30% of an individual's daily kilojoule intake with saturated fat <10% and cholesterol no more than 300 $mg.day^{-1}$.

Labelling regulations surrounding processed meat products are strict and they differ from country to country. The regulations are in place to help consumers make an informed decision regarding the product they want to purchase. According to Decker & Park (2010) certain countries, such as the USA, state that any additional fortification that may lead to nutritional benefits cannot be claimed on the label. The implication being that the communication of the health benefits is not readily available to the consumer and eliminating the advantage of having a fortified product, as there is no way to differentiate it from an unfortified processed meat product. Regulations as the above-mentioned can deter manufacturers from improving products. Under the EU Regulation 1924/2006 a manufacturer produced a frankfurter sausage with less than 30% fat and was able to have a nutritional claim reading "reduced fat content" on the label (European Commission, 2006).

Developing countries are the majority that suffer financial stress however, Africans are beginning to eat more meat. This has resulted in a rise in production of meat and meat products for several African countries, most notably populous South Africa (Meat Atlas, 2014). In the past nine years the BRICS group comprising of five developing countries Brazil, Russia, India, China and South Africa have had good economic growth. According to Meat Atlas (2014), meat consumption increased 6.3 per cent year per year between 2003 and 2012. Thus, forecasting that there will be a further increase of 2.5 per cent year per year between 2013 and 2022. South Africa's meat consumption has increased by 45.7% the past decade (BFAP, 2011). The South African Food Based Dietary Guidelines recommended that individuals consume fats sparingly however, there are studies

that showed that oil consumption has increased (Steyn *et al.*, 2003; Kearney, 2010). According to FAOSTAT (2013), vegetable oils are responsible for the increased oil consumption in South Africa. The increase in vegetable oil consumption has influenced the animal fat consumption to the point where it has declined (Kearney, 2010). The studies above indicate that dietary guidelines in a country can have an influence on what consumers buy with regard to health however, income might hold a greater influence with regard to price differences. Sans & Combris (2015) noted that meat consumption increased as an individual's income increased, furthermore, Popkin (2006) added that as the dietary structure changed the more expensive food items such as meat, fruit and vegetables, increased in consumption due to the rise in income. There is a trend where dietary guidelines influence consumers with a higher income which has a positive impact on the economic growth of important food sectors within the country.

2.6 Effect of processing on product quality

When producing a processed meat products there are various factors that can affect human health and the quality of the product. Table 2.4 provides a basic breakdown of factors that can potentially be detrimental to the product, as well as the consumer. Jiménez-Colmenero *et al.* (2001) constructed a table to identify different elements in the production of processed meat products that could potentially be harmful to humans. The risk factors of fats and cholesterol (category 1) were discussed in the above-mentioned literature. The risk factors (categories 2, 3 and 4) will be discussed further in this section.

Table 2.4 Elements in meat products that are potentially harmful (Jiménez-Colmenero *et al.*, 2001)

Categories	Potential harmful elements	Factors
1	Constituents (natural or otherwise) present in live animals	• Fat • Cholesterol
2	Elements added to the product during processing for technological, microbiological or sensory reasons	• Salt • Nitrite
3	Elements produced by technological treatment	• Toxic compounds formed during cooking
4	Elements developed – particularly in the storage/commercialisation phase	• Pathogenic bacteria • Formation of certain lipid oxidation products

2.6.1 The effect of lipid oxidation in processed meat products

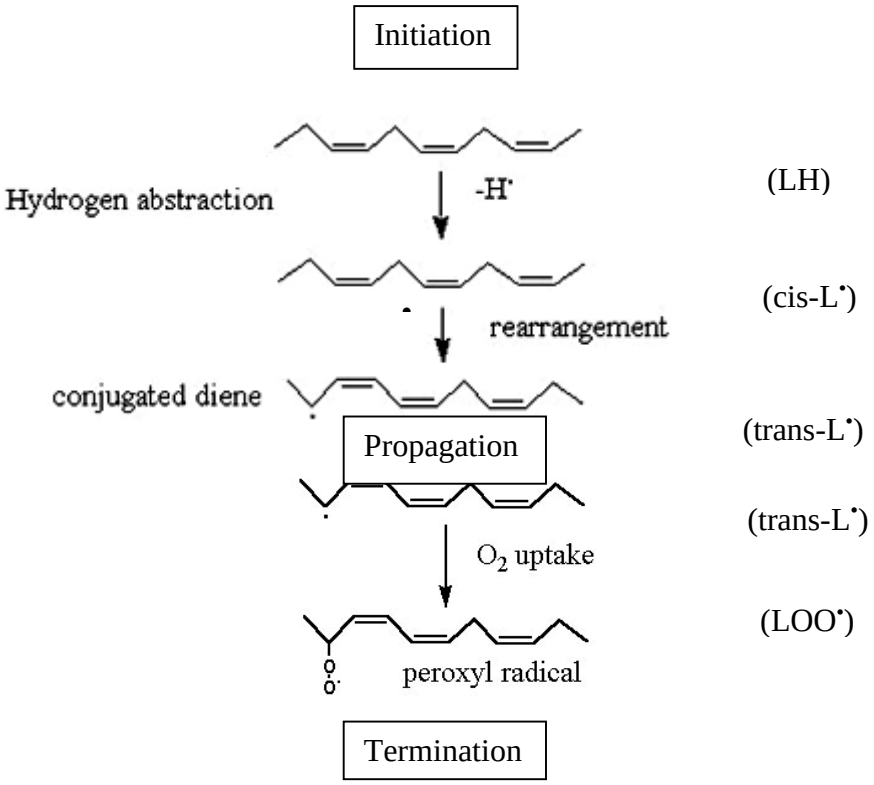
The lipid oxidation reaction can cause fatty acid structures to alter resulting in the formation of different fatty acid compounds (Decker & Park, 2010). The use of vegetable oils (olive oil or canola oil) in processed meat products can present the risk of higher lipid oxidation values, however, utilising

oils high in oleic acid could reduce the risk lipid oxidation as oleic acid is 10 times more oxidative stability than vegetable oils that are higher in poly-unsaturated fatty acids (McClements & Decker, 2008). The free radical chain mechanism for lipid oxidation undergoes three stages: initiation, propagation and termination (Figure 2.1) (Gray & Monahan, 1992). Initiation of lipid oxidation can occur via autoxidation, enzymatic oxidation and singlet oxygen Wheatley (2000). There are several autoxidation mechanisms that can occur but all are in the presence of initiators such as metal ions (M^+) and/ or reactive oxygen species (ROS) (Berton-Carabin *et al*., 2014). The initiators (M^+ and/ or ROS) extract a hydrogen in the allylic position to the double bond on an unsaturated fatty acid or acyl group (LH) to form a alkyl free radical ($L^•$) or lipoyl (Berton-Carabin *et al*., 2014). The structure rearranges from cis to a trans conjugated diene in order to increase structural stability. Propagation occurs when the alkyl radicals ($L^•$) react with oxygen to form peroxyl radicals ($LOO^•$) which are unstable therefore, they extract hydrogen atoms from adjacent unsaturated fatty acids to form hydroperoxides (LOOH) (Figure 2.1) (Berton-Carabin *et al*., 2014). Hydroperoxides are the primary products that are formed although, hydroperoxides can be broken down to form secondary products (carbonyls, alcohols, aldehydes and hydrocarbons) which can also be tested due to their contribution to off-flavours and odours in food. Termination occurs when two peroxyl radicals ($LOO^•$) react to form a nonradical product (Figure 2.1). The primary product of lipid oxidation is hydroperoxides which are colourless, odourless and tasteless. These primary products can be broken down to form low molecular compounds such as alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids that can impart rancid and pungent off-flavours in meat and meat products (Love & Pearson, 1971; Gray & Monahan, 1992). There are several methods that test for primary and secondary products which can be used to determine lipid oxidation. Lipid oxidation of uncooked meat stored at low temperatures can be accurately determined by analysing primary products such as oxygen uptake, loss of poly-unsaturated fatty acids and the formation of hydroperoxides (Gray & Monahan, 1992). In processed meat products the rate of lipid oxidation is increased which leads to the rapid development of stable secondary compounds such as carbonyls, alcohols, aldehydes, hydrocarbons and fluorescent products (Gray & Monahan, 1992; Berton-Carabin *et al*., 2014).

The thiobarbituric acid (TBA) test should be used to determine the general extent of lipid oxidation in a product rather than quantifying malondialdehyde thus, the TBA value is referred to as the thiobarbituric acid-reactive substances (TBARS) value. The detection of the malondialdehyde (three-carbon dialdehyde) occurs when poly-unsaturated fatty acids are oxidized and bind to TBA forming a coloured complex with absorption of 530-532 nm (Gray, 1978; Gray & Monahan, 1992). Lipid oxidation occurs when reactive free radicals are present. The susceptibility of meat products to lipid oxidation depends on the manufacturing process such as composition, heating, grinding, chopping, deboning, temperature abuse, oxygen availability and prolonged storage as well as the

addition of additives such as salt, nitrite and spices (Min & Ahn, 2005) The rate of oxidation in meat and meat products can be retarded when frozen but it cannot be stopped or prevented. Previous studies have shown that there are limitations to the TBA test, one of which is the decrease in the TBARS value with time (Tarladgis *et al*., 1960; Kosugi *et al.*, 1985; Hoyland & Taylor, 1991). One of these limitations was that malondialdehyde (MDA) was unstable and breaks down while the product is stored for a long period of time. MDA can be further oxidised during storage to form organic alcohols and acids that the TBARS test was unable to measure (Tarladgis *et al*., 1960; Fernández *et al.*, 1997). MDA produced from poly-unsaturated fatty acids are highly reactive and are able to bind to other food ingredients which is the reason the meat product undergoes acid/heat treatment in order for the MDA to be released for analysis (Ulu, 2004). Therefore, these secondary products are thought to contribute to the decline in TBARS values.

Wheatley (2000) noted that the TBARS test is insensitive to oleic oxidation and that MDA is labile, while Shahidi (1998) stated that cooked muscle foods (processed meat products) reach their maximum TBARS value during storage and then decline. Ulu (2004) noted that residual nitrite present in the meat product sample could react with MDA leading to nitrosation of the MDA which could cause all or a portion of the MDA to be unreactive leading to lower TBARS values. Feiner (2006) noted that once a processed meat product was stored, the nitrate was no longer converted to nitrite as the enzyme nitrate reductase has been denatured. In addition, the residual nitrate and nitrite act as strong antioxidants to preserve flavour in processed meat products that are stored for long periods (Sebranek, 2009).



LOO•+LH → hydroperoxide (LOOH) + L•

And/ Or

LOO•+ LOO• → nonradical products

Figure 2.1 The initiation, propagation and termination for the lipid oxidation process.

2.6.2 Fatty acid composition alteration in processed meat products

The human body cannot synthesise all essential fatty acids and therefore, need to consume these through a healthy diet. The most abundant mono-unsaturated fatty acid in red meat is oleic acid (C18:1n9c), however, it is not essential as the body is able to synthesise the fatty acid (Aidoo & Haworth, 1995; Bézard *et al.,* 1994). Linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) are essential fatty acids and are necessary for the growth and the development of important cognitive functions (Bézard *et al.*, 1994). It is possible to change the fatty acid profile of animals through genetic and environmental factors which alters the meat and meat products fatty acid composition (Raes *et al*., 2004). Hanczakowska *et al.* (2015) and Wiklund *et al.* (2001) noted that vegetable oils contain high levels of PUFA's and that animals consuming pasture or fed diets could contain components of these rich vegetable oils that could lead to the development of elevated levels of PUFA's in their fat. The direct addition of vegetable oils to meat products can display similar findings. Utrilla *et al.* (2014) noted when the fat was replaced with increasing amounts of olive oil in salchichon sausages, the

percent composition of oleic acid increased. PUFA's such as arachidonic acid (C20:4n6) are an important omega-6 fatty acid and can be found in red or white meat. Arachidonic acid can also be derived from linoleic acid which could increase its content once in the human body (Li *et al*., 1998). Schönfeldt & Gibson (2008) noted that oleic acid (C18:1n9), linoleic acid (C18:2n6c) and arachidonic acid (C20:4n6) are able to lower cholesterol levels in humans. Due to the omega-6 and omega-3 fatty acids role in the human body the n-6:n-3 ratio is the focus of many debates. The n-6:n-3 ratio has been in focus for years as food industries try to increase the omega-3 (n-3) composition due to the demand from the consumer (Alvarenga *et al*., 2015). Dunbar *et al.* (2014), as well as several other authors, have noted the rising n-6:n-3 ratio could have an impact on human health. The n-6:n-3 ratio has developed into a tool for indicating the nutritional value of dietary fat for human consumption (Valencak *et al*., 2015).

2.6.3 Effect of packaging on processed meat products lipid stability

As manufacturers make healthier alternatives to traditional processed meat products by increasing the MUFA and PUFA, the risk of oxidation increases (Rubio et al., 2008). For this exact reason these products have taken time to develop as several strategies need to be implemented to try to prevent oxidation. The more unsaturated the fatty acid, the more susceptible it is to lipid oxidation, once the fatty acid is oxidized the sensory characteristics as well as the shelf-life are compromised (Sheard *et al.,* 2000). Rubio *et al.* (2008) noted that a difference in TBA values were found when salchichón sausages were stored in aerobic, vacuum and gas packaging; a trend was found where the TBA values decreased as the oxygen content decreased in the packaging. Gas packaging showed the lowest TBA value, however, it was not significantly different to the vacuum packaging (Zanardi *et al*., 2002). According to Rubio *et al.* (2008) vacuum packaging will have no great impact on lipid oxidation during storage and can be used successfully in the storage of cabanossi. O'Sullivan & Kerry (2010) noted that vacuum packaging can be effective with low permeability, as it reduces the oxygen content to <1% with the remaining residual oxygen being used up by the available enzymes in the meat. The packaging is utilised as an additional hurdle to prevent the growth of microorganisms by reducing the oxygen content in the packaging. Walsh & Kerry (2002) noted that reducing the oxygen <1% can help prevent the growth of microorganisms.

2.6.4 The microbiology of processed meat products and ways of preventing growth

Food safety has been on the rise for a number of years due to the increased supply and demand by a forever increasing population. This has put pressure on manufacturers to produce greater volumes of

food. The problem with the growth of these unwanted microorganisms is that they produce undesirable characteristics such as off-flavours, off-odours, gas production and fluctuating pH with severe cases containing toxins. Fresh meat products can be susceptible to microbial spoilage due to the high water activity and pH values which creates an environment conducive for the growth of unwanted spoilage and pathogenic bacteria (Jayasena & Jo, 2013). In addition, processed meat products are able to be shelf-stable and have a long shelf-life due to the processes (curing, cooking, smoking and drying) used in the production that have inhibitory effects on microorganisms (Figure 2.2).

The nitrites that are added to this type of product do not only contribute to colour but have a preservative and bacteriostatic impact (Jay *et al.*, 2005a; Feiner, 2006). The mechanism for bacterial inhibition is not well understood and may very between bacterial species (Sindelar & Milkowski, 2011). Microorganisms such as *Salmonella* spp., *Listeria* monocytogenes, Staphylococcus *aureus* and *Clostridium botulinum* are inhibited in the presence of nitrite. However, nitrite has been found to have little to no effect on gram-negative *Escherichia coli* (Sebranek, 2009). Although, *L. monocytogenes* can survive in the presence of nitrite Xi *et al.* (2011) noted that *L. monocytogenes* still grew in cured cooked products however, the growth was lower ($P < 0.05$) than cooked products containing no nitrite. Golden *et al.* (2014) found similar results where the *L. monocytogenes* growth in deli-style turkey breast was lower ($P < 0.05$) with nitrite present than in samples without nitrite. Nitrite is an effective antimicrobial agent, however, some microorganisms are resistant and can only be eliminated using alternative preservation techniques.

Although, intrinsic factors (nitrite, salt and a_w) are able to reduce microorganisms in meat products, however, combining it with extrinsic factors (heating, smoking and drying) will reduce the in microorganisms by a larger scale. This use of one or more of these processes is known as hurdle technology or the combination prevention technique (Jay *et al.* 2005a; Chawla *et al.*, 2006). In RTE products such as processed meat products the heating step is critical. Heat treatment is widely used and effective as the high temperatures are able to in reduce microorganisms in processed meat products. Microorganisms are able to grow at different temperatures however, heating a product to a core temperature of 70°C can eliminate a high percentage of bacteria (Feiner, 2006; Jiang & Xiong, 2015). At this temperature spores can still survive but the pathogenic bacteria are destroyed (Farkas, 1997).

Wood-smoked products are a result of woods such as oak or beech that is partially burned under restricted oxygen delivery. The geographical area and treatment of wood can cause the wood to develop unique characteristics when used to smoke a processed meat product. Smoking was initially used as a preservation technique, however, it has become less of a preservation method as manufacturers use it primarily for flavour. Smoke is widely used for colour, flavour and taste it does

provide certain antimicrobial properties that can act as technological hurdle for microbes (Sikorski & Kolakowski, 2010). Although, it is still an effect preservation method due to the polycyclic aromatic hydrocarbons (PAH) produced during the smoking process (Fasano *et al.* 2016). The PAH compounds attach to the surface of the product but have poor diffusivity due to the fat and casing of the processed meat product (Fasano *et al.* 2016). This illustrates that smoking is only effective in preventing microbial growth on the outside of the product, however, hot smoking can cause the products internal temperature to increase to the acceptable 65-70^{o}C range (Tyburcy & Kozyra, 2010). Although, PAH content is a health issue but according to Codex Alimentarius Codex (CAC) the indirect smoking method applied in the manufacture of reduced fat game cabanossi's reduces the PAH content on the final product. Drying of the product between processing steps or after the cooking process can aid in the reduction of water activity (a_w) to between 0.95-0.9 which inhibit microorganisms' ability to grow (Figure 2.2) (Jay *et al.*, 2005b).

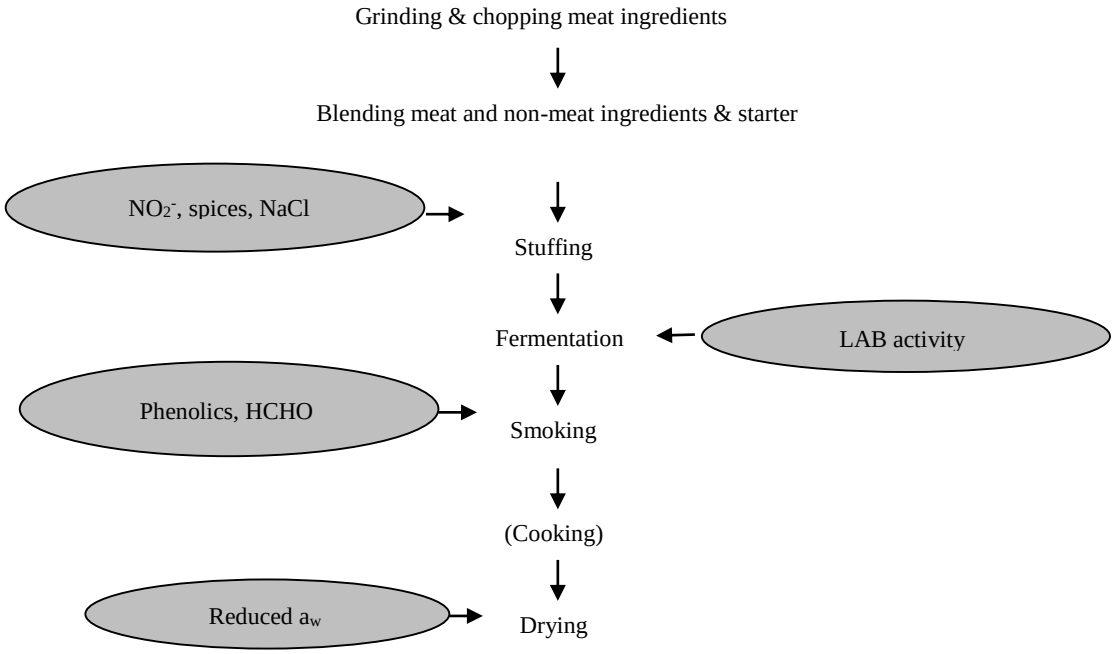


Figure 2.2 Antimicrobial hurdles in meat fermentation (Adams & Mitchell, 2002).

2.7 Conclusions

The literature from previous studies has indicated many trends as well as gaps in the scientific knowledge with regards to the use of fat replacers in cabanossi as well as game meat. Studies have focused on the reduction of pork fat and replacement thereof with healthier vegetable oils, due to the added benefits the oils could contribute to the consumer's diet. Over the past two decades it has been

noted that vegetable oils consumption has increased and that there has been a decline in animal fat consumption. The inclusion of alternative ingredients (fat replacers, game meat and vegetable oils) would aid in improving processed meat products' nutritional profile. A trend can be observed shifting towards low fat reformulated processed meat products. Therefore, it would be of interest to determine the effect of a protein-based gel containing canola oil on the chemical properties, shelf-life stability, sensory attributes and physical characteristics of a game cabanossi.

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Chapter 3

The effect of a fat replacer on the chemical properties, lipid stability and fatty acid profile of blesbok (*Damaliscus pygargus Phillipsi*) cabanossi

3.1 Abstract

The replacement of animal fat in a processed product can be challenging due to the complexity in mimicking the properties such as texture and flavour of fat but at the same time producing a healthier product. This study evaluated the effect of a canola oil-based fat replacer (FR) at three different concentrations 10% (*FR1*), 20% (*FR2*) and 30% (*FR3*) with no pork back fat added as an alternative to a *Control* with pork back fat in blesbok cabanossi. The moisture, protein and ash content were higher ($P \leq 0.05$) in all three FR treatments. As expected, the fat content was lower ($P \leq 0.05$) in all three *FR* treatments. However, the lipid oxidation results were lower than expected after 60 days and the *Control* and all three *FR* treatments did not differ ($P > 0.05$) from one another; a result possibly due to the added nitrates' antioxidant ability. The fatty acid profiles did not differ ($P > 0.05$) from the day 0 levels after 60 days storage at 4°C. However, there were differences ($P \leq 0.05$) in the fatty acid composition between the *Control* and the three *FR* treatments on day 0 and day 60. The fatty acid ratios especially the omega-6:omega-3 (n-6:n-3) ratio can be used to indicate the nutritional value of dietary fat. At day 0 and day 60, the *FR2 and FR3* had larger ($P \leq 0.05$) PUFA:SFA ratios (0.8-1.0) than the *Control* and *FR1*. Furthermore, at day 0 and day 60 all three *FR* treatments had lower ($P \leq 0.05$) n-6:n-3 ratios (2.8-3.1) than the *Control*. The overall chemical analysis showed that the combination of a fat replacer containing canola oil provided a healthy alternative to animal fat that is traditionally added to blesbok (*Damaliscus pygargus Phillipsi*) cabanossi.

3.2 Introduction

The development of semi-dried and fermented meat products may be centuries old, however, some of the basic processing are still relevant and valuable when processed meat products are stored for long periods of time (Vandendriessche, 2008). Transforming raw meat (low quality or off-cuts) into processed meat products can repurpose and add value to an otherwise low quality, cheaper raw material (Hoffman, 2008). Consumers are, however, becoming increasingly concerned with the saturated fat content present in animal fat, especially when used in processed meat products (Grasso *et al*., 2014). It has been indicated that this is one of the factors influencing consumer's decisions when purchasing processed meat products (Resurreccion, 2003; Del Nobile *et al*., 2009). On the other hand, game species have been found to contain an average of less than 3% intramuscular fat which could help decrease the fat content in processed meat products if this meat was to be used (Hoffman & Wiklund, 2006; Valencak *et al*., 2015). The fat replacement of dry fermented and emulsified

sausages has been well documented. In the past few years' research has been done on the addition of fat replacers in frankfurters, chorizo, ostrich patties and many more processed meat products. The fat replacers used in the latter products include modified starch, soy isolate, hydrocolloids and olive oil (Hoffman & Mellett, 2003; Ferandez-Gines *et al*., 2005; Garcia-Garcia & Totosaus, 2008; Olmedilla-Alonso *et al.*, 2013).

The objective of adding a fat replacer is to reduce the amount of animal fat used and at the same time trying to keep the sensory characteristics of the product consistent with that of the original product. Brewer (2012) noted when reformulating a processed meat product it is important to retain the characteristics of the original product, therefore, a large characteristic change could prevent consumers from purchasing the reformulated product. Replacing animal fat with a fat replacer would definitely affect the moisture, protein, fat and ash content of the product. The fat to protein ratio is important for structural and textural stability of a processed meat product (Wu *et al.*, 2009). Fat acts as a binder, as well as aiding in lubricity which adds to textural stability (De Hoog *et al.*, 2012). Therefore, these characteristics need to be analysed. Lipid oxidation and fatty acid analysis are important as it could impact on the flavour and aroma of processed meat products. Foodstuffs deterioration depends on several factors, one of which can be the occurrence of lipid oxidation which is an undesirable attribute in food as the off-flavours produced can deter the public from consuming the product (Gray, 1978). In processed meat products the rates of lipid oxidation tend to increase, largely due to the larger surface area to volume ratio caused by the process itself and this can lead to the rapid development of secondary oxidation compounds such as carbonyls, alcohols, aldehydes, hydrocarbons and fluorescent products (Gray & Monahan, 1992; Berton-Carabin *et al*., 2014). In processed meat products, the degree of lipid oxidation would be better determined measuring one of the above-mentioned secondary compounds. The 2-thiobarbituric acid-reactive substances (TBARS) test is frequently used to determine lipid oxidation in meat and meat products (Gray, 1978; Gray & Monahan, 1992).

The majority of fats contain one glycerol molecule attached to three fatty acid chains and the combination of fatty acid chains differentiates fats from one another (Baghurst, 2004; Ospina *et al.*, 2012). The fatty acid profile of game meat is similar to other red meat types in that palmitic acid (C16:0) and stearic acid (C18:0) are the predominant saturated fatty acids (SFA) with oleic acid (C18:1n9) as the predominant mono-unsaturated fatty acid (MUFA) (Aidoo & Haworth, 1995; Neethling *et al*., 2014). The saturated fatty acid composition of processed meat products need to be reduced as they contribute to the total low-density lipoprotein (LDL) cholesterol content which can lead to cardiovascular disease (Krauss *et al.* 2000; Baghurst, 2004; Scollan *et al.*, 2006; Yoo *et al*.,2007). The fat content and therefore fatty acid composition of a processed product is typically determined by that of the meat used, as well as that of the fat. There is increasing interest to use game

meat and/or venison in processed meat products due to their low fat content. For example Blesbok (*Damaliscus pygargus phillipsi*) meat is known to have a low fat content (<2.5%) and its fatty acid profile can change in composition depending on the gender and season (Neethling *et al.*, 2014). When these low fat meat sources are used in processed products, the composition of the fat, or fat replacer, becomes important in determining the sensory and quality attributes of the processed products. Utrilla *et al.* (2014) noted that the addition of a vegetable oil increased and decreased a number of fatty acids in a processed meat product such as salchichón. Beriain *et al.* (2011) showed that the addition of olive oil with emulsified alginate showed a different fatty acid profile in Pamplona-style chorizo produced with pork back fat. The substitution of pork back fat in processed meat products has been in focus due to its unfavourably high saturated fatty acid and cholesterol content. Therefore, through the addition of vegetable oils (canola oil) and a fat mimicker it is possible to alter the fatty acid content of the processed meat product (Ospina *et al.*, 2012).

Cabanossi is becoming an important processed/cooked meat product in the South African market where it is traditionally made from beef trimmings and pork fat. In this study the replacement of pork fat with a fat replacer containing canola oil as a fat source was investigated in a typical game meat based cabanossi and the effect thereof on the chemical quality of the product determined. Previous background on fat replacers has shown that the chemical profile of processed meat products can be improved. Strategies used to improve the chemical profile of fat replaced processed meat products can develop positive, but also negative characteristics therefore the analysis thereof provides insight into developing a product with a positive nutrient profile. The objective of this study was thus to add the fat replacer containing canola oil to a cooked semi-dry processed meat product such as cabanossi to reduce the fat content and improve the fatty acid composition. Therefore, the aim of the study was to analyse the chemical profile (proximate, lipid oxidation and fatty acid composition) of a blesbok cabanossi containing a fat replacer with added canola oil and pork fat.

3.3 Materials and methods

3.3.1 Cabanossi production

Fat replacer: Scanpro CE 40 (Danlink product, P. O. Box 707, Fourways North, 2086 Johannesburg, South Africa) an animal protein and alginate blended powder was used as the basis for the fat replacer gel. The gel was made by adding 8% of the fat replacer powder and 5% canola oil to 87% potable water. The mixture was mixed in a high-speed food mixer (Kenwood, Model no KM220, Britain) at 3000-5000 rpm until a thick emulsion formed. The emulsion was poured into a container to harden

and stored at 4˚C until used. Although the gel can set at room temperature in order to help prevent microbial growth it was maintained at 4˚C.

Cabanossi production: Six replications with four cabanossi treatments were produced (N=24). The recipe produced 3 kg batches (Table 3.1). Previously frozen blesbok meat and pork back fat was placed into a refrigerator (4˚C) to thaw. The fat replacer gel was cut into 5x5 cm blocks for ease of use and for a more even distribution during processing. Due to the use of a smaller sized bowl, the bowl chopper was able to distribute the fat more evenly throughout the batter. The blesbok meat was weighed and placed into the bowl chopper (Manica, Equipamientos Carnicos S.L., Model no. CM-21, Barcelona, Spain). Once the meat was cut into ~3 mm pieces a commercial cabanossi spice (Freddy Hirsch GM Cabanossi spice 96030C & 42521A, 0.6 kg; Maitland East, PO Box 2554, Cape Town, South Africa) which contained salt, cereal (wheat bran), spices, phosphates, curing agent (sodium nitrite), starch, MSG, ascorbic acid and flavour enhancers which was added, as well as water and the mixture further processed to mix the spice. The meat and fat was mixed until a homogenous mixture had formed. The meat-fat mixture was placed into a sausage filler (Tulsa model, DMD Foodtec Code T-0102 5-89, Europe) and filled into 24 mm diameter sheep casings (Freddy Hirsch, Maitland East, PO Box 2554, Cape Town, South Africa). The cabanossi was twisted into 20 cm lengths and placed in a maturation chamber (Reich Airmaster® UKF 2000 BE, Reich Klima-Räuchertechnik, Urbach, Germany) with a SmartSmoker and TradiSmoker LS 500 HP electronic system that was controlled automatically by a Microprocessor (Unicontrol 2000). The process flow is outlined in Figure 3.1 with the blesbok cabanossi manufactured in a HACCP-approved (Hazard Analysis and Critical Control Points) processing area with procedures to ensure good manufacturing practices (GMP).

Table 3.1 Cabanossi formulation for the *Control* and *FR* treatment to make 3 kg product

	Meat (kg)	Pork back fat (g)	Fat replacer (g)	Spice (g)	Water (g)
***Control* (20%)**	2.4	600	-	126	80
***FR1* (10%)**	2.7	-	300	126	80
***FR2* (20%)**	2.4	-	600	126	80
***FR3* (30%)**	2.1	-	900	126	80

Control (contains 20% pork fat), *FR1* (contains 10% fat replacer), *FR3* (contains 20% fat replacer) and *FR3* (contains 30% fat replacer).

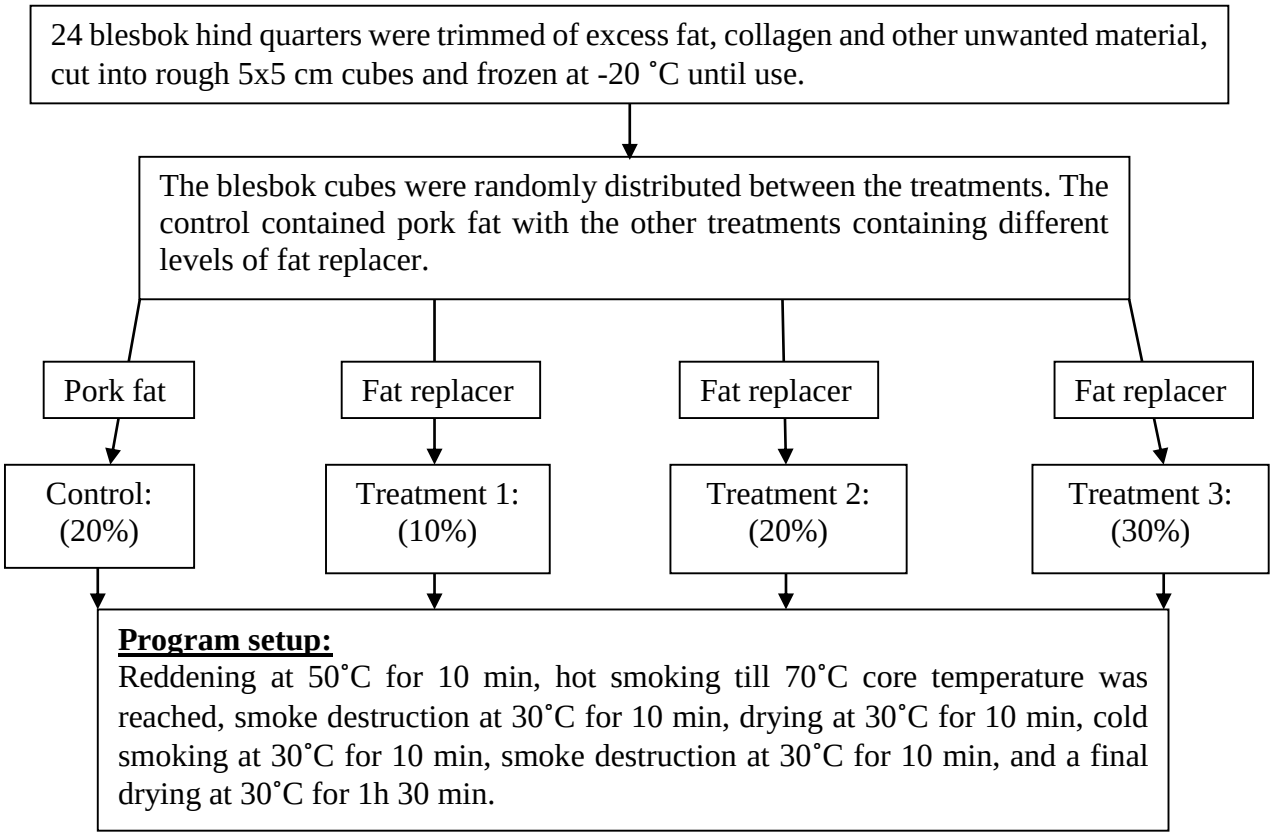

Figure 3.1 Flow diagram showing the process flow of how the cabanossi was made.

3.3.2 Sample preparation

After production the six replications were separated into two groups, day 0 and day 60, for analysis which included chemical, sensory, physical and microbial quality. The cabanossi sausages were randomly selected for analysis according to the replication and treatment number. On the sampling dates (day 0 and day 60), the cabanossi sausages were homogenised for 3 min, placed into a vacuum bag and stored at -20˚C until analysed for proximate composition (moisture, protein total fat, ash). Fatty acid and lipid oxidation samples were placed in pill containers and stored at -80˚C until analysis.

3.3.3 Proximate analysis

The following chemical analyses were conducted: Moisture was analysed using the method AOAC 934.01 (2002), crude protein using the method AOAC 992.15 (2002) and ash using the method AOAC 942.05 (2002). However, total fat was determined using the chloroform/methanol 2:1 method in accordance to Lee *et al*. (1996). All analyses were done in duplicate. For protein content, dried and defatted samples were ground with a pestle in a mortar until a fine powder was obtained. Subsequently 0.1 mg of the powder was used per sample and inserted into a foil wrap designed for the LECO protein analyser (Model software: FP-528 Determinator). The protein concentration in the sample was determined as nitrogen x 6.25. The moisture content was analysed by drying 2.5 g sample at 100ºC for a period of 24 h and ashing was done at 500ºC for a period of 6 h.

3.3.4 Lipid oxidation analysis

Cabanossi samples (0.5 g) were added to 0.15 M potassium chloride (KCl) solution and homogenised for 20 s. In accordance to Neethling *et al.* (2015), the thiobarbituric acid reactive substances (TBARs) were extracted and absorbance (532 nm) was measured using a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd). The TBA values are expressed as mg malondialdehyde (MDA) per kg product.

3.3.5 Fatty acid analysis

The fatty acid profile was determined using gas chromatography (GC). The lipids were extracted according to a modification of Folch *et al.* (1957). Approximately 2 g of sample was weighed and added together with a chloroform:methanol solution (CM 2:1 $v.v^{-1}$) containing 0.01% Butylated hydroxytoluene (BHT, antioxidant, Cat no. B-1378, Sigma Aldrich) to prevent oxidation and an internal standard Heptadecanoic acid, C17:0, 10 $mg.mL^{-1}$ made up in CM 2:1 (Sigma Aldrich Inc., 3050 Spruce str., St. Louis, MO, 63103, USA, Cat no. H3500, 98%) and used to quantify the fatty acids. A Polytron (Kinematica AG PT 2500 E, speed 7-8 x 1000 rpm) was used to homogenise the

sample for approximately 10 s and the mixture transferred to an extraction funnel, where the filtrate was separated. For esterification, 250 µL of the filtrate was transferred to a Kilmax tube (125 x 16 mm) with a Teflon lined screw cap and dried for ±10 min under nitrogen in a 45°C water bath. For methylation, 2 mL transmethylating reagent (TMR) was added to the sample and placed into a water bath at 70°C for 2 h. For separation, 1 mL dH_2O and 2 mL hexane were added once the sample had cooled, the top layer was transferred to a clean Kilmax tube (125 x 16 mm) using a glass Pasteur pipette. The sample was dried under nitrogen for a further ±30 min in a 45°C water bath. Thereafter, 100 µL hexane was added and the dissolved mixture was transferred using a glass Pasteur pipette to a GC vial containing an insert. Thereafter the fatty acid methyl esters (FAME) were identified on a GC (Trace 1300 Series; Thermo Scientific; S/N 712100906; Thermo Fisher Scientific S.p.A.; Strada Rivoltana; 20090 Rodano, Milan – Italy) equipped with a GC auto-sampler (CTC Analytics; Combi PAL**;** Product No: G6500-CTC**;** Serial No: CH00127681**;** Waldbronn; Germany) and GC column (TR FAME; Length: 30 m; ID (internal diameter): 0.25 mm; Film: 0.25 um; P/N 260M142P). The carrier gas used was hydrogen (0.7 $mL \cdot min^{-1}$). The temperature settings were as follows: the initial temperature 50°C, the final temperature 240°C, the injector temperature 250°C and the detector temperature 280°C. The rate of temperature increase was 12°C per min.

3.3.6 Statistical analysis

The data obtained from the chemical analysis were statistically analysed with STATISTICA (StatSoft, Inc. 2013, version 12). The chemical data were analysed using analysis of variance (ANOVA); for the ANOVA of the proximate analysis data the days were regarded as fixed factors and the treatments as random effect(s). Similarly, for the analysis of the lipid oxidation and fatty acid composition data the treatments, days and interactions were regarded fixed factors and the sample as random effect(s). The chemical analyses were treated as a random block design, with four treatments, six replications and done in duplicate. A 5% significance level was used as a guideline for determining significant effects. The values are reported as means ± standard error (SE) of the means.

3.4 Results

3.4.1 Proximate results

Chemical analysis was done on both the control and fat replacer (*FR*) treatments at day 0. Moisture, protein, fat and ash were determined on wet basis. The fat replacer contained: moisture (86.2%), protein (5.0%), fat (2.6%) and ash (1.9%). The average percent weight loss after production for the *Control* (22.1%) differed ($P \leq 0.05$) to all three *FR* treatments (~25%) (Table 3.2). The moisture content of the *Control* (52.0%) was lower ($P \leq 0.05$) than the three *FR* treatments (Table 3.3). *FR1*

(63.8%) had a lower ($P \leq 0.05$) moisture content than the other *FR* treatments with *FR3* (66.8%) exhibiting the highest moisture content (Table 3.3). The *Controls* (19.8%) protein content was lower ($P \leq 0.05$) than all three of the *FR* treatments. Moreover, the protein content of the three *FR* treatments differed ($P \leq 0.05$) from one another with *FR1* (26.6%) having the highest protein content as was expected as it contained the largest quantity of blesbok meat (Table 3.1). The *Controls* (23.5%) had the highest fat content which differed ($P \leq 0.05$) from that of the three *FR* treatments (Table 3.3). The fat content of the three *FR* treatments were similar ($P > 0.05$), i.e. <3.4%. The *FR* treatments all had higher ($P \leq 0.05$) ash contents than the *Control* (4.0%) (Table 3.3).

Table 3.2 Mean (%) weight and moisture loss (%) for each cabanossi treatment

	Weight before smoking (kg)	**Weight after smoking (kg)**	**Weight loss (%)**
Control	2.57^{a}	2.00^{a}	22.1^{b}
FR1	2.58^{a}	1.93^{a}	25.0^{a}
FR2	2.56^{a}	1.91^{a}	25.4^{a}
FR3	2.60^{a}	1.91^{a}	26.4^{a}

$*^{a,b}$ Means in a column with different superscripts are significantly different ($P \leq 0.05$).
Control, *FR1*, *FR1* and *FR1* contained 20% pork fat, 10% fat replacer, 20% fat replacer and 30% fat replacer, respectively.

Table 3.3 Mean (%) and standard errors for proximate analysis of cabanossi

	Control **(20% PF)**	***FR1*** **(10% FR)**	***FR2*** **(20% FR)**	***FR3*** **(30% FR)**
Moisture	$52.0^{c} \pm 0.6$	$63.8^{b} \pm 0.5$	$66.2^{a} \pm 0.5$	$66.8^{a} \pm 0.5$
Protein	$19.8^{d} \pm 0.4$	$26.6^{a} \pm 0.3$	$24.4^{b} \pm 0.5$	$23.0^{c} \pm 0.3$
Fat	$23.5^{a} \pm 0.7$	$3.4^{b} \pm 0.3$	$2.8^{b} \pm 0.2$	$3.2^{b} \pm 0.3$
Ash	$4.0^{b} \pm 0.1$	$4.7^{a} \pm 0.1$	$4.7^{a} \pm 0.1$	$5.0^{a} \pm 0.2$
Total	$99.2^{a} \pm 0.2$	$98.6^{ab} \pm 0.1$	$98.1^{b} \pm 0.4$	$98.0^{b} \pm 0.2$

$*^{a,b,c,d}$ Means in a row with different superscripts are significantly different ($P \leq 0.05$).
PF = Pork fat, *Control*, *FR1*, *FR1* and *FR1* contained 20% pork fat, 10% fat replacer, 20% fat replacer and 30% fat replacer, respectively.

3.4.2 Lipid oxidation

At day 0, all three fat replacer treatments had higher TBA values compared to the *Control*, however the former three treatments means did not differ (P > 0.05). The same trend was observed at day 60 between the *Control* and all three *FR* treatments. However, there were differences shown in the levels of lipid oxidation during the 60 day shelf-life evaluation. The control at day 0 and day 60 exhibited mean TBA values of 1.40 and 1.49, respectively, which did not differ (P > 0.05; Fig 3.2; Table 3.4). The three *FR* treatments at day 0 obtained mean TBA values >1.86 with the three *FR* treatments at day 60 exhibiting decreased (P ≤ 0.05) mean TBA values of <0.98 (Figure 3.2; Table 3.4). All three of the *FR* treatments measured no difference (P > 0.05) in lipid oxidation at day 0 or on day 60 (Table 3.4; Figure 3.2).

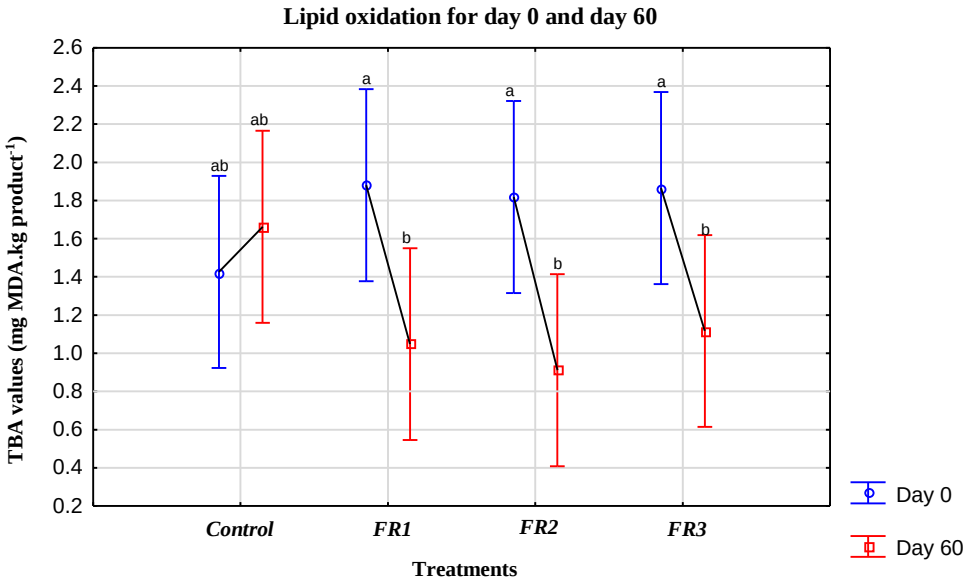

Figure 3.2 Lipid oxidation means (mg MDA.kg product^{-1}) for the treatments *Control* sample (20% PF), and the three experimental treatments with different percentages of fat replacer [***FR1*** (10% FR), *FR2* (20% FR) and *FR3* (30% FR)] measured on day 0 and day 60.

3.4.3 Fatty acid composition

No interaction ($P > 0.05$) was observed for the fatty acids between treatment and day (results not shown). There was a difference ($P \leq 0.05$; Table 3.4) exhibited for day 0 and day 60 between docosahexaenoic acid (DHA; C22:6n3), erucic acid (C22:1n9) and docosadienoic acid (C22:2n6). At day 0, the myristic acid (C14:0) composition for the *Control* was lower ($P \leq 0.05$) than all three *FR* treatments. *FR2* and *FR3* differed ($P \leq 0.05$) from one another, however, *FR3* did not differ ($P > 0.05$) from the other *FR* treatments (Table 3.4). At day 60, the trend continued as the *Control* was lower than all three *FR* treatments for myristic acid. Moreover, all three *FR* treatments did not differ ($P > 0.05$) from one another for myristic acid (C14:0) (Table 3.4). The palmitic acid (C16:0) composition of the *Control* (22.7%) at day 0 was higher ($P \leq 0.05$) than that of the three *FR* treatments. Furthermore, the C16:1 concentration of *FR1* (16.9%) differed ($P \leq 0.05$) from *FR2* (12.4%) and *FR3* (13.1%). Day 0 and day 60, showed the same trend between the *Control* and all three *FR* treatments for C16:0 with no notable differences ($P > 0.05$). Stearic acid (C18:0) showed the same trend between day 0 and day 60 with the *FR1* (>22.05%) having a higher ($P \leq 0.05$) composition in comparison to the *Control*, *FR2* and *FR3* with the latter three treatments having no differences ($P > 0.05$; Table 3.4). *FR1* contained the most blesbok meat with the game species containing a C18:0 composition of 35.5% compared to the pork back fat containing 22.4% (Table 3.5).

At day 0, oleic acid (C18:1n9c) was the highest ($P \leq 0.05$) in the *Control* (41.1%) with the *FR* treatment compositions increasing (>27.4%) as more of the fat replacer was added (Table 3.4). Erucic acid (C22:1n9) adds little value to the total mono-unsaturated fatty acid composition of the product, however, it did differ ($P \leq 0.05$) between day 0 and day 60 (Table 3.4).

At day 0, the linoleic acid (C18:2n6c) composition for *FR1* was lower ($P \leq 0.05$) than the *Control* and *FR2*. However, *FR3* did not differ ($P > 0.05$) in composition when compared to the *Control* and the other *FR* treatments (Table 3.4). At day 60, the linoleic acid (C18:2n6c) composition for *FR1* was lower ($P \leq 0.05$) than *FR2* and *FR3* with the *Control* having no difference ($P > 0.05$) in composition when compared to all three *FR* treatments. The α-linolenic acid (C18:3n3) composition between the *Control* and all three *FR* treatments showed the same trend between day 0 and day 60 (Table 3.4); *FR2* and *FR3* had the highest composition and differed ($P \leq 0.05$) from the *Control* and *FR1*. Moreover, the control had the lowest content and differed ($P \leq 0.05$) from *FR1*. At day 0 and day 60, the arachidonic acid (C20:4n6) composition was lower ($P \leq 0.05$) in the *Control* compared to all three *FR* treatments. Also, at day 0 the *FR3* had lower ($P \leq 0.05$) concentrations of C20:4n6 than *FR2* but has similar levels ($P > 0.05$) as *FR1* (Table 3.4). At day 60, the *FR3* had lower ($P \leq 0.05$) concentrations of C20:4n6 than *FR1* and had similar ($P > 0.05$) levels when compared to *FR2* (Table 3.4). At day 0, the docosahexaenoic acid (DHA; C22:6n3) composition did not differ ($P > 0.05$)

between the *Control* and all the *FR* treatments. In addition, at day 60 the DHA compositions decreased for the *Control* and all three *FR* treatments. Furthermore, at day 60, the *Control* differed ($P \leq 0.05$) to *FR2* and *FR3* but did not differ ($P > 0.05$) to *FR1* in terms of DHA. Moreover, *FR1* did not differ ($P > 0.05$) to *FR2* and *FR3* (Table 3.4). The *FR1* differed ($P \leq 0.05$) from the other treatments with the SFA composition decreasing as the fat replacer percentage increased (Table 3.4). In contrast the MUFA and PUFA compositions increased as the fat replacer percentage increased (Table 3.4).

At day 0 and day 60, the same trend was exhibited for the total poly-unsaturated fatty acid and saturated fatty acid ratios (PUFA:SFA) between the *Control* and all three *FR* treatments (Table 3.4). The *Control* and *FR1* had lower ($P \leq 0.05$) PUFA:SFA ratios at day 0 and day 60 than *FR2* and *FR3* with the latter two *FR* treatments not differing ($P > 0.05$). Similarly, the omega-6 (n-6) and omega-3 (n-3) ratio (n-6:n-3) at day 0 and day 60 illustrated the same trends when comparing the *Control* and all three *FR* treatments (Table 3.4). At day 0 and day 60, the *Controls* n-6:n-3 ratio was higher ($P \leq 0.05$) than that of all three *FR* treatments, furthermore *FR1* was higher ($P \leq 0.05$) than *FR3*, however *FR2* and *FR3* indicated no difference ($P > 0.05$).

Table 3.4 Mean compositions (%) and standard errors of the fatty acids identified in the processed meat products

		Day 0				Day 60			
		Control	***FR1***	***FR2***	***FR3***	**Control**	***FR1***	***FR2***	***FR3***
TBA values		$1.4^{ab} \pm 0.25$	$1.9^{a} \pm 0.25$	$1.9^{a} \pm 0.25$	$1.9^{a} \pm 0.25$	$1.5^{ab} \pm 0.25$	$0.8^{b} \pm 0.25$	$0.9^{b} \pm 0.25$	$1.0^{b} \pm 0.25$
SFA	C14:0	$1.6^{c} \pm 0.04$	$4.3^{b} \pm 0.44$	$6.1^{a} \pm 0.29$	$5.3^{ab} \pm 1.08$	$1.7^{b} \pm 0.05$	$4.7^{a} \pm 0.29$	$6.1^{a} \pm 0.25$	$5.5^{a} \pm 1.07$
	C15:0	$0.1^{b} \pm 0.01$	$0.4^{a} \pm 0.04$	$0.3^{a} \pm 0.03$	$0.3^{a} \pm 0.04$	$0.1^{c} \pm 0.01$	$0.4^{a} \pm 0.03$	$0.3^{b} \pm 0.01$	$0.3^{b} \pm 0.02$
	C16:0	$22.7^{a} \pm 0.54$	$16.9^{b} \pm 0.83$	$12.4^{c} \pm 0.75$	$13.1^{c} \pm 1.12$	$23.1^{a} \pm 0.29$	$16.7^{b} \pm 0.89$	$12.8^{c} \pm 0.64$	$11.7^{c} \pm 0.36$
	C18:0	$11.6^{b} \pm 1.01$	$23.9^{a} \pm 2.05$	$15.4^{b} \pm 1.39$	$15.4^{b} \pm 1.72$	$11.4^{b} \pm 1.08$	$22.1^{a} \pm 1.79$	$14.7^{b} \pm 1.14$	$11.7^{b} \pm 0.88$
	C20:0	$0.3^{b} \pm 0.01$	$0.4^{a} \pm 0.05$	$0.5^{a} \pm 0.07$	$0.5^{a} \pm 0.03$	$0.2^{d} \pm 0.01$	$0.3^{c} \pm 0.01$	$0.4^{b} \pm 0.02$	$0.5^{a} \pm 0.02$
	C21:0	$0.8^{a} \pm 0.02$	$0.4^{b} \pm 0.04$	$0.3^{c} \pm 0.03$	$0.3^{c} \pm 0.02$	$0.8^{a} \pm 0.02$	$0.4^{b} \pm 0.03$	$0.3^{b} \pm 0.04$	$0.3^{b} \pm 0.02$
	C22:0	$0.1^{c} \pm 0.01$	$0.2^{b} \pm 0.02$	$0.4^{a} \pm 0.04$	$0.3^{ab} \pm 0.03$	$0.1^{c} \pm 0.01$	$0.3^{b} \pm 0.02$	$0.3^{a} \pm 0.04$	$0.3^{a} \pm 0.03$
	C24:0	$0.2^{b} \pm 0.02$	$0.5^{a} \pm 0.04$	$0.6^{a} \pm 0.04$	$0.5^{a} \pm 0.03$	$0.2^{b} \pm 0.01$	$0.5^{a} \pm 0.03$	$0.6^{a} \pm 0.05$	$0.5^{a} \pm 0.03$
MUFA	C14:1	$0.1^{c} \pm 0.002$	$0.3^{a} \pm 0.03$	$0.2^{b} \pm 0.02$	$0.2^{ab} \pm 0.03$	$0.1^{c} \pm 0.004$	$0.3^{a} \pm 0.02$	$0.2^{b} \pm 0.01$	$0.2^{b} \pm 0.01$
	C16:1	$2.0^{a} \pm 0.09$	$1.1^{b} \pm 0.05$	$0.8^{c} \pm 0.06$	$0.9^{c} \pm 0.08$	$2.1^{a} \pm 0.05$	$1.2^{b} \pm 0.05$	$0.9^{c} \pm 0.04$	$0.8^{c} \pm 0.04$
	C18:1n9c	$41.1^{a} \pm 0.62$	$27.4^{d} \pm 1.23$	$32.8^{c} \pm 0.55$	$35.8^{b} \pm 0.52$	$40.6^{a} \pm 0.55$	$27.0^{c} \pm 1.14$	$33.6^{b} \pm 0.94$	$38.4^{a} \pm 0.97$
	C20:1	$0.7^{a} \pm 0.02$	$0.4^{c} \pm 0.03$	$0.5^{b} \pm 0.04$	$0.5^{b} \pm 0.04$	$0.6^{a} \pm 0.02$	$0.4^{c} \pm 0.04$	$0.6^{b} \pm 0.03$	$0.7^{a} \pm 0.03$
	C24:1	$0.1^{c} \pm 0.01$	$0.3^{b} \pm 0.03$	$0.4^{a} \pm 0.05$	$0.3^{ab} \pm 0.02$	$0.1^{b} \pm 0.01$	$0.3^{a} \pm 0.02$	$0.3^{a} \pm 0.03$	$0.4^{a} \pm 0.02$

*[a,b,c,d] Means in the same row with different superscript are significantly different ($P \leq 0.05$). TBA values = mg MDA.kg product^{-1}
Control, *FR1*, *FR2* and *FR3* contained 20% pork fat, 10% fat replacer, 20% fat replacer and 30% fat replacer, respectively.

Table 3.4 Continued mean compositions (%) and standard errors of the fatty acids identified in the processed meat products

		Day 0				Day 60			
		Control	***FR1***	***FR2***	***FR3***	**Control**	***FR1***	***FR2***	***FR3***
PUFA	C18:2n6c	$16.0^{a} \pm 0.29$	$12.9^{b} \pm 1.35$	$16.5^{a} \pm 0.92$	$15.4^{ab} \pm 1.09$	$16.2^{ab} \pm 0.46$	$14.0^{b} \pm 0.98$	$16.6^{a} \pm 0.72$	$17.1^{a} \pm 0.76$
	C18:3n3	$1.4^{c} \pm 0.04$	$4.3^{b} \pm 0.43$	$6.1^{a} \pm 0.29$	$6.2^{a} \pm 0.37$	$1.7^{c} \pm 0.05$	$4.7^{b} \pm 0.29$	$6.1^{a} \pm 0.25$	$6.7^{a} \pm 0.26$
	C20:3n6	$0.2^{b} \pm 0.004$	$0.3^{a} \pm 0.03$	$0.3^{a} \pm 0.03$	$0.2^{a} \pm 0.03$	$0.1^{c} \pm 0.002$	$0.3^{a} \pm 0.03$	$0.3^{ab} \pm 0.03$	$0.2^{b} \pm 0.02$
	C20:4n6	$0.7^{c} \pm 0.04$	$4.6^{ab} \pm 0.55$	$4.9^{a} \pm 0.41$	$3.5^{b} \pm 0.50$	$0.7^{c} \pm 0.03$	$4.9^{a} \pm 0.44$	$4.5^{ab} \pm 0.47$	$3.5^{b} \pm 0.31$
	C20:5n3	$0.2^{c} \pm 0.01$	$1.0^{a} \pm 0.11$	$0.9^{a} \pm 0.07$	$0.7^{b} \pm 0.06$	$0.2^{c} \pm 0.01$	$1.0^{a} \pm 0.11$	$0.9^{ab} \pm 0.07$	$0.8^{b} \pm 0.04$
	C22:2n6	$0.2^{b} \pm 0.02$	$0.4^{a} \pm 0.07$	$0.4^{a} \pm 0.04$	$0.4^{a} \pm 0.05$	$0.2^{c} \pm 0.01$	$0.5^{a} \pm 0.03$	$0.5^{ab} \pm 0.04$	$0.4^{b} \pm 0.03$
	C22:6n3	$0.1^{a} \pm 0.01$	$0.2^{a} \pm 0.03$	$0.2^{a} \pm 0.03$	$0.1^{a} \pm 0.02$	$0.1^{a} \pm 0.01$	$0.1^{ab} \pm 0.01$	$0.1^{b} \pm 0.01$	$0.1^{b} \pm 0.01$
Totals	SFA	$37.3^{b} \pm 0.69$	$47.0^{a} \pm 2.32$	$35.9^{b} \pm 1.76$	$35.7^{b} \pm 2.08$	$37.5^{b} \pm 0.98$	$45.3^{a} \pm 2.23$	$35.^{5bc} \pm 1.49$	$30.8^{c} \pm 1.82$
	MUFA	$43.9^{a} \pm 0.63$	$29.5^{d} \pm 1.17$	$34.9^{c} \pm 0.58$	$37.9^{b} \pm 0.55$	$43.4^{a} \pm 0.57$	$29.2^{d} \pm 1.14$	$35.7^{c} \pm 0.93$	$40.4^{b} \pm 0.98$
	PUFA	$18.8^{c} \pm 0.35$	$23.6^{bc} \pm 2.54$	$29.2^{a} \pm 1.67$	$26.5^{ab} \pm 2.07$	$19.1^{b} \pm 0.49$	$25.6^{a} \pm 1.83$	$28.9^{a} \pm 1.41$	$28.8^{a} \pm 1.36$
	PUFA:SFA	$0.5^{b} \pm 0.02$	$0.5^{b} \pm 0.09$	$0.8^{a} \pm 0.08$	$0.8^{a} \pm 0.09$	$0.5^{b} \pm 0.03$	$0.6^{b} \pm 0.08$	$0.8^{a} \pm 0.07$	$1.0^{a} \pm 0.11$
	n-6	$16.9^{b} \pm 0.31$	$18.1^{ab} \pm 1.99$	$22.1^{a} \pm 1.34$	$19.^{5ab} \pm 1.65$	$17.2^{b} \pm 0.44$	$19.7^{ab} \pm 1.45$	$21.9^{a} \pm 1.19$	$21.2^{a} \pm 1.09$
	n-3	$1.9^{c} \pm 0.05$	$5.4^{b} \pm 0.56$	$7.2^{a} \pm 0.37$	$7.0^{a} \pm 0.43$	$1.9^{c} \pm 0.06$	$5.8^{b} \pm 0.40$	$7.0^{a} \pm 0.29$	$7.5^{a} \pm 0.27$
	(n-6)/(n-3)	$8.8^{a} \pm 0.16$	$3.3^{b} \pm 0.08$	$3.1^{bc} \pm 0.09$	$2.8^{c} \pm 0.09$	$8.8^{a} \pm 0.17$	$3.4^{b} \pm 0.08$	$3.1^{bc} \pm 0.13$	$2.8^{c} \pm 0.07$

∗[a,b,c,d] Means in the same row with different superscript are significantly different ($P \leq 0.05$).
Control, *FR1*, *FR2* and *FR3* contained 20% pork fat, 10% fat replacer, 20% fat replacer and 30% fat replacer, respectively.

Table 3.5 Mean compositions (%) of the fatty acids identified in the ingredients

		Ingredients		
		Blesbok meat	**Pork back fat**	**Fat replacer**
SFA	C14:0	1.0	1.4	8.9
	C15:0	0.5	0.1	0.1
	C16:0	17.9	24.6	5.4
	C18:0	35.5	22.4	2.7
	C20:0	3.1	0.7	0.5
	C21:0	0.3	0.4	0.2
	C22:0	0.8	0.1	0.3
	C24:0	0.7	0.1	0.3
MUFA	C14:1	0.3	0.0	0.0
	C16:1	1.1	1.7	0.5
	C18:1n9c	15.9	35.9	53.2
	C20:1	0.1	0.4	1.0
	C24:1	2.4	0.1	0.2
PUFA	C18:2n6c	10.2	11.0	17.6
	C18:3n3	3.6	0.8	8.9
	C20:3n6	0.3	0.0	0.0
	C20:4n6	4.4	0.1	0.1
	C20:5n3	1.1	0.0	0.0
	C22:2n6	0.5	0.0	0.1
	C22:6n3	0.2	0.0	0.0
Totals	SFA	59.6	49.8	18.3
	MUFA	20.0	38.1	55.0
	PUFA	20.4	12.1	26.8
	PUFA:SFA	0.3	0.2	1.5
	n-6	15.5	11.2	17.9
	n-3	4.9	0.9	8.9
	(n-6)/(n-3)	3.1	12.7	2.0

No standard errors were indicated as the test was only conducted on the original mixed ingredient.

3.5 Discussion

3.5.1 Proximate results

The fat replacer added significant moisture to the fat replacer treatments and thus impacted on the overall moisture of the experimental products as it contained 86.2% moisture. The fat replacer was made from an animal protein (gelatine) and hydrocolloid (alginate) with both ingredients having a large water binding capacity (Gómez-Guillén *et al.*, 2011; Lee & Mooney, 2012). Although, the protein content of the three *FR* treatments differed ($P \leq 0.05$) from one another, a trend was observed

where the protein content decreased as the fat replacer increased due to the decreased volume of meat used (Table 3.3). The average protein content of blesbok is between 22-24% (Hoffman & Wiklund, 2006; Hoffman *et al.*, 2008) compared to the fat replacer which contained 5.2% (Table 3.3). The *Control* and *FR2* samples contained the same amount of blesbok meat, however, *FR2* had a higher ($P \leq 0.05$) protein content which indicates that the fat replacer has a positive effect on the protein content of the product. The *Control's* fat content was far higher than that of the three *FR* treatments (by approximately 20%). However, cabanossi traditionally contains high levels of pork fat, therefore the high fat content in the *Control* was typical for this product. The three *FR* treatments did not differ ($P \leq 0.05$) from one another as pertaining to their total fat content; this may be due to blesbok meat containing low levels of fat (Neethling *et al.*, 2014). The large reduction in fat content could appeal to consumers who consume large amounts of processed meat products. The *Control* had the smallest ash content due to the pork back fat that contained approximately 0.1% ash (Jones *et al.*, 2015). This would suggest that the fat replacer contained larger amounts of inorganic molecules and therefore the *FR* treatments had larger amounts of ash than the *Control*.

3.5.2 Lipid oxidation

The blesbok cabanossi was tested at day 0 and day 60 using the thiobarbituric acid-reactive substances (TBARS) method which tests for the presence of malondialdehyde (MDA), as well as other reactive substances (hydroperoxides and conjugated aldehydes). It was observed that the *Control* did not differ ($P > 0.05$) from the three *FR* treatments at day 0 and day 0 (Table 3.4). At day 0, the mean TBA values for the three *FR* treatments were higher ($P \leq 0.05$) compared to day 60 (Table 3.4). In addition, the *Control* had a lower ($P > 0.05$) mean TBA value at day 0 than at day 60. The curing salts added to the cabanossi's antioxidant capacity which could potentially have a great impact on lipid oxidation over time. Unreacted nitrite is able to react with MDA leading to the nitrosation of MDA which creates an unreactive product. In addition, MDA is a liable compound when it is stored for long periods of time (Wheatley, 2000; Ulu, 2004). Therefore, it can been postulated that the decrease in mean TBA values for all three *FR* treatments over a period of 60 days could be due to the nitrosation of MDA or the decrease in MDA as it broke down to form other unreactive products. Furthermore, it was postulated that the *Control's* lipid oxidation did not decrease due to the *Control* containing larger amounts of fat.

3.5.3 Fatty acid composition

It was expected that the *Control* (containing 20% pork back fat) would have the highest SFA as animal fat naturally comprises of a high SFA content (Krauss *et al.* 2000). The *Control's* higher C16:0 values can be attributed to the pork back fat added. The pork back fat contained 24.6% in comparison to the blesbok meat which contained 17.9% (Table 3.5). However, at day 0 and day 60 the *FR1* (contains 10% FR) had the largest SFA composition (47.0%; $P \leq 0.05$; Table 3.4), i.e. although the *FR1* had the largest quantity of blesbok meat (Table 3.1). Therefore, the contribution to the high SFA composition found in *FR1* was due to the high stearic acid (C18:0) composition in the blesbok meat, as well as the high myristic acid (C14:0) found in the fat replacer (Table 3.5).

Vegetable oils such as canola oil are abundant in oleic acid (C18:1n9) which can increase the MUFA content of meat and processed meat products (Siebert *et al.*, 1993; Senanayake *et al.*, 2014). Oleic acid was the most abundant MUFA present in the *Control* and all three *FR* treatments. The fat replacer contained high levels of canola oil therefore, it would be expected that the oleic acid composition increased as the amount of fat replacer increased due to the increased content of canola oil in the fat replacer (Table 3.5). At day 0 and day 60, the *Control* had the highest oleic acid composition with the *FR* treatments' composition increasing ($P \leq 0.05$) as more of the fat replacer was added (Table 3.4). Furthermore, it would be expected that the oleic composition would be greater in *FR3* (contains 30% fat replacer) than in *FR1* (contains 10% fat replacer).

At day 0 and day 60, the total PUFA content comprised mainly of linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) for the *Control* and all three *FR* treatments (Table 3.4). The fat replacer itself had the largest composition of these two fatty acid (17.6% and 8.9%), respectively (Table 3.5) and as the fat replacer increased in the cabanossi, the linoleic and α-linolenic acid percentages increased. Furthermore, at day 0 and day 60 the linoleic and α-linolenic acids contributed the highest amount to the omega-6 (71-94%) and omega-3 (79-89%) percentages, respectively (Table 3.4). Arachidonic acid (AA; C20:4n6) is commonly found in meat (Table 3.5), and it was found at day 0 and day 60 that the composition decreased as the meat content decreased. Docosahexaenoic acid (DHA) is of importance as it is an essential omega-3 fatty acid which also contributes towards the n-6:n-3 ratio (Wertz, 2009). DHA has a susceptibility to oxidise due to its poly-unsaturated structure, therefore the decrease ($P \leq 0.05$) in the composition between day 0 (0.14%) and day 60 (0.09%) was expected (Table 3.4) (Meadus *et al.*, 2010).

The concern with the "Western diet" revolves around the excessive consumption and eventual consequences of consuming foods with a high n-6:n-3. However, the n-6:n-3 ratio can be positively affected by the addition of linoleic and α-linolenic acids alongside other long chain PUFA's such as eicosapentaenoic acid (EPA) and DHA. Scollan *et al.* (2006) and Ospina *et al.* (2012) noted that the

recommended PUFA:SFA ratio was >0.4 and the n-6:n-3 ratio should be <4 with Raes *et al*. (2004) recommending a PUFA:SFA ratio of >0.7 and an n-6:n-3 ratio of <5. At day 0 and day 60, the *Control* and *FR1* did not differ (P > 0.05) with both having a PUFA:SFA ratio >0.4 but >0.7. In contrast, *FR2* and *FR3* exceeded the 0.7 recommendation. At day 0 and day 60, the *Control* had an n-6:n-3 ratio of >8 which exceeded the recommendation, however, *FR1* and *FR2* had an n-6:n-3 ratio of <3.4 and *FR3* a n-6:n-3 ratio <2.9 (Table 3.4). Therefore, all the *FR* treatments n-6:n-3 ratios were within the recommended dietary guidelines which would appeal to the health conscious consumer.

3.6 Conclusions

The reduction of fat in processed meat products can be challenging due to complexity in mimicking the animal fat properties in the cabanossi. The objective was to reduce the fat content and improve the unsaturated fatty acid composition of a game cabanossi using a fat replacer containing canola oil. The fat content was lower ($P \leq 0.05$) in all three *FR* treatments when compared to the *Control*. The proximate analyses revealed that the fat replacer reduced the fat content by ~20%. In addition, the protein for all three *FR* treatments were higher ($P \leq 0.05$) than the *Control*. Lipid stability indicated that the lipid oxidation values did not differ ($P > 0.05$) between the *Control* and the three *FR* treatments, however, the three *FR* treatments differed ($P \leq 0.05$) between day 0 and day 60 which could be due to the nitrite providing an antioxidant effect. The fatty acid results revealed that a period of 60 days storage showed no difference ($P > 0.05$) in fatty acid composition. In this research only 5% canola oil was added to the fat replacer. Although such a low percentage of canola oil was used, the PUFA:SFA and n-6:n-3 ratios for the *FR* treatments were considerably greater than that of the *Control*. The canola oil seemed to provide a balanced MUFA and PUFA content aiding in the improved fatty acid ratios of the *FR* treatments. The PUFA:SFA and n-6:n-3 ratios indicated that the canola oil had a positive impact on the fatty acid composition. The overall comparison showed that the *Control* obtained an undesirable high fat content and poor fatty acid ratio where the *FR* treatments, especially *FR2* and *FR3*, showed a favourably low fat content and acceptable fatty acid ratios. Overall, the *FR* treatment that showed the most desirable chemical profile (proximate analysis, lipid oxidation and fatty acid composition) was *FR2* (contains 20% fat replacer). Furthermore, a comparison to the *Control* highlighted *FR2's* low fat content and improved fatty acid composition.

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Chapter 4

The effect of a fat replacer on the sensory profile of blesbok (*Damaliscus pygargus Phillipsi*) cabanossi

4.1 Abstract

Traditionally, processed meat products contain large amounts of animal fat (~25%) that consist of saturated fatty acids which could impact negatively on human health; therefore an increasing trend in the meat industry is to reduce the fat by means of fat replacers. A blesbok cabanossi was made using a protein-hydrocolloid gel which acted as the fat replacer. The *Control* (20% pork back fat) was compared to three fat replacer (*FR*) treatments, i.e. 10% (*FR1*), 20% (*FR2*) and 30% (*FR3*). The latter three treatments contained no pork back fat. Descriptive sensory analysis was performed alongside instrumental texture profile analysis. Within the study the sensory attributes fatty aroma, fatty flavour and fattiness all had strong correlations ($r > 0.985$). At day 0 and day 60, it was evident that the *Control* scored higher ($P \leq 0.05$) for all the fatty attributes. In terms of the cabanossi flavour, there was a strong correlation ($r > 0.981$) with the fatty attributes, as well as the smoky aroma and flavour attributes ($r > 0.935$) at day 0 and day 60. At day 0 and day 60, the *Control* had higher ($P \leq 0.05$) cabanossi flavour and smoky attribute scores compared to the three *FR* treatments. The reduced fat content of the three *FR* treatments resulted in higher intensity scores ($P \leq 0.05$) at day 0 and day 60 for meat spice flavour and saltiness compared to the *Control*. At day 60, the three *FR* treatments had a bitter taste which correlated reasonably strong ($r = 0.764$) with the texture attribute gelatinous texture.

The discriminant analysis (DA) plot at day 0, showed that the attributes chewiness, fat colour, fatty flavour, n-6:n-3 and PUFA:SFA had the greatest influence on the classification of the treatments with only one *FR2* replicate sharing similar characteristics as *FR3*. In a further DA plot at day 0, without considering the chemical attributes, one *FR2* replicate shared similar characteristics with *FR1*. The DA plot without the *Control* sample at day 0, showed that the attributes chewiness, instrumental hardness and saturated fatty acids had an influence on the classification. In addition, the DA plot without the chemical attributes, the plot indicated that one *FR1* replicate shared similar characteristics with *FR2*. The DA plot at day 60 showed that the attributes chewiness, gelatinous, fatty flavour and dispersion of fat had the greatest influence on the classification of the replicates. In this instance, one replicate from both *FR1* and *FR3* shared similar characteristics with *FR2*. At day 60, without considering the *Control*, the attributes meat spice flavour, bitter taste, chewiness, juiciness and gelatinous had the greatest influence on the classification and two replicates from *FR3* shared similar characteristics as *FR2* and *FR1*. In terms of physical characteristics at day 0 and day 60, the *Control* and *FR1* scored the highest value ($P \leq 0.05$) for instrumental hardness, instrumental

gumminess and instrumental cohesiveness. In contrast, *FR2* and *FR3* scored the lowest ($P \leq 0.05$) for these instrumental attributes. These results indicated that the texture of the fat replaced blesbok cabanossi was good however, the sensory profile needs to be addressed, especially given the fact that a perceptible bitter taste developed during storage. To conclude, the sensory profile of the *Control* was different ($P \leq 0.05$) to that of all three *FR* treatments which indicated that the fat replacer was not able to wholly mimic the appearance and texture characteristics of animal fat. The aroma and flavour of the *FR* treatments were also significantly different to that of the *Control*. This research adds valuable knowledge to the field of processed meat products and how fat replacers impact on the sensory profile of processed meat products such as cabanossi.

4.2 Introduction

Processed meat products can repurpose low quality or off-cut pieces of meat and add value to an otherwise low quality raw material. However, saturated fat content present in animal fat have raised health concerns among the general public and has impacted negatively on the image of processed meat products (Grasso *et al*., 2014). Therefore, processed meat products such as dry fermented and emulsified sausages can be regarded as being nutritionally undesirable as they contain animal fat with a high percentage of saturated fatty acids (SFA), i.e. rather than the healthier mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids. Unfortunately products with undesirable nutrition profiles influence the consumer's decision when purchasing processed meat products (Resurreccion, 2003; Del Nobile *et al*., 2009).

Based on the above-mentioned concerns there is a demand for reduced fat processed meat products. Reducing or replacing fat in these meat products has become a challenge as the substitute products need to mimic the appearance, texture and overall functionality that animal fat provides in processed meat products. An important criterion for reducing fat in processed meat products is that the sensory attributes such as aroma, appearance and texture of the "new" reformulated product need to be unaffected. If not, consumers will be hesitant in purchasing such products (Bech-Larsen & Grunert, 2003; Brewer, 2012). Other processed meat products' fats have however, been replaced successfully. Salcedo-Sandoval *et al.* (2013) partially substituted pork back fat with vegetable oil (olive oil) by combining the oil with konjac gel (polysaccharide produced from *Amorphophallus konjac*). The results showed that even though the hardness had increased, the sensory quality was not affected. Garcia-Garcia & Totosaus (2008) added carrageenan and locust bean gum to low fat sausages which improved the texture and water retention with only minor effects on the colour of the product. Unfortunately, no sensory tests were conducted in the latter research therefore, there are no results on the sensory quality of these products.

There are a variety of processed meat products containing fat replacers, but there is little literature on the fat replacement of cabanossi. Cabanossi is a semi-dry smoked product that is slightly spiced. The product can be made in several different ways; generally the mixture consists of beef and pork (60:40 meat to fat ratio), as well as bacon (50:50 meat to fat ratio) which increases the fat content of the product to approximately 25% (Feiner, 2006; Schoon, 2012). Conducting descriptive sensory analysis (DSA) is a useful tool when assessing the full sensory profile of a new product such as a fat replaced game cabanossi. DSA uses trained panellists to quantify the flavour, texture, appearance and aroma of a product, thereby evaluating the similarity of treatments in terms of specific sensory attributes (Lawless & Heymann, 2010). DSA can also be used for detecting changes, such as rancidity, which can easily occur during shelf-life studies of processed meat products (Love & Pearson, 1971; Gray, 1978; Gray & Monahan, 1992; Min & Ahn, 2005). Studies such as these can assist a manufacturer to indicate the sensory drivers of the original product.

Physical tests are another way in which a product's physical properties can be measured, primarily to assess a product's structural stability over a period (shelf-life), again a vital quality control tool when it is important to determine the physical drivers of sensory quality in processed meat products, e.g. the firmness of meat products as in emulsified meat products (Schutte, 2008; Mapanda *et al*., 2015). It is well-known that the use of alternative ingredients in processed meat products can compromise the structural stability of a product. Some ingredients make the product hard and others soft, while other ingredients can easily result in the onset of oxidation, and thus the development of unwanted aromas/flavours (Mapanda *et al.*, 2015).

Instrumental texture analysis, especially texture profile analysis (TPA), is usually conducted when it is important to define a product's textural quality, whereas sensory analysis can be used to quantify a product's flavour and texture profile (Bourne, 2002; Lawless & Heymann, 2010). The data of these two methods of analysis can furthermore be correlated, primarily to determine instrumental drivers of sensory quality. Both analyses are able to provide good descriptive sensory and textural profiles of processed meat products and could definitely add knowledge to the database of processed meat products.

In view of this, the aim of this research project is to use descriptive sensory analysis as well as texture profile analysis to develop a full profile (appearance, aroma, flavour and texture) for the fat replaced cabanossi and compare the results to that of traditional cabanossi containing animal fat.

4.3 Materials and methods

4.3.1 Fat replacer

The cabanossi production, fat replacer and treatment recipes are described in Chapter 3.

4.3.2 Textural analysis

The texture properties of the respective cabanossi treatments were established using the Instron Universal Testing Machine and the texture profile analysis (TPA) Bluehill software (Instron UTM, Model 3345). A compression test was performed on a piece of cabanossi with a height of 15 mm and width of 20 mm. A circular platen of 25 mm was attached to a 5000 N load cell and the sample was compressed to 50% of its original height at a crosshead speed of 100 mm.min^{-1} twice in two cycles (Desmond & Troy, 2001). The following TPA parameters were tested: Hardness (N) = the maximum force required to compress the sample, cohesiveness (A_1/A_2), where A_1 = total energy of first compression and A_2 = total energy of second compression; an indication of the extent to which the sample could be deformed prior to rupture and, gumminess (N/cm^2) = the force required to disintegrate a semisolid meat sample for swallowing (Mendoza *et al.*, 2001).

4.3.3 Descriptive sensory analysis

The aroma, flavour and texture of the processed meat treatments were analysed at day 0 and day 60 using descriptive sensory analysis (DSA). A panel of 11 analysts were chosen, based upon previous experience with sensory analysis of meat and meat products, to analyse the respective treatments on day 0 and 10 assessors at day 60, of which 9 of the panel members were present at day 0. The panellists were trained according to the guidelines for sensory analysis of meat by the American Meat Science Association (AMSA, 1995), as well as using descriptive analysis techniques in accordance with Lawless & Heymann (2010). Specific reference standards (Table 4.1) were used during the four training sessions to train the panel in recognising the primary sensory descriptors (Table 4.2). Panel consistency was tested using the software programme PanelCheck (Version 1.3.2, www.panelcheck.com).

The panel received a 3 cm in length piece from each treatment for the training and testing sessions to analyse for aroma, appearance, flavour and texture. Attributes for the descriptors were scored on an unstructured 100 mm line scale with 0 = *No intensity* and 100 = *Extreme intensity* (Table 4.2). Training consisted of four sessions lasting approximately 90 min each. In the training sessions the terminology was discussed, as well as the scores given to each attribute. Blind test sessions consisted of six replicate sessions, the cabanossi pieces were served in glass ramekins, labelled with

random 3-digit codes, and presented to each panellist in a random order. For all the blind testing sessions the panellists sat in booths that contained *Compusense® five software* (Compusense, Guelph, Canada). 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 samples, the panellists received distilled water (21˚C), apple quarters and water biscuits (Carr, UK).

4.3.4 Statistical analysis

The study consisted of a randomised block design with four processed meat treatments and six replications per treatment. PanelCheck software (Version 1.3.2, www.panelcheck.com) was used to monitor DSA panel performance. The collected sensory data were also pre-processed for further application in multivariate analyses. The sensory, physical and chemical data were firstly subjected to test–retest analysis of variance (ANOVA) using SAS® software (Statistical Analysis System 2006, Version 9.2, SAS Institute Inc., Cary, NC, USA). . The Shapiro–Wilk test was performed to test for non-normality of residuals (Shapiro & Wilk, 1965). Correlation coefficients were calculated for the sensory, physical and chemical data by means of the Pearson's correlation coefficient (r) (Snedecor & Cochran, 1980). Principal component analysis (PCA), using the correlation matrix, was performed and used in conjunction with discriminant analysis (DA) in order to indicate and clarify the relationships between the sensory, physical and chemical data (Næs, Brockhoff, & Tomic, 2010). The latter multivariate analyses were conducted using XLSTAT software (Version 2012, Addinsoft, New York, USA).

Table 4.1 References and definitions used to validate the terms for each attribute during DSA training

Attributes	Term	Reference standard	Scale
Appearance	**Meat colour**	Intensity of red-brown meat colour where *Spar* cabanossi = 70	0 = Light red-brown 100 = Dark red-brown
	Shiny	The amount of light reflection from the surface of the sample where *Hartlief cabanossi* = 60	0 = Dull 100 =Shiny
	Fat colour	Associated with fat where fresh pork fat = 100	0 = Dark 100 =White
	Dispersion of fat	Associated with small even/emulsified particle shape and size where *Ricomondo frankfurter* = 0; Associated with large particle shape and size where *Spar beef droëwors* = 100	0 = Even/ Emulsified 100 = Coarse
Aroma	**Smoky_A**	Associated with oak smoked meat products where *Ricomondo frankfurters* = 30, Oak stave and trimmings = 100	*0 = None *100 = Extreme
	Gamey_A	Associated with game/wild animal where *Spar kudu droëwors* = 100	
	Fatty_A	Associated with fat where fresh pork fat = 100	
	Rancid_A	Associated with oxidised fat	
Flavour	**Smoky_F**	Associated with oak smoked meat products where *Ricomondo frankfurters* = 30	*0 = None *100 = Extreme
	Fatty_F	Associated with fat where *Hartlief cabanossi* = 100	
	Meat spice_F	Flavour associated with a solution containing 2% spice	
	Gamey_F	Associated with game/wild animal where *Spar kudu droëwors* = 100	
	Cabanossi_F	Associated with a smoked cooked meat product, meat spice and semi-dried where *Hartlief cabanossi* = 50	
	Rancid_F	Associated with oxidised fat	
	Saltiness	Associated with sodium chloride solution	
	Bitter taste	Associated with caffeine solution	
Texture	**Chewiness**	The ease which the *wors* falls apart when biting	0 = Soft 100 = Chewy
	Coarseness	The perception of coarse pieces in the product while chewing	0 = Not coarse 100 = Very coarse
	Juiciness	Amount of fluid exuded upon chewing / perception of moisture release in product when chewing where *Hartlief cabanossi* = 100	0 = Dry 100 = Very juicy
	Fattiness	Associated with fat where *Hartlief cabanossi* = 100	0 = None 100 = Extreme
	Gelatinous	Associated with a jelly-like consistency where 10 g gelatine per 250 mL water = 100	0 = None 100 = Abundant
	Crumbly	The way the product disintegrates upon chewing, where crumbly = disintegrates into abundant small pieces. Coarse *mieliepap* = 100	0 = Not crumbly 100 = Very crumbly

*Line scale range applies to aroma and flavour attributes (0 = No intensity, 100 = Extreme intensity).

Table 4.2 Attributes and descriptions used for descriptive sensory analysis

	Attributes	Descriptions	Scale
Appearance	**Meat colour**	Look at the outside surface	0 = Light Red-brown 100 = Dark Red-Brown
	Shiny	Light reflection	0 = Dull 100 = Shiny
	Fat colour	Look at fat on the cut surface of the sample	0 = Darker 100 = White
	Dispersion of fat	How the fat is dispersed in the meat	0=Even/Emulsified 100 = Coarse
Aroma	**Smoky_A**	Associated with an oak smoked product	*0 = None *100 = Extreme
	Gamey_A	Associated with game/wild animal	
	Fatty_A	Associated with fat	
	Rancid_A	Associated with oxidised fat	
Flavour	**Smoky_F**	Associated with wood smoked meat product	*0 = None *100 = Extreme
	Fatty_F	Associated with fat	
	Meat spice_F	Flavour associated with added spice	
	Gamey_F	Associated with game/wild animal	
	Rancid_F	Associated with oxidised fat	
	Cabanossi_F	Associated with a smoked cooked meat product, meat spice and semi-dried	
	Saltiness	Associated with sodium chloride solution	
	Bitter taste	Associated with caffeine solution	
Texture	**Chewiness**	The ease which the *wors* falls apart when biting	0= Soft 100= Chewy
	Coarseness	The perception of coarse pieces in the product while chewing	0 = Not coarse 100 = Very coarse
	Juiciness	The perception of moisture in the product while chewing	0 = Dry 100 = Very juicy
	Fattiness	Leaves a fatty mouthfeel on the palate	0= None 100= Extreme
	Gelatinous	Having a jelly-like consistency	0 = None 100 = Abundant
	Crumbly	Old dry bread that crumbles	0 = Not crumbly 100 = Very crumbly

*Line scale range applies to aroma and flavour attributes (0 = None, 100 = Extreme).

4.4 Results

The sensory profile results for day 0 and day 60 are showed in Table 4.3 and Table 4.4, respectively. The PCA bi-plot for day 0 (Figure 4.1) showed a distinct difference between the *Control* (with pork back fat) and the three fat replacer (*FR*) treatments. The attributes on the right side of the PCA bi-plot (F1) are strongly associated with the *Control*. The attributes that associate with these high fat content products are primarily fatty aroma ("Fatty_A"), fatty flavour ("Fatty_F"), and Fattiness, these sensory attributes were all strongly correlated ($P \leq 0.05$; $r > 0.99$; Table 4.5). Table 4.3 showed that all these attributes were higher in intensity for the *Control* sample ($P \leq 0.05$) for day 0, i.e. "Fatty_A" (34.8), "Fatty_F" (36.7) and Fattiness (27.1) when compared to that of the three *FR* treatments ("Fatty_A" <7.7; "Fatty_F" <5.1; "Fattiness" <4.5). The fatty acid attributes (SFA, MUFA and PUFA) on the right side of the PCA bi-plot (F1) at day 0 (Figure 4.1) showed a strong correlation ($P \leq 0.05$) with the high fat percentage ($r > 0.994$) of the *Control*, as well as the "Fatty_A", "Fatty_F" and Fattiness attributes ($r > 0.979$) typically associated with high fat content products (Table 4.5). In addition, the PCA bi-plot without the chemical attributes at day 0 (Figure 4.2), showed no positional change in any of the sensory attributes. The PCA bi-plot for day 60 (Figure 4.3) showed the same strong correlations ($r > 0.985$) between the "Fatty_A", "Fatty_F" and Fattiness attributes (Table 4.6). As indicated in Table 4.4 the *Control* also illustrated higher scores ($P \leq 0.05$) for day 60 ("Fatty_A" = 32.0, "Fatty_F" = 36.0; Fattiness = 32.7) than the three *FR* treatments ("Fatty_A" <2.8, "Fatty_F" <1.1; Fattiness <0.8). This tendency was anticipated as the *Control* contained 23.50% fat and all three of the *FR* treatments had a fat percentage of <3.5% (Chapter 3). At day 60, the PCA bi-plot with and without the chemical attributes, showed the same trend as previously seen in day 0 (Figures 4.2 & 4.4, respectively).

Cabanossi flavour ("Cabanossi_F"), represents the typical overall flavour associated with cabanossi as an example of a processed meat product (Table 4.2). "Cabanossi_F" for day 0 (Figure 4.1) and day 60 (Figure 4.3) both associated strongly with the *Control* on F1, similarly this attribute correlated strongly ($P \leq 0.05$) with the fatty and smoky aromas and flavours ($r > 0.981$; > 0.935, respectively) (Tables 4.5 & 4.6). Table 4.3 showed that the smoky aroma ("Smoky_A") and flavour ("Smoky_F") for *Control* had higher ($P \leq 0.05$) scores for day 0 ("Smoky_A" = 82.9 and "Smoky_F" = 76.1) than the three *FR* treatments ("Smoky_A" <72.9 and "Smoky_F" <56.2). Moreover, the *Control* for day 60 (Table 4.4) showed higher ($P \leq 0.05$) scores for "Smoky_A" (85.4) and "Smoky_F" (76.6) than for the three *FR* treatments ("Smoky_A" <73.1 and "Smoky_F" <58.6).

The three *FR* treatments (Figure 4.1; 4.3) were associated with "Meat spice_F" and Saltiness, as well as Gelatinous and Crumbly texture. The attributes "Meat spice_F" and Saltiness had a moderate correlation ($P \leq 0.05$; $r = 0.67$) for day 0, however, on day 60 it had a significantly stronger

correlation ($P \leq 0.05$; $r = 0.904$) (Tables 4.5 & 4.6, respectively). The three *FR* treatments at day 0 and day 60 (Tables 4.3; 4.4) showed higher ($P \leq 0.05$) scores for "Meat spice_F" (<18.9; <23.5, respectively) compared to the *Control* (15.5; 19.1, respectively). Saltiness at day 0 (Table 4.3) had similar scores for all three *FR* treatments (Saltiness <19.1) which were higher ($P \leq 0.05$) than those of the *Control* (Saltiness 18.1), thus, Saltiness was definitely associated with the *FR* treatments (Table 4.3). The day 60 PCA bi-plot (Figure 4.3) showed the same trend for Saltiness, however, the *FR* treatment scores for Saltiness were greater (Table 4.4; <21.7) than their counterparts on day 0.

Crumbly for day 0 and day 60 (Figure 4.1; 4.3) as indicated in the PCA bi-plots showed an association with all three *FR* treatments, indicating that this is an inherent textural attribute of *FR* treatments. In addition, at day 60 (Table 4.6) a good correlation ($P \leq 0.05$) between Crumbly and Gelatinous ($r = 0.923$) was found. Chewiness scores for day 0 and day 60 (Table 4.3; 4.4) were quite similar, and both shelf-life days showed that *FR1* obtained the largest score and *FR3* the lowest score for this attribute. The PCA bi-plot for day 60 (Figure 4.3) showed an additional attribute, Bitter taste. Table 4.4 showed that although at day 60 the Bitter taste was present in the *FR* treatments (<4.4), the scores were low indicating that this basic taste attribute was barely, but just detectable. The Bitter taste was thus only associated with the three *FR* treatments and had a good correlation ($P \leq 0.05$; $r = 0.764$) with the sensory attribute Gelatinous (Table 4.6).

Discriminative analysis (DA) is used by researchers as a classification technique (Lawless and Heymann, 2010), primarily to ascertain which attributes drive classification. In this research project stepwise model selection (forward) was used to ascertain which variables were selected. The DA variables plot at day 0 (Figure 4.5a) showed a clear distinction between the sensory and chemical attributes that influenced the *Control* and the three FR treatments. The sensory attributes Chewiness, Fat colour, "Fatty_F", and with the addition of the omega-6 and omega-3 ratio (n-6:n-3), had the greatest influence in the *Control's* position with the poly-unsaturated and saturated fatty acid ratio (PUFA:SFA) influencing the *FR* treatment's position (Figure 4.5a). The DA replicates plot showed a clear grouping between the *Control* and all three of the FR treatments (Figure 4.5b) with the cross-validation table indicating that one of the *FR2* replicates shared characteristics with *FR3*. The same trend was observed for the DA variables plot without chemical attributes at day 0 (Figure 4.6a; 4.6b), however, the cross-validation table indicated that one of the *FR2* replicates shared characteristics with *FR1*. *FR2* was thus not classified correctly in all instances. This resulted in an overall 95.83% correct classification. The shift could have been influenced by the chemical attribute "PUFA:SFA" that had been removed from the DA variables plot (Figure 4.6a) during stepwise model selection.

The *Control* sample was then removed from the DA in order to separately identify which attributes associated and had the greatest influence on each of the three FR treatments. The DA variables plot without the control at day 0 (Figure 4.7a) showed a clear distinction between sensory

and chemical attributes that influenced each *FR* treatment. The attributes Chewiness, Instrumental Hardness ("Inst. Hardness") and SFA all had an influence on the FR treatments' position and were greatly associated with *FR1* (Table 4.3 & Chapter 3, Table 3.4). The DA replicates plot without the *Control* at day 0 (Figure 4.7b) showed a clear distinction between *FR1* with 10% fat replacer and *FR3* with 30% fat replacer, however, *FR2* was situated in between as indicated on Figure 4.7b. However, the cross-validation table indicated a 100% correctness of classification, showing that all the replicates were associated with their corresponding treatments. The same trend was observed for the DA variables plot without the *Control*, as well as the chemical attributes at day 0 (Figure 4.8a; 4.8b) with the attributes in similar positions, however, the cross-validation showed a shift in one *FR1* replicate which shared characteristics with *FR2* and thus a final correctness of classification value of 94.44%. The shift could have been influenced by the fact that the stepwise model selection was conducted considering only the sensory and physical attributes.

The DA variables plot at day 60 (Figure 4.9a) showed a clear distinction between sensory and chemical attributes that influenced the *Control* and the three *FR* treatments. The attributes Chewiness, Gelatinous, "Fatty_F" and Dispersion of fat had the greatest influence on the treatments' positions. The DA replicates plot at day 60 (Figure 4.9b) showed a clear distinction between the *Control* and the three *FR* treatments. In addition, the cross-validation table (91.7%) showed that one replicate from both *FR1* and *FR3* shared similar characteristics with *FR2*. The same trend was observed for the DA variables plot, DA replicates plot and accompanying cross-validation table without the chemical attributes at day 60 (Figure 4.10a, 4.10b).

Once again, the *Control* was removed in order to separately identify which attributes associated and had the greatest influence on each of the three *FR* treatments for day 60. In the DA variables plot without the *Control* at day 60 (Figure 4.11a), the attributes "Meat spice_F", Bitter taste, Chewiness, Juiciness and Gelatinous had the greatest influence on the positions of the treatments. The DA replicates plot without the *Control* at day 60 (Figure 4.11b) showed a grouping between *FR2* and *FR3* with *FR1* standing separately. Furthermore, the cross-validation table, indicating a correctness of classification of 88.9%, showed that two replicates from *FR3* were associated with *FR2* and that *FR1* had no association with any of the other *FR* treatments. The same trend was observed for the DA variables plot, DA replicates plot and the accompanying cross-validation table without the *Control* and the chemical attributes at day 60 (Figure 4.12a; 4.12b).

The physical attributes of the *Control* and treatments at day 0 and day 60 were tested using a compression test. Instrumental hardness, gumminess and cohesiveness were the parameters that were tested (Table 4.7 gives the breakdown for all P-values obtained for the main and interaction effects). Table 4.8 showed a difference ($P \leq 0.05$) between the treatments at day 0 and day 60 with regard to the physical parameters tested. Instrumental hardness ("Inst. Hardness") had a reasonably good

correlation ($P \leq 0.05$) with Chewiness ($r = 0.718$; 0.500) for day 0 and day 60, respectively where "Inst. Hardness" showed the same trend as Chewiness (Table 4.5; 4.6). Figure 4.13 indicated that at day 0 and day 60 the "Inst. Hardness" for the *Control* and *FR1* were similar but both were significantly different from *FR2* and *FR3* with the latter having obtained the lowest score (Table 4.8); Day had an influence ($P \leq 0.05$) irrespective of treatment (Figure 4.14). Instrumental gumminess (Inst. Gumminess) differed ($P \leq 0.05$) between day 0 and day 60 but not between the different treatments (Table 4.8) therefore, a combined means graph was not included. In addition, the same trend was observed were there was a difference ($P \leq 0.05$) between day 0 and day 60 irrespective of treatment (Figure 4.15). Instrumental cohesiveness (Inst. Cohesiveness) differed ($P \leq 0.05$) between treatments, as well as at day 0 and day 60 (Table 4.8); "Inst. Cohesiveness" for the *Control* and *FR1* were similar but both were different ($P \leq 0.05$) from *FR2* and *FR3* with the latter having the lowest score (Figure 4.16). Furthermore, the same trend that was observed for "Inst. Hardness" and "Inst. Gumminess" where the difference ($P \leq 0.05$) between day 0 and day 60 were significant irrespective of treatment (Figure 4.17).

Table 4.3 Mean ± SE for the sensory attributes analysed from blesbok cabanossi (Day 0) with a pork fat (PF) control and three increasing levels of fat replacer (FR)

	Attributes	*Control* (20% PF)	*FR1* (10% FR)	*FR2* (20% FR)	*FR3* (30% FR)
Appearance	**Meat colour**	$70.1^{a} \pm 0.4$	$58.9^{b} \pm 1.3$	$57.3^{bc} \pm 1.1$	$53.7^{c} \pm 1.2$
	Shiny	$74.1^{a} \pm 1.1$	$34.0^{b} \pm 2.6$	$37.6^{b} \pm 2.5$	$36.4^{b} \pm 2.4$
	Fat colour	$89.1^{a} \pm 0.4$	$45.3^{c} \pm 1.3$	$48.5^{bc} \pm 1.2$	$49.3^{b} \pm 1.1$
	Dispersion of fat	$83.2^{a} \pm 1.1$	$48.8^{d} \pm 2.0$	$54.3^{c} \pm 1.5$	$60.6^{b} \pm 1.6$
Aroma	**Smoky_A**	$82.9^{a} \pm 1.2$	$72.9^{b} \pm 1.1$	$72.1^{b} \pm 1.0$	$71.2^{b} \pm 1.1$
	Gamey_A	$17.0^{a} \pm 1.2$	$8.4^{b} \pm 0.5$	$8.4^{b} \pm 0.5$	$8.3^{b} \pm 0.5$
	Fatty_A	$34.8^{a} \pm 1.0$	$6.1^{b} \pm 0.5$	$7.7^{b} \pm 0.9$	$6.5^{b} \pm 0.5$
Flavour	**Smoky_F**	$76.1^{a} \pm 0.8$	$56.2^{b} \pm 1.3$	$55.1^{bc} \pm 1.2$	$52.1^{c} \pm 1.3$
	Fatty_F	$36.7^{a} \pm 1.0$	$5.1^{b} \pm 0.8$	$4.2^{b} \pm 0.7$	$5.1^{b} \pm 0.7$
	Meat spice_F	$15.5^{b} \pm 0.7$	$18.6^{a} \pm 0.8$	$18.9^{a} \pm 0.7$	$18.2^{a} \pm 0.8$
	Gamey_F	$8.6^{a} \pm 1.1$	$3.4^{b} \pm 0.6$	$3.3^{b} \pm 0.6$	$3.3^{b} \pm 0.6$
	Cabanossi_F	$84.5^{a} \pm 1.1$	$44.0^{b} \pm 2.3$	$40.6^{bc} \pm 1.9$	$38.4^{c} \pm 2.0$
	Saltiness	$18.1^{b} \pm 0.5$	$19.1^{ab} \pm 0.5$	$19.8^{a} \pm 0.5$	$19.1^{ab} \pm 0.5$
Texture	**Chewiness**	$45.1^{ab} \pm 1.4$	$47.7^{a} \pm 1.3$	$43.4^{b} \pm 1.2$	$36.5^{c} \pm 0.8$
	Coarseness	$58.1^{a} \pm 1.7$	$45.3^{b} \pm 1.2$	$43.5^{b} \pm 1.3$	$43.1^{b} \pm 1.5$
	Juiciness	$64.4^{a} \pm 0.8$	$39.1^{b} \pm 1.5$	$41.4^{b} \pm 1.4$	$40.2^{b} \pm 1.3$
	Fattiness	$27.1^{a} \pm 0.8$	$4.4^{b} \pm 0.7$	$4.2^{b} \pm 0.7$	$4.5^{b} \pm 0.7$
	Crumbly	$17.5^{c} \pm 1.5$	$35.7^{b} \pm 1.6$	$36.4^{b} \pm 1.8$	$42.6^{a} \pm 1.8$

$*^{a,b,c,d}$ Means in a row with different superscripts are significantly different ($P \leq 0.05$). SE = Standard error. *Control* (contains 20% pork fat), *FR1* (contains 10% fat replacer), *FR3* (contains 20% fat replacer) and *FR3* (contains 30% fat replacer).

Table 4.4 Mean ± SE for the sensory attributes analysed from blesbok cabanossi (Day 60) with a pork fat (PF) control and three increasing levels of fat replacer (FR)

	Attributes	*Control* (20% PF)	*FR1* (10% FR)	*FR2* (20% FR)	*FR3* (30% FR)
Appearance	**Meat colour**	$69.3^{a} \pm 0.6$	$54.2^{b} \pm 1.5$	$54.0^{b} \pm 1.2$	$53.4^{b} \pm 1.2$
	Shiny	$74.3^{a} \pm 0.9$	$39.36^{c} \pm 1.6$	$44.7^{b} \pm 1.9$	$45.3^{b} \pm 1.7$
	Fat colour	$88.1^{a} \pm 0.5$	$45.6^{c} \pm 0.9$	$47.8^{bc} \pm 1.1$	$49.1^{b} \pm 1.0$
	Dispersion of fat	$78.8^{a} \pm 1.3$	$44.3^{d} \pm 1.2$	$53.4^{c} \pm 1.2$	$61.2^{b} \pm 1.9$
Aroma	**Smoky_A**	$85.4^{a} \pm 0.7$	$73.1^{b} \pm 0.7$	$73.0^{b} \pm 0.7$	$72.6^{b} \pm 0.8$
	Gamey_A	$9.9^{a} \pm 1.1$	$3.5^{b} \pm 0.6$	$3.6^{b} \pm 0.7$	$3.3^{b} \pm 0.6$
	Fatty_A	$32.0^{a} \pm 1.3$	$2.7^{b} \pm 0.7$	$2.2^{b} \pm 0.4$	$2.8^{b} \pm 0.7$
Flavour	**Smoky_F**	$76.6^{a} \pm 1.4$	$58.5^{b} \pm 1.4$	$58.6^{b} \pm 1.1$	$56.2^{b} \pm 1.1$
	Fatty_F	$36.0^{a} \pm 0.9$	$0.4^{b} \pm 0.2$	$1.0^{b} \pm 0.4$	$1.1^{b} \pm 0.4$
	Meat spice_F	$19.1^{b} \pm 0.6$	$23.3^{a} \pm 0.7$	$23.5^{a} \pm 0.7$	$23.1^{a} \pm 0.7$
	Bitter taste	$0.0^{c} \pm 0.0$	$2.4^{b} \pm 0.4$	$3.0^{b} \pm 0.6$	$4.4^{a} \pm 0.8$
	Cabanossi_F	$84.9^{a} \pm 0.8$	$43.6^{b} \pm 2.1$	$43.6^{b} \pm 2.0$	$42.2^{b} \pm 1.7$
	Saltiness	$17.6^{b} \pm 0.6$	$21.7^{a} \pm 0.4$	$21.3^{a} \pm 0.5$	$21.3^{a} \pm 0.5$
Texture	**Chewiness**	$43.7^{cb} \pm 1.5$	$49.0^{a} \pm 1.0$	$46.0^{ab} \pm 0.8$	$41.2^{c} \pm 1.0$
	Coarseness	$59.0^{a} \pm 1.2$	$41.4^{c} \pm 1.2$	$44.4^{bc} \pm 1.0$	$45.6^{b} \pm 1.4$
	Juiciness	$63.8^{a} \pm 1.4$	$34.6^{c} \pm 1.1$	$38.2^{b} \pm 1.2$	$37.0^{bc} \pm 1.2$
	Fattiness	$32.7^{a} \pm 1.0$	$0.02^{b} \pm 0.01$	$0.4^{b} \pm 0.3$	$0.8^{b} \pm 0.4$
	Gelatinous	$0.1^{c} \pm 0.1$	$8.7^{ab} \pm 1.3$	$7.2^{b} \pm 1.2$	$10.3^{a} \pm 1.2$
	Crumbly	$9.6^{c} \pm 1.6$	$23.8^{b} \pm 1.9$	$23.7^{b} \pm 1.8$	$28.1^{a} \pm 1.7$

$*^{a,b,c,d}$ Means in a row with different superscripts are significantly different ($P \leq 0.05$). SE = Standard error. *Control* (contains 20% pork fat), *FR1* (contains 10% fat replacer), *FR3* (contains 20% fat replacer) and *FR3* (contains 30% fat replacer).

Table 4.5 Pearson's correlation coefficients (r) displaying the relationship between the chemical parameters and positive aroma, flavour and texture attributes of all samples (N = 24) for day 0

	Smoky_A	**Fatty_A**	**Smoky_F**	**Fatty_F**	**Saltiness**	**Fattiness**	**Hardness**	***Fat %***
Fatty_A	0.920	**1**	0.963	**0.993**	-0.563	**0.992**	0.315	**0.982**
Cabanossi_F	**0.935**	**0.981**	**0.971**	**0.985**	-0.570	**0.983**	0.405	**0.983**
Meat spice_F	-0.797	-0.832	-0.815	-0.843	**0.670**	-0.809	-0.335	-0.845
Chewiness	0.373	0.229	0.384	0.230	-0.087	0.229	**0.718**	0.231
Fat %	0.892	0.982	0.950	**0.990**	-0.578	**0.986**	0.307	1.000
SFA	0.889	**0.979**	0.956	**0.987**	-0.624	**0.986**	0.336	**0.995**
MUFA	0.890	**0.981**	0.946	**0.988**	-0.576	**0.983**	0.287	**0.999**
PUFA	0.891	**0.979**	0.933	**0.987**	-0.529	**0.981**	0.294	**0.994**

Values in bold are different from 0 with a significance level alpha = 0.05. Chemical parameters are highlighted in purple.

Table 4.6 Pearson's correlation coefficients (r) displaying the relationship between the chemical parameters and positive aroma, flavour and texture attributes of all samples (N = 24) for day 60

	Smoky_A	Fatty_A	Smoky_F	Fatty_F	Saltiness	Fattiness	Gelatinous	Hardness
Fatty_A	0.953	**1**	0.923	**0.985**	-0.909	**0.988**	-0.905	0.312
Cabanossi_F	**0.968**	**0.987**	**0.948**	**0.996**	-0.921	**0.996**	-0.934	0.315
Meat spice_F	-0.853	-0.894	-0.845	-0.879	**0.904**	-0.884	0.832	-0.281
Chewiness	-0.188	-0.241	-0.085	-0.244	0.261	-0.252	0.018	**0.500**
Crumbly	-0.934	-0.909	-0.943	-0.927	0.850	-0.927	**0.923**	-0.404
Bitter taste	-0.721	-0.722	-0.709	-0.750	0.657	-0.742	**0.764**	-0.521
SFA	0.957	**0.980**	0.936	**0.987**	-0.882	**0.986**	-0.911	0.362
MUFA	0.948	**0.971**	0.910	**0.990**	-0.894	**0.985**	-0.908	0.311
PUFA	0.940	**0.962**	0.901	**0.987**	-0.887	**0.980**	-0.903	0.307

Values in bold are different from 0 with a significance level alpha = 0.05. Chemical parameters are highlighted in purple.

Table 4.7 P-values for main and interaction effects for all physical tests on day 0 and day 60

	Hardness (N)	**Gumminess (N/cm^2)**	**Cohesiveness (A_1/A_2)**
Treatment	**0.000044**	0.344278	**0.0002**
Day	**0.00022**	**0.000047**	**0.000056**
Treatment*Day	0.538247	0.542723	0.663235

The significant main and interaction effects ($P \leq 0.05$) are indicated in **bold.**

Table 4.8 Means (%) and standard error for physical analysis of cabanossi day 0 and day 60

	Treatment*Day		
	Hardness (N)	**Gumminess (N/cm^2)**	**Cohesiveness (A_1/A_2)**
Control	$61.7^{a} \pm 2.2$	$27.6^{a} \pm 4.7$	$0.60^{a} \pm 0.01$
***FR1* (10%)**	$65.7^{a} \pm 3.5$	$27.7^{a} \pm 5.0$	$0.58^{a} \pm 0.01$
***FR2* (20%)**	$52.1^{b} \pm 1.8$	$19.6^{a} \pm 3.2$	$0.54^{b} \pm 0.01$
***FR3* (30%)**	$40.7^{c} \pm 1.5$	$14.9^{a} \pm 2.5$	$0.51^{b} \pm 0.01$

*^{a,b,c} Means in a column with different superscripts are significantly different ($P \leq 0.05$). *Control* (contains 20% pork fat), *FR1* (contains 10% fat replacer), *FR3* (contains 20% fat replacer) and *FR3* (contains 30% fat replacer).

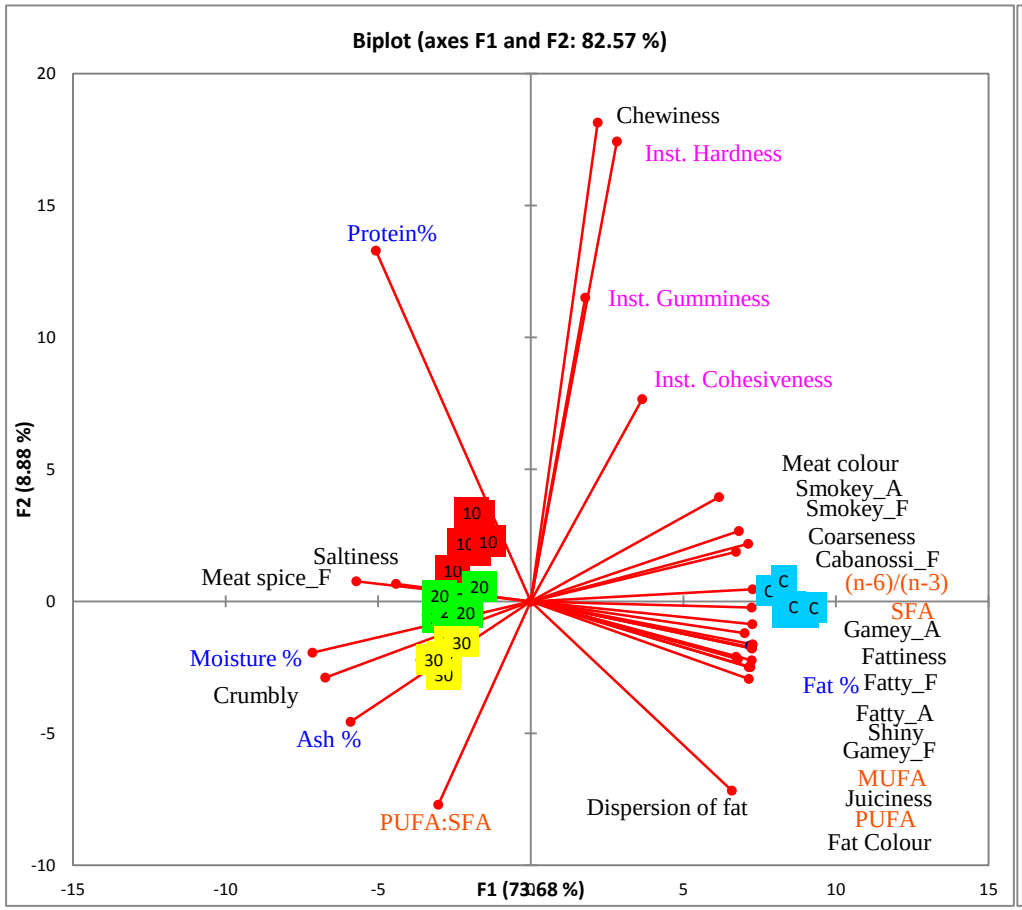


Figure 4.1 PCA bi-plot illustrating the sensory attributes, physical and chemical properties associated with the different cabanossi treatments at day 0. The abbreviations SFA, MUFA and PUFA refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, respectively, Inst. = Instrumental, 10 = Treatment 1 (10% FR); 20 = Treatment 2 (20% FR); 30 = Treatment 3 (30% FR); A = Aroma; F = Flavour and FR = Fat replacer.

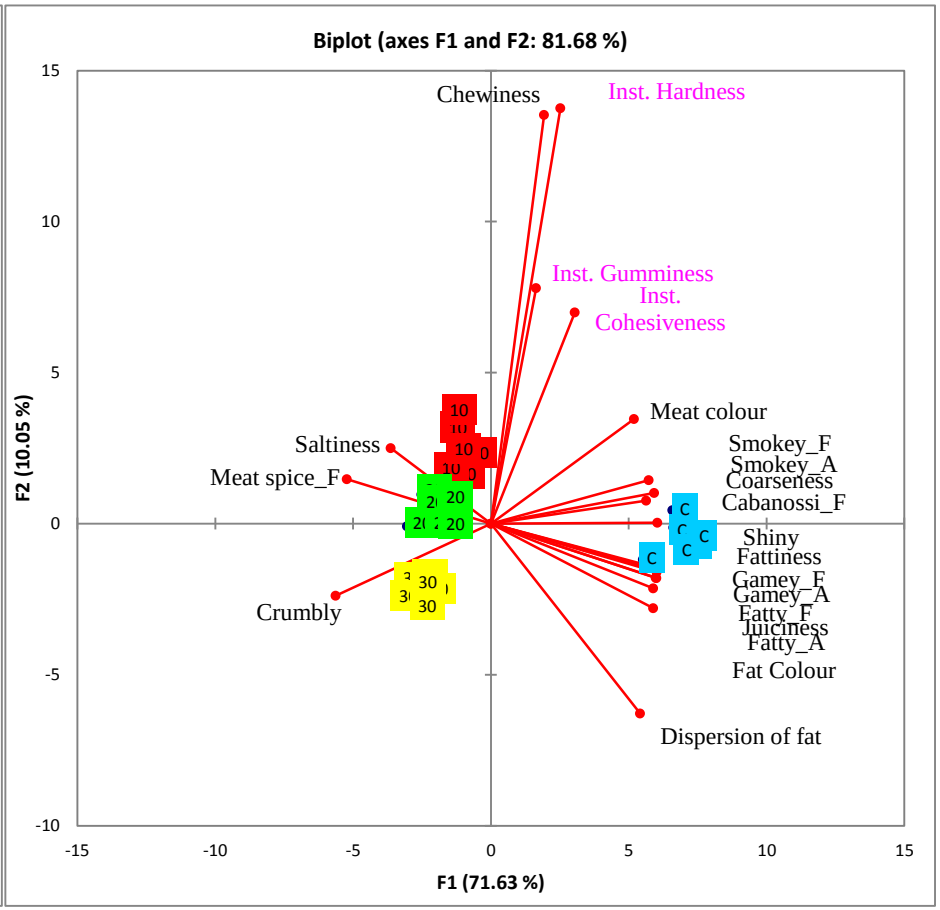


Figure 4.2 PCA bi-plot illustrating the sensory attributes and physical properties associated with the different cabanossi treatments at day 0. The abbreviations Inst. = Instrumental; 10 = Treatment 1 (10% FR); 20 = Treatment 2 (20% FR); 30 = Treatment 3 (30% FR); A = Aroma; F = Flavour and FR = Fat replacer.

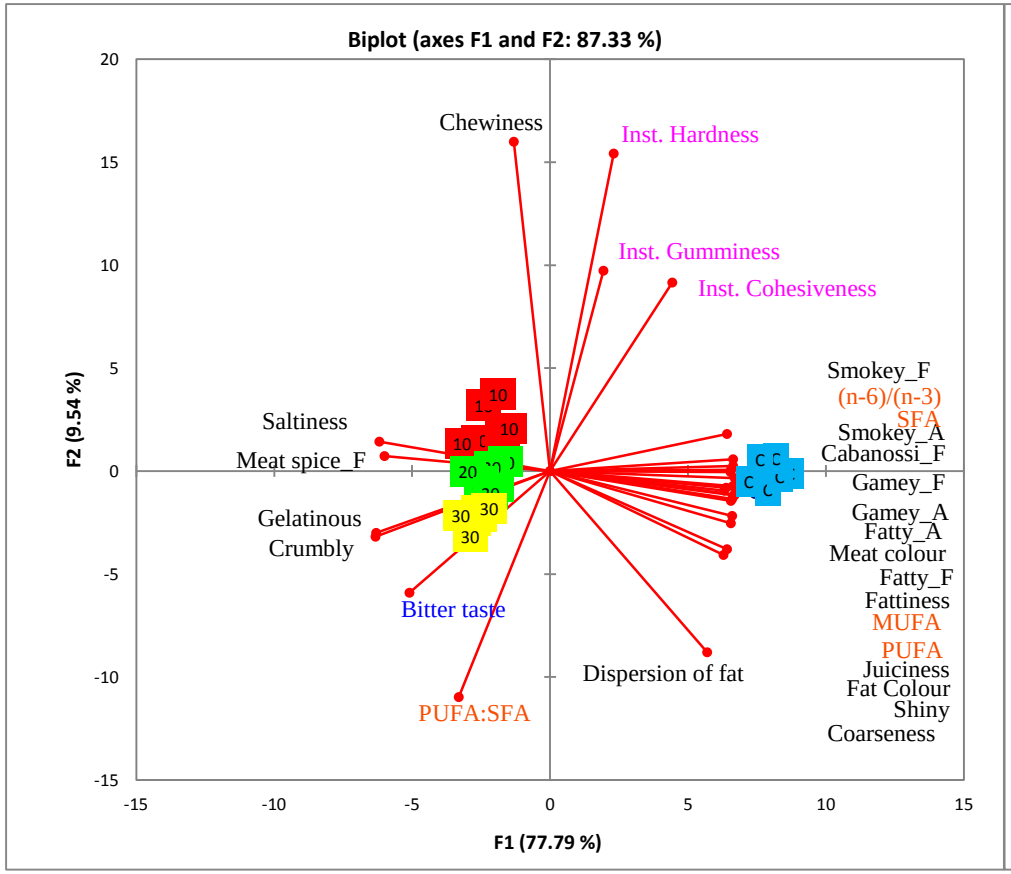


Figure 4.3 PCA bi-plot illustrating the sensory attributes, physical and chemical properties associated with the different cabanossi treatments at day 60. The abbreviations SFA, MUFA and PUFA refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, respectively, Inst. = Instrumental, 10 = Treatment 1 (10% FR); 20 = Treatment 2 (20% FR); 30 = Treatment 3 (30% FR); A = Aroma; F = Flavour & FR = Fat replacer.

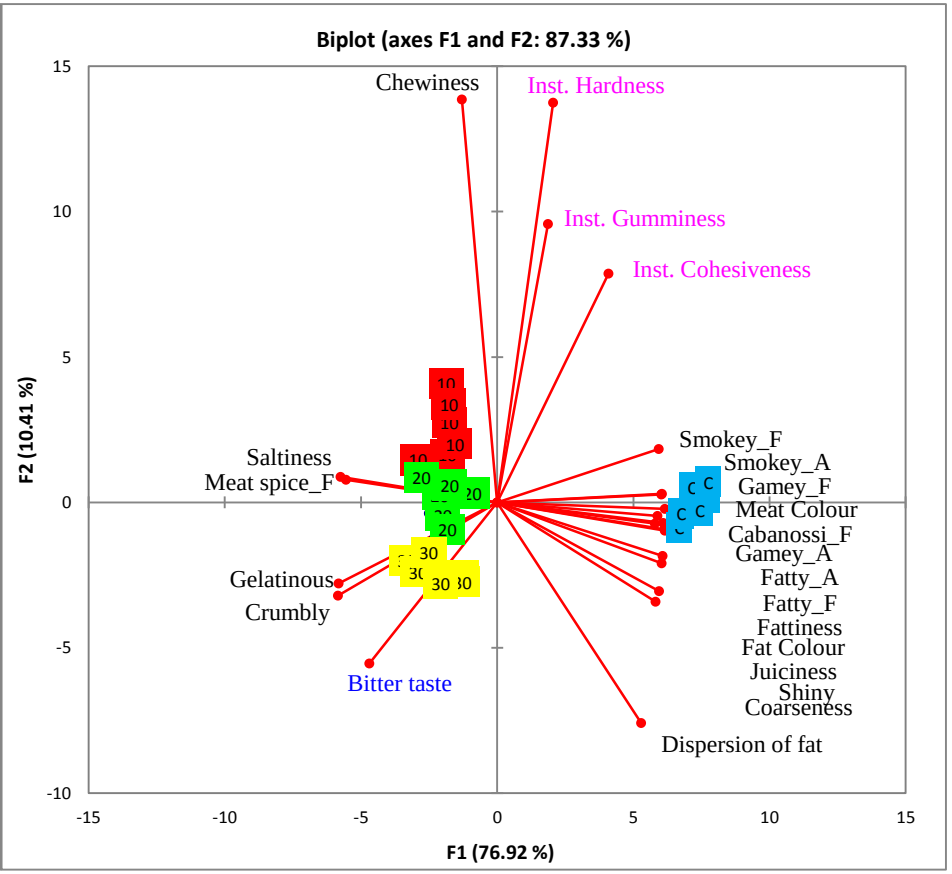
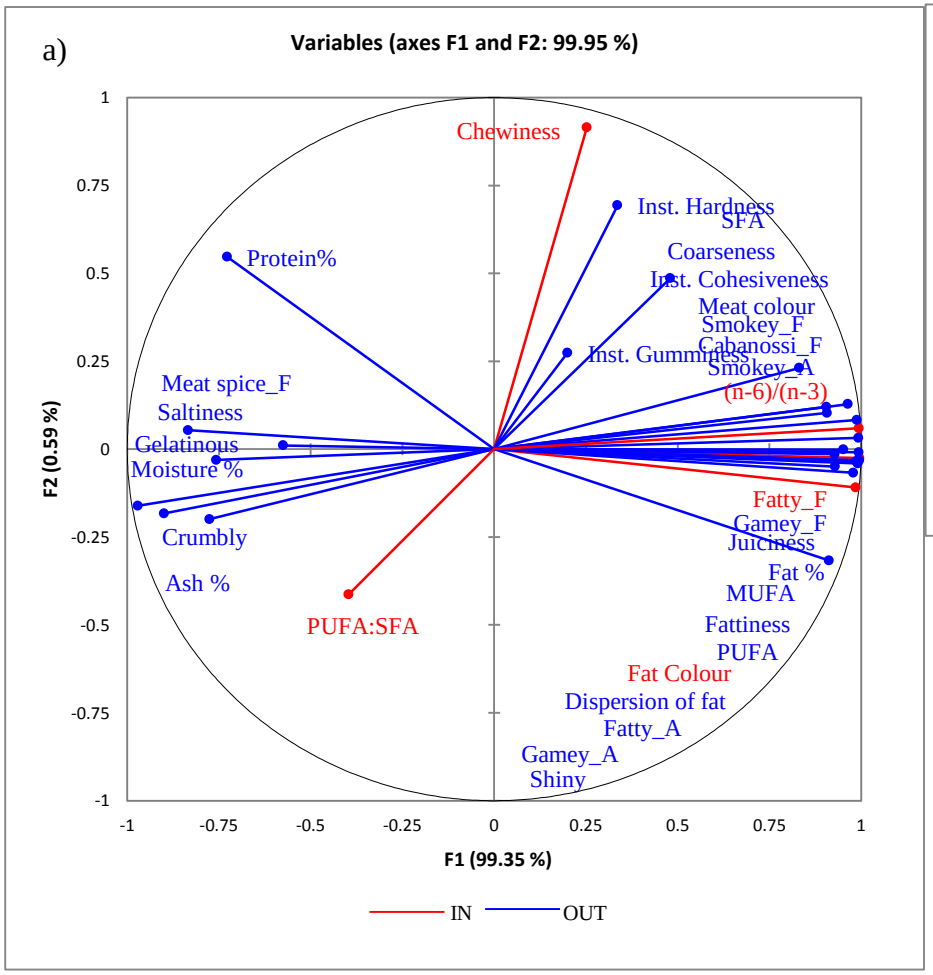
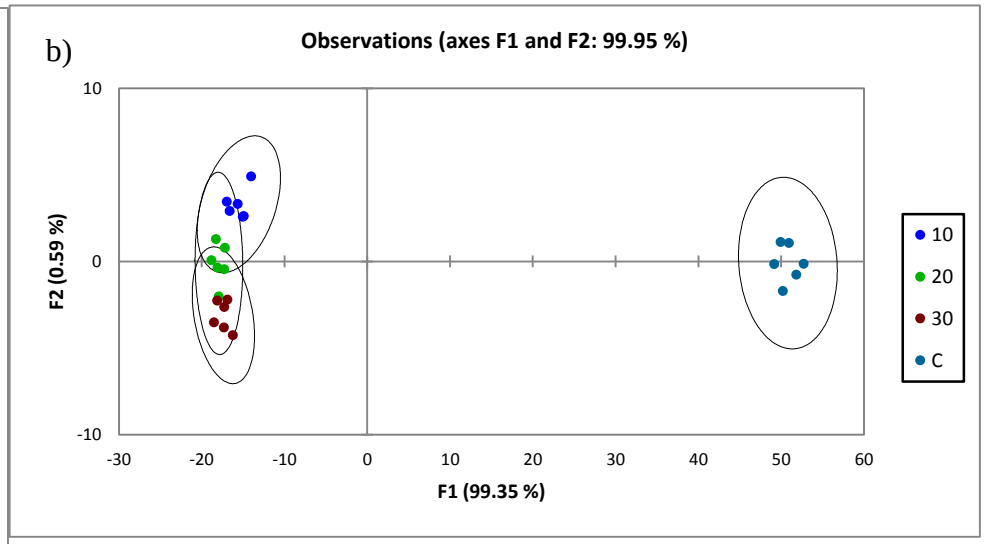


Figure 4.4 PCA bi-plot illustrating the sensory attributes and physical properties associated with the different cabanossi treatments at day 60. The abbreviations Inst. = Instrumental, 10 = Treatment 1 (10% FR); 20 = Treatment 2 (20% FR); 30 = Treatment 3 (30% FR); A = Aroma; F = Flavour and FR = Fat replacer.

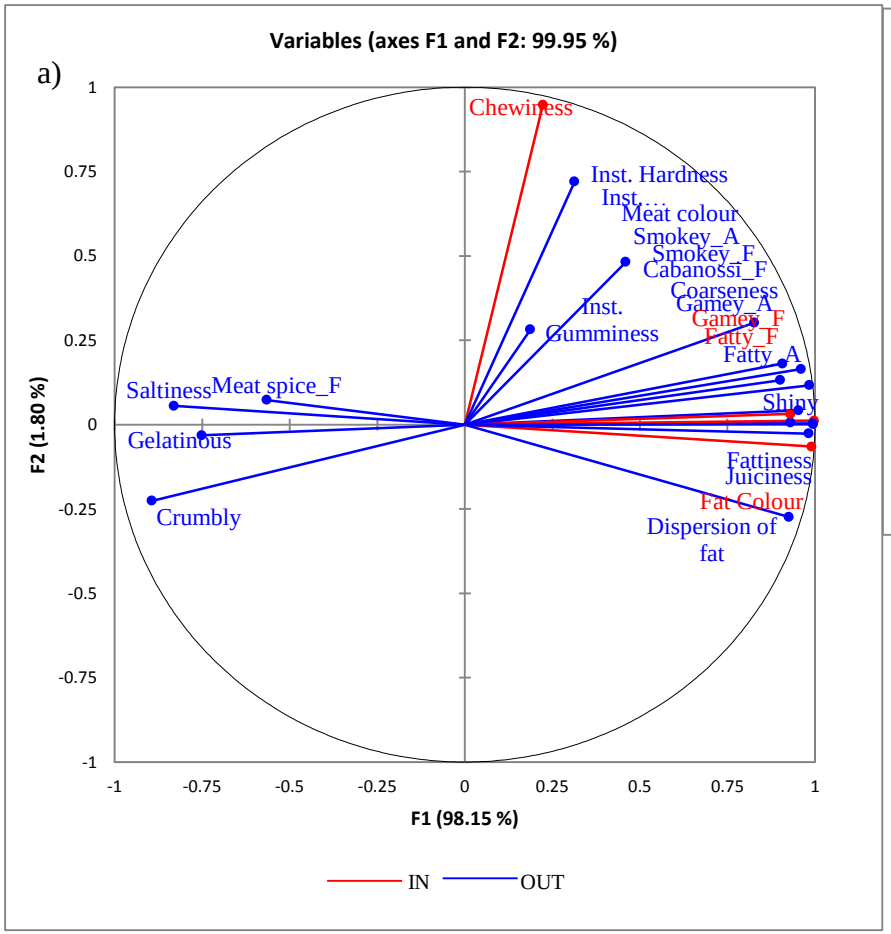


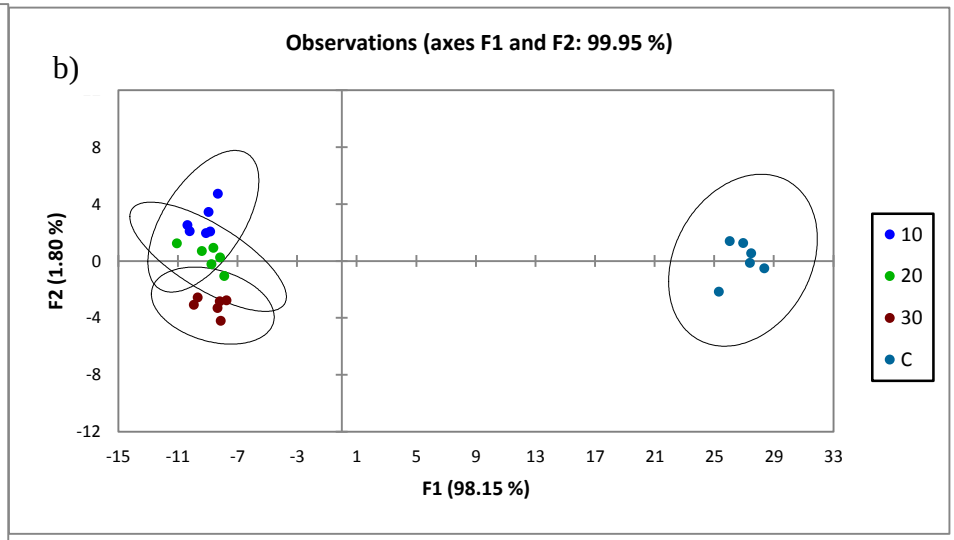


Cross-validation table for control and FR treatments at day 0: classification model

from \ to	10	20	30	C	Total	% correct
10	6	0	0	0	6	100.00%
20	0	5	1	0	6	83.33%
30	0	0	6	0	6	100.00%
C	0	0	0	6	6	100.00%
Total	6	5	7	6	24	95.83%

Figure 4.5 a) DA variables plot at day 0 showing position of the chemical, physical and positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot illustrating groupings of the control and three fat replacer treatments according to similar sensory profiles at day 0. The abbreviations SFA, MUFA, PUFA, Inst., C, 10, 20 and 30 refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

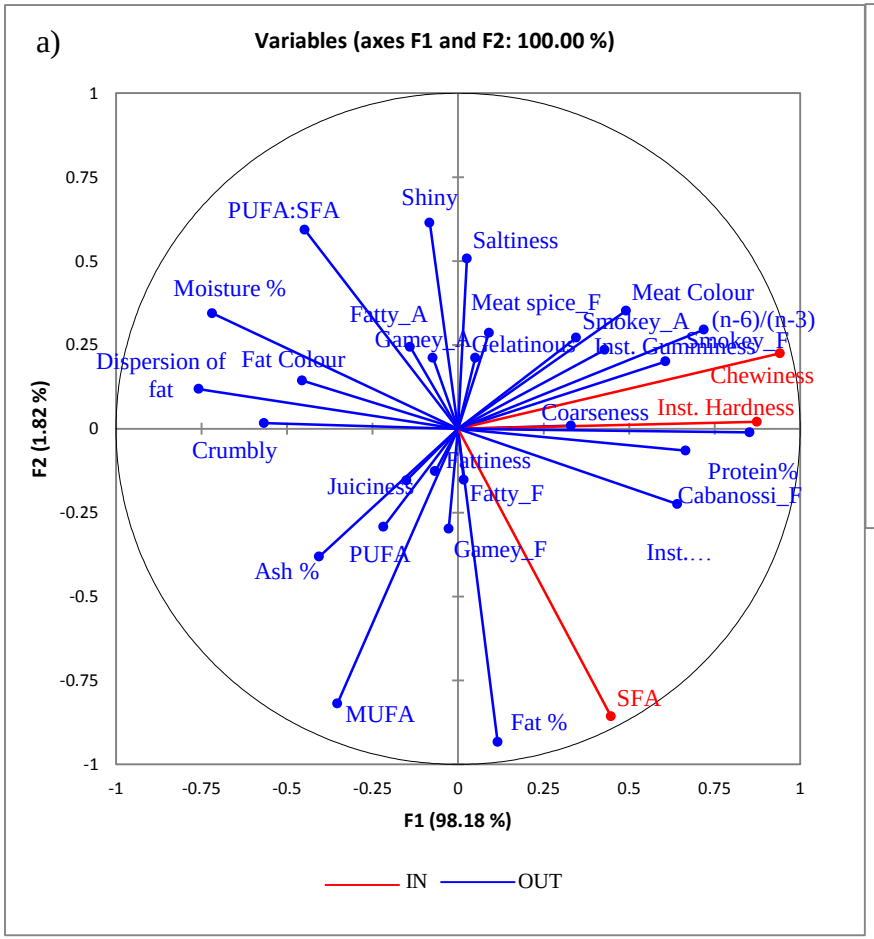


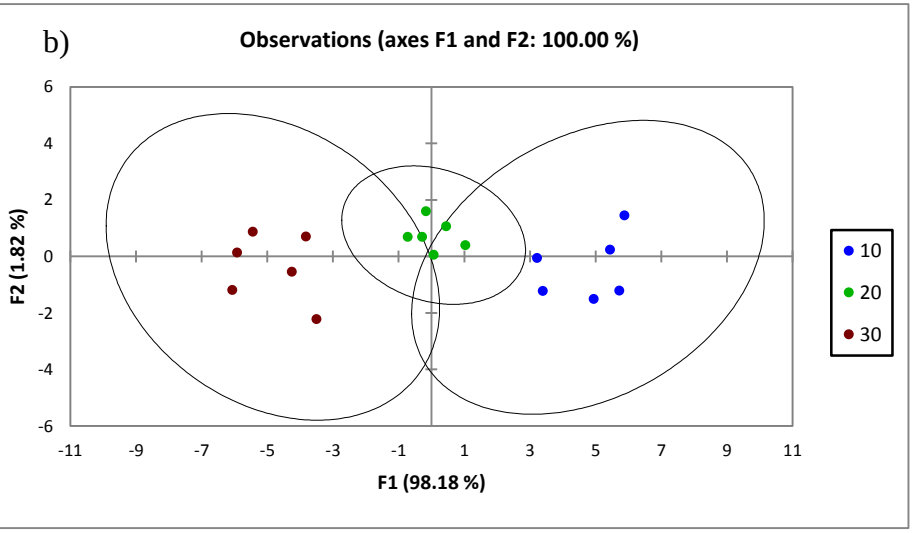


Cross-validation table for the control and FR treatments at day 0: classification model

from \ to	10	20	30	C	Total	% correct
10	6	0	0	0	6	100.00%
20	1	5	0	0	6	83.33%
30	0	0	6	0	6	100.00%
C	0	0	0	6	6	100.00%
Total	7	5	6	6	24	95.83%

Figure 4.6 a) DA variables plot without chemical results at day 0 showing position of physical attributes as well as the positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot illustrating groupings of the control and three fat replacer treatments according to similar sensory profiles at day 0. The abbreviations Inst., C, 10, 20 and 30 refer to Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

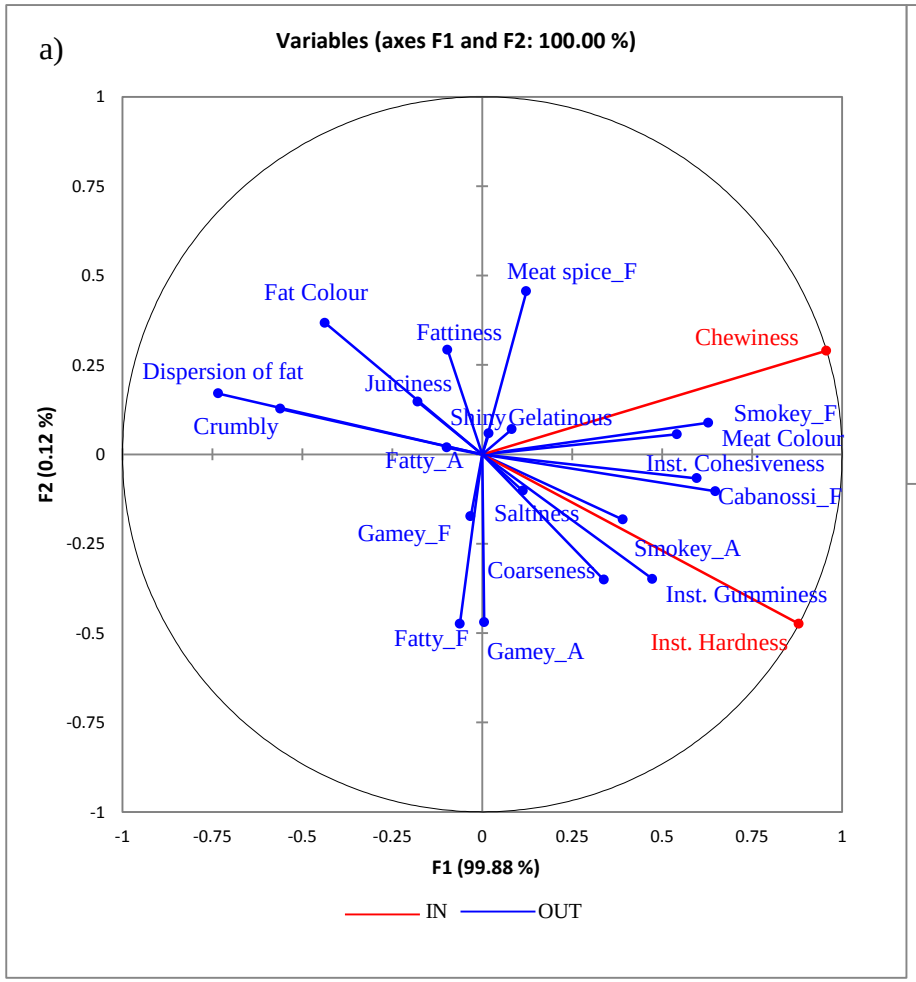


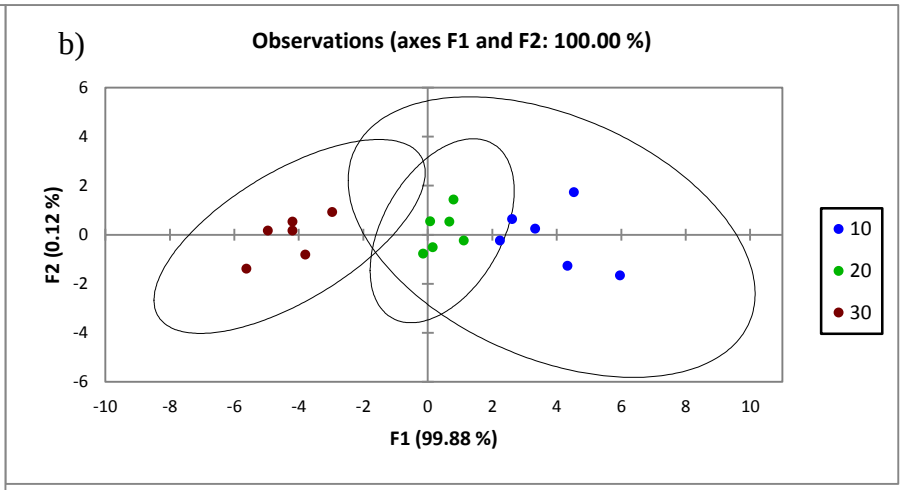


Cross-validation table for fat replacer treatments at day 0: classification model

from \ to	10	20	30	Total	% correct
10	6	0	0	6	100.00%
20	0	6	0	6	100.00%
30	0	0	6	6	100.00%
Total	6	6	6	18	100.00%

Figure 4.7 a) DA variables plot at day 0 without the control showing position of the chemical, physical and positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot without the control illustrating groupings of the three fat replacer treatments according to similar sensory profiles at day 0. The abbreviations SFA, MUFA, PUFA, Inst., C, 10, 20 and 30 refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

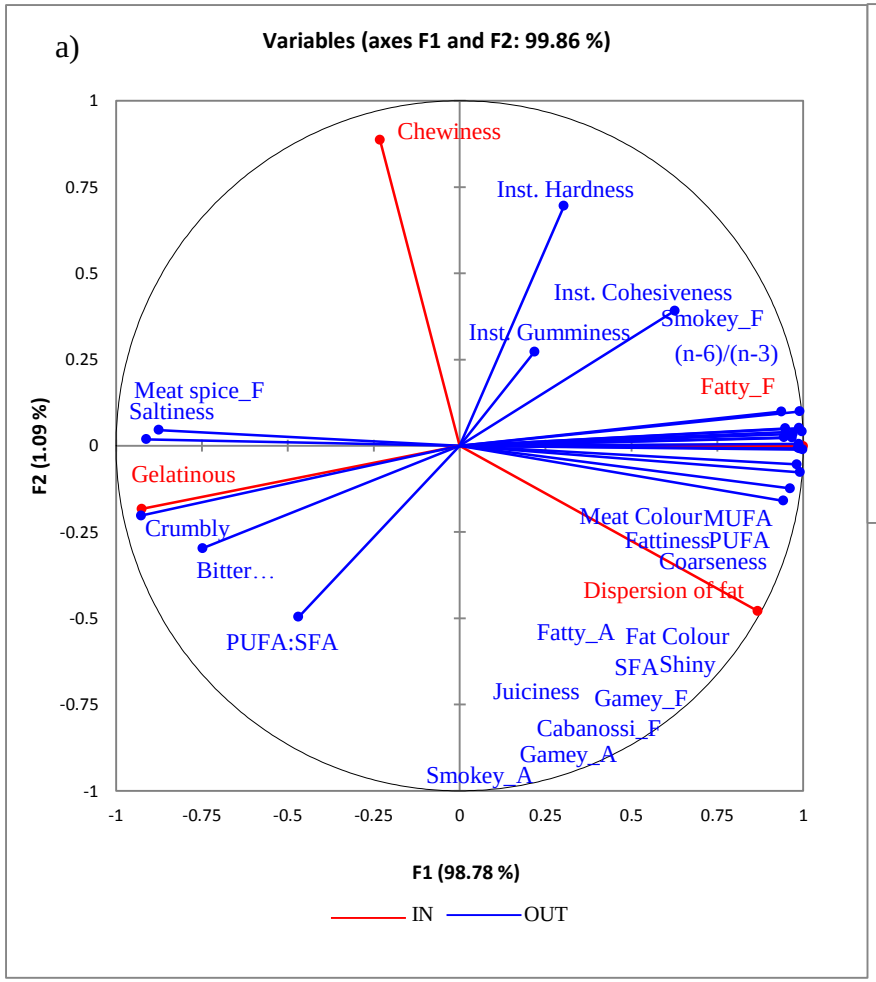


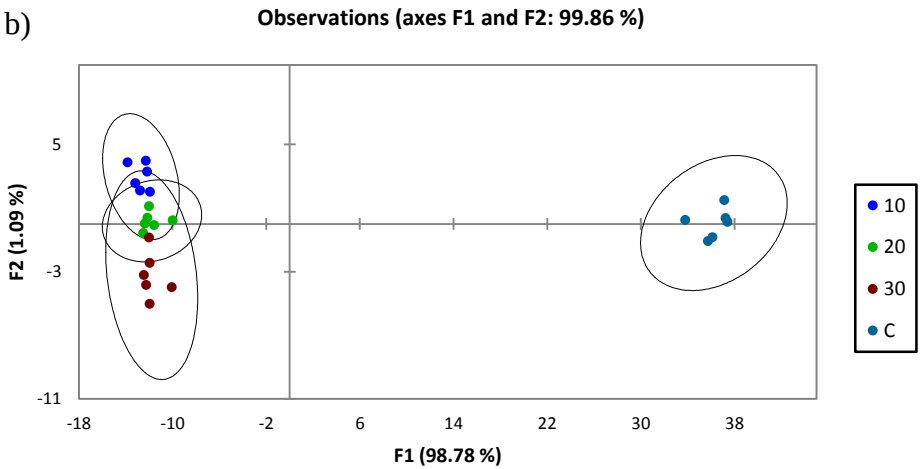


Cross-validation table for fat replacer treatments at day 0: classification model

from \ to	10	20	30	Total	% correct
10	5	1	0	6	83.33%
20	0	6	0	6	100.00%
30	0	0	6	6	100.00%
Total	5	7	6	18	94.44%

Figure 4.8 a) DA variables plot without the control and chemical results at day 0 showing position of the physical attributes as well as the positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot without the control illustrating groupings of the three fat replacer treatments according to similar sensory profiles at day 0. The abbreviations Inst., 10, 20 and 30 refer to Instrumental, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

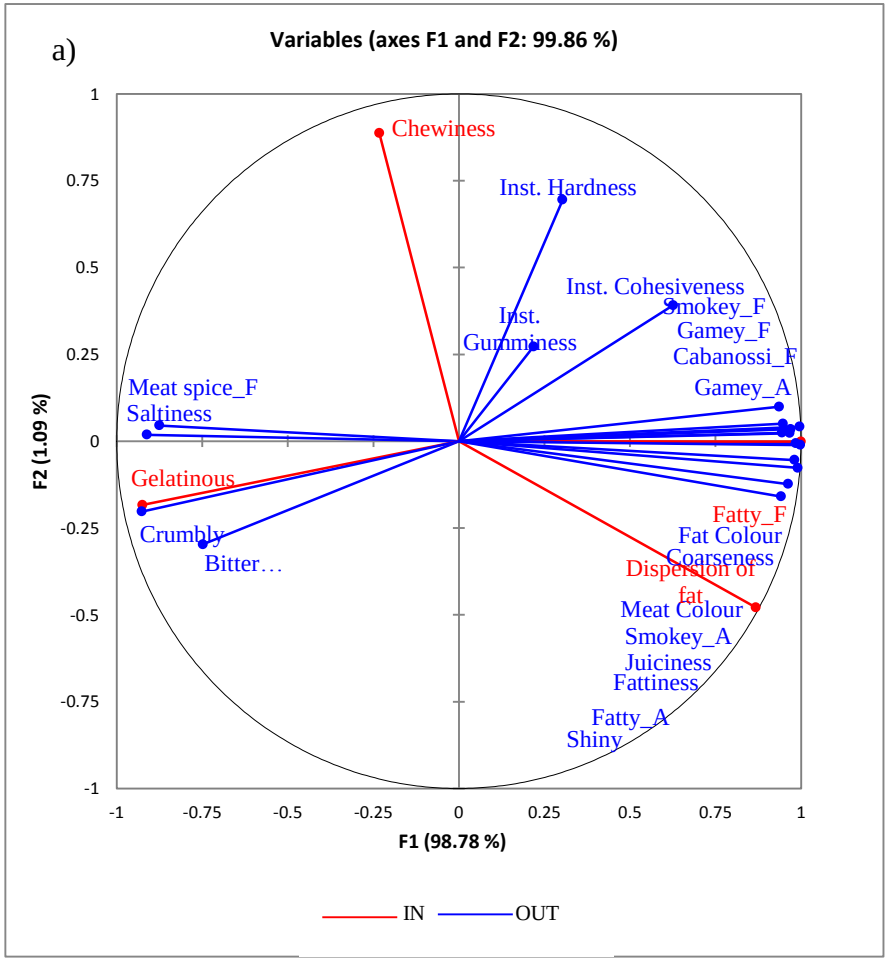


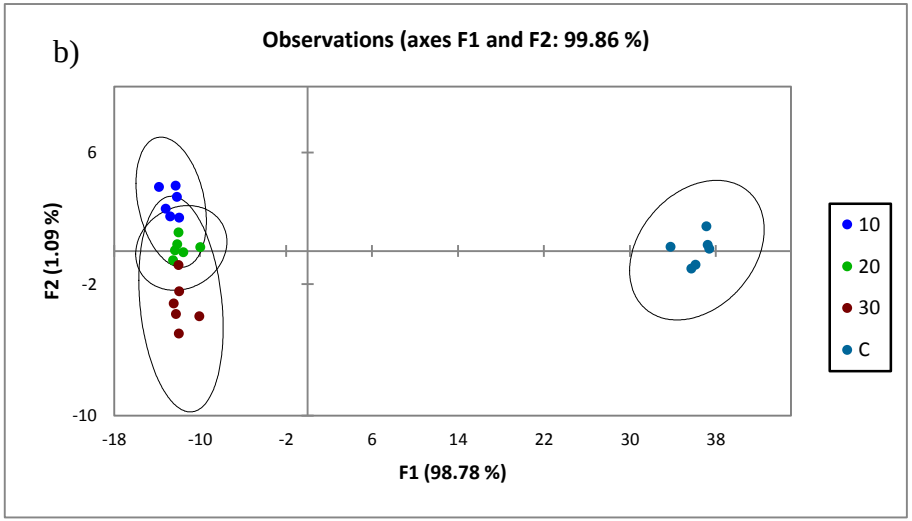


Cross-validation table for the control and FR treatments at day 60: classification model

from \ to	10	20	30	C	Total	% correct
10	5	1	0	0	6	83.33%
20	0	6	0	0	6	100.00%
30	0	1	5	0	6	83.33%
C	0	0	0	6	6	100.00%
Total	5	8	5	6	24	91.67%

Figure 4.9 a) DA variables plot at day 60 showing position of the chemical, physical and positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot illustrating groupings of the control and three fat replacer treatments according to similar sensory profiles at day 60. The abbreviations SFA, MUFA, PUFA, Inst., C, 10, 20 and 30 refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

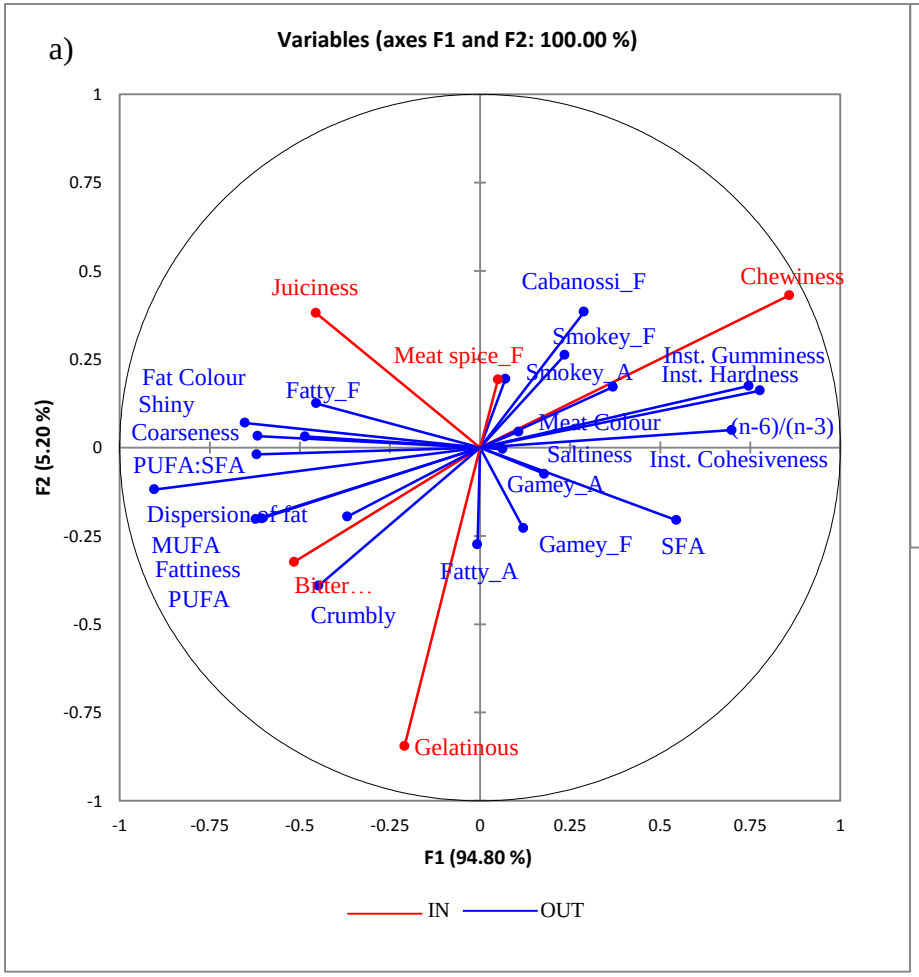


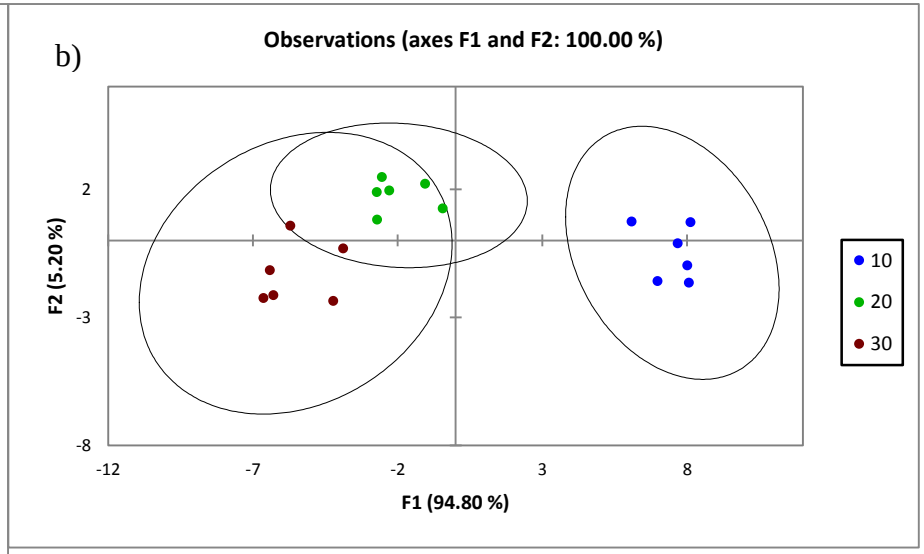


Cross-validation table for the control and FR treatments at day 60: classification model

from \ to	10	20	30	C	Total	% correct
10	5	1	0	0	6	83.33%
20	0	6	0	0	6	100.00%
30	0	1	5	0	6	83.33%
C	0	0	0	6	6	100.00%
Total	5	8	5	6	24	91.67%

Figure 4.10 a) DA variables plot without chemical results at day 60 showing position of the physical attributes as well as the positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot illustrating groupings of the control and three fat replacer treatments according to similar sensory profiles at day 60. The abbreviations Inst., C, 10, 20 and 30 refer to Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.

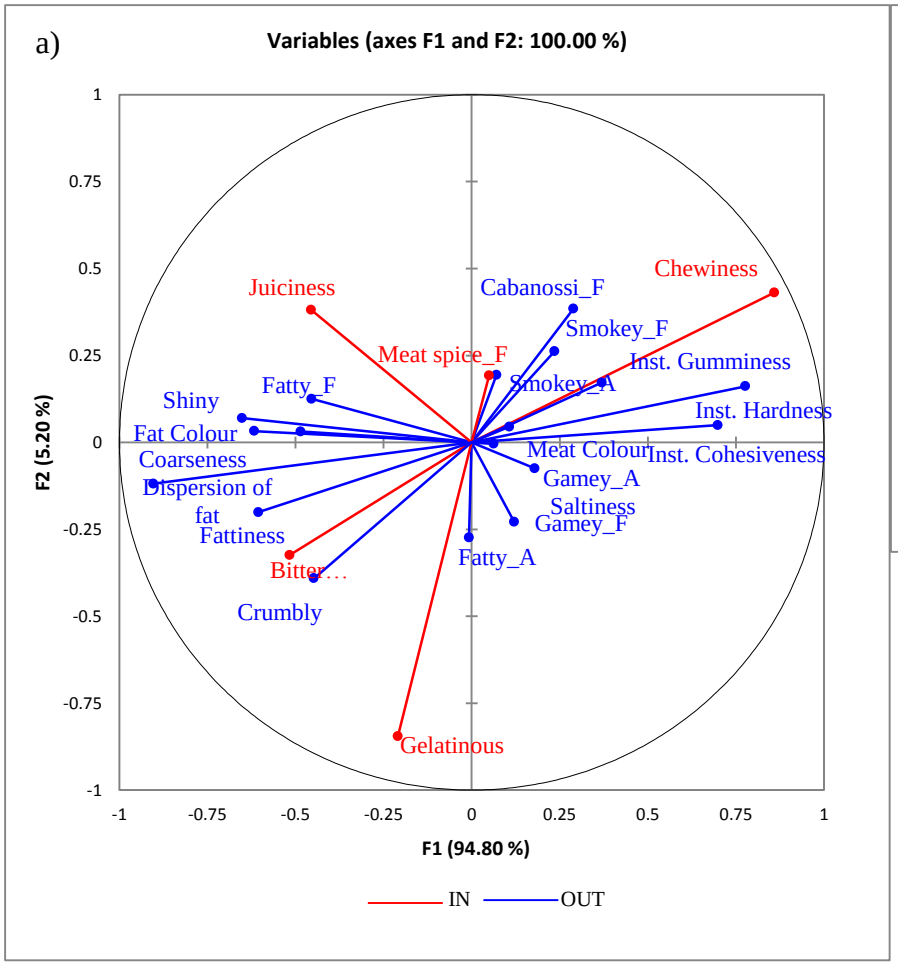


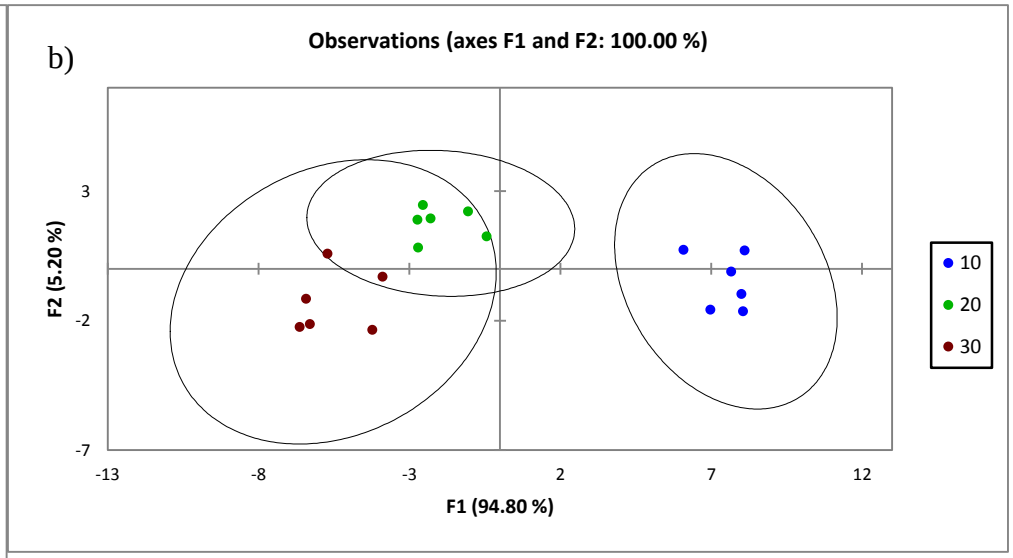


Cross-validation table for fat replacer treatments at day 60: classification model

from \ to	10	20	30	Total	% correct
10	6	0	0	6	100.00%
20	0	6	0	6	100.00%
30	0	2	4	6	66.67%
Total	6	8	4	18	88.89%

Figure 4.11 a) DA variables plot at day 60 showing position of the chemical, physical and positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot illustrating groupings of the three fat replacer treatments according to similar sensory profiles at day 0. . The abbreviations SFA, MUFA, PUFA, Inst., C, 10, 20 and 30 refer to the saturated fatty acids, mono-unsaturated fatty acids and poly-unsaturated fatty acids, Instrumental, Control, Treatment 1 (10% FR); Treatment 2 (20% FR); Treatment 3 (30% FR), respectively.





Cross-validation table for fat replacer treatments at day 60: classification model

from \ to	10	20	30	Total	% correct
10	6	0	0	6	100.00%
20	0	6	0	6	100.00%
30	0	2	4	6	66.67%
Total	6	8	4	18	88.89%

Figure 4.12 a) DA variables plot without chemical results at day 60 without the control showing position of the physical attributes as well as the positive and negative appearance, aroma, flavour and texture attributes. Attributes highlighted in red associated strongly with the treatments. The abbreviations "A" and "F" at the end refers to aroma and flavour, respectively. b) DA replicates plot without the control illustrating groupings of the three fat replacer treatments according to similar sensory profiles at day 60. The abbreviations Inst., 10, 20 and 30 refer to Instrumental, Treatment 1 (10% *FR1*); Treatment 2 (20% *FR2*); Treatment 3 (30% *FR3*), respectively.

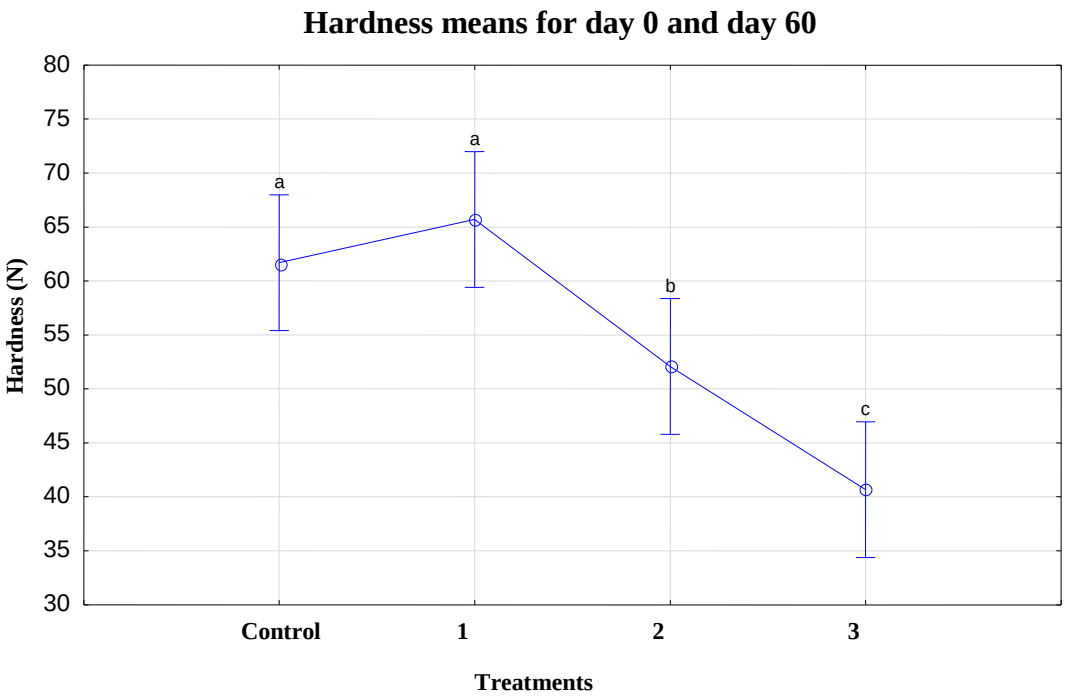


Figure 4.13 Pooled hardness means ($P \leq 0.05$) for day 0 and day 60. The *Control* (contains 20% PF). The abbreviations 1, 2 and 3 refer to *FR1* (10% FR), *FR2* (20% FR); *FR3* (30% FR), respectively.

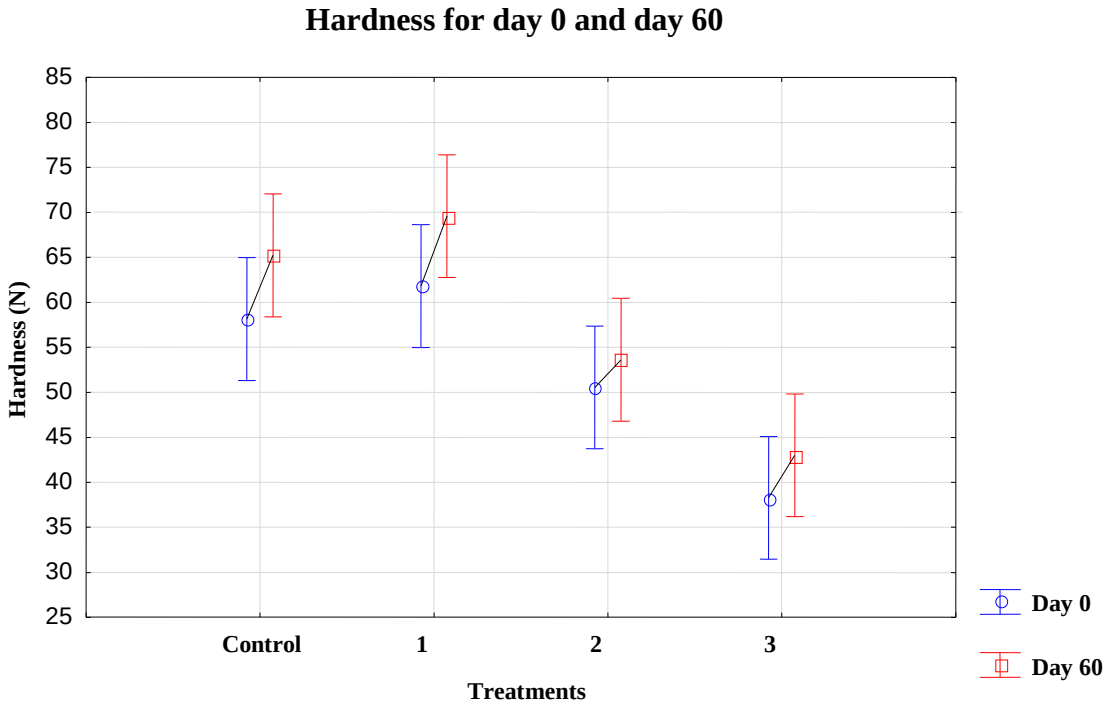


Figure 4.14 Mean hardness values ($P \leq 0.05$) for day 0 and day 60. The *Control* (contains 20% PF). The abbreviations 1, 2 and 3 refer to *FR1* (10% FR), *FR2* (20% FR); *FR3* (30% FR), respectively.

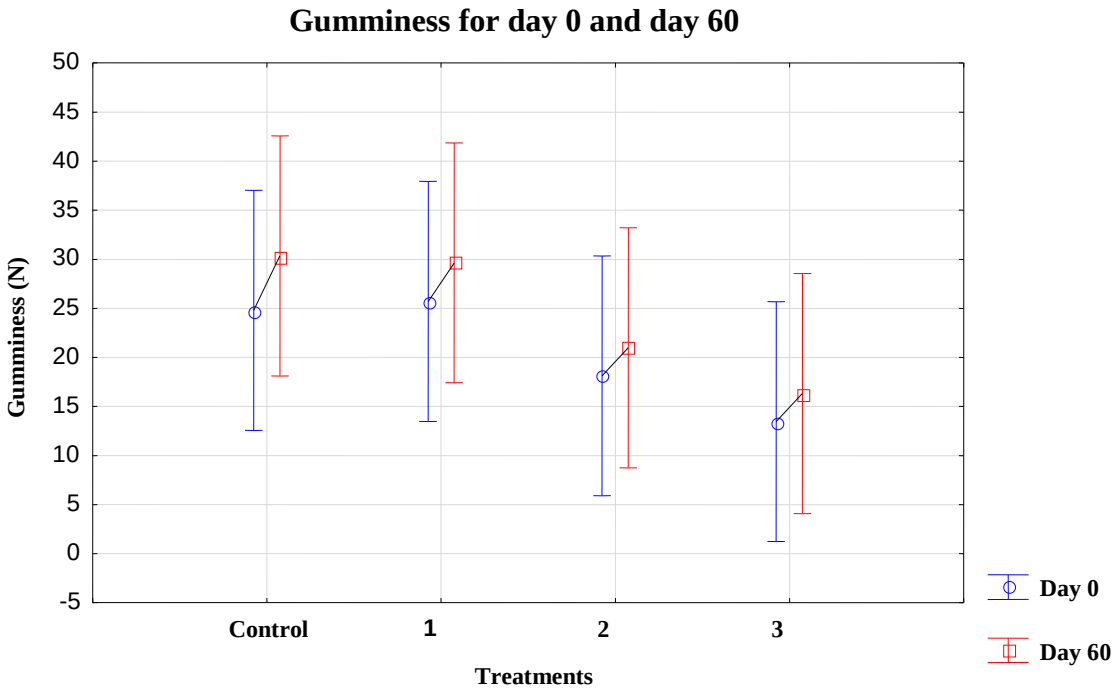


Figure 4.15 Gumminess values ($P \leq 0.05$) for day 0 and day 60. The *Control* (contains 20% PF). The abbreviations 1, 2 and 3 refer to *FR1* (10% FR), *FR2* (20% FR); *FR3* (30% FR), respectively.

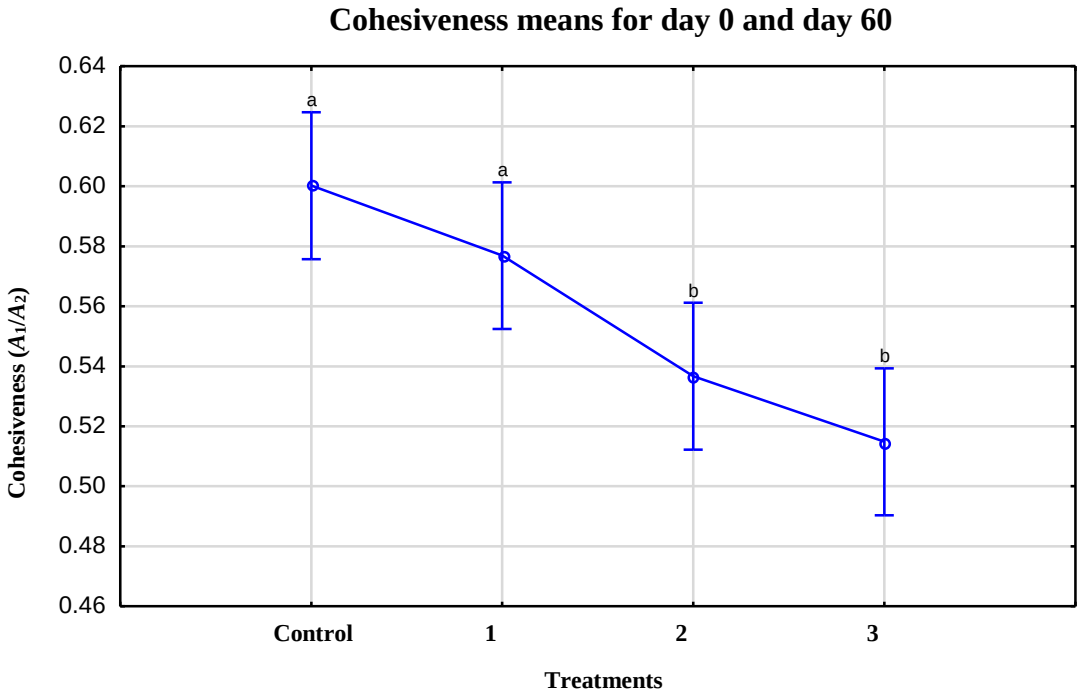


Figure 4.16 Cohesiveness means ($P \leq 0.05$) for day 0 and day 60. The *Control* (contains 20% PF). The abbreviations 1, 2 and 3 refer to *FR1* (10% FR), *FR2* (20% FR); *FR3* (30% FR), respectively.

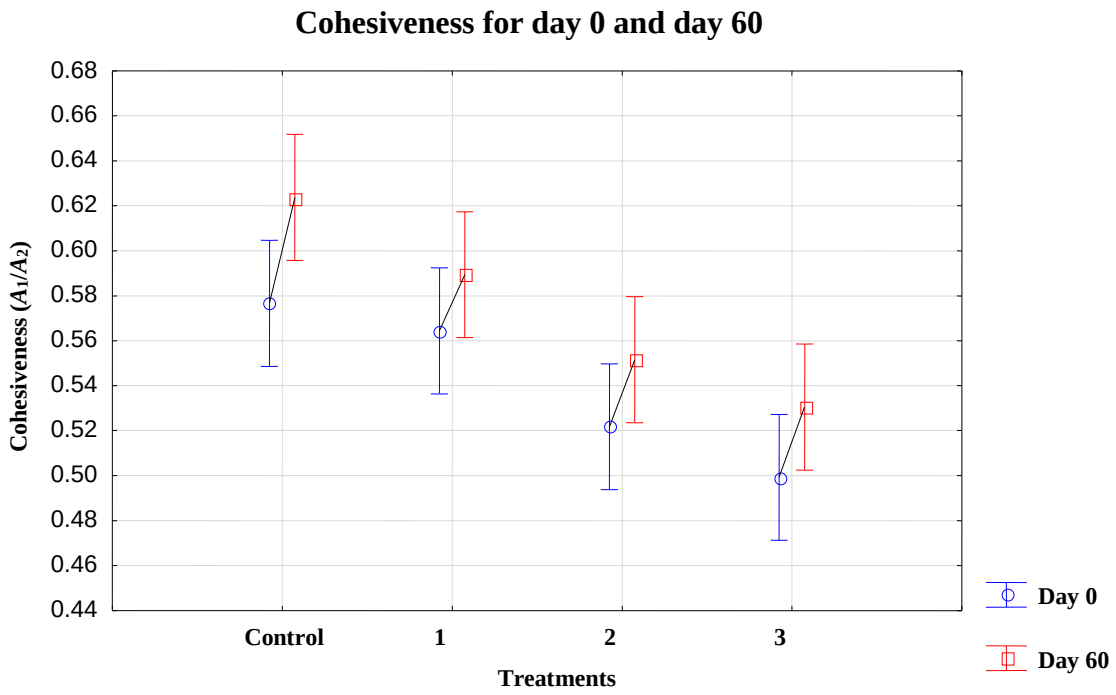


Figure 4.17 Cohesiveness values ($P \leq 0.05$) for day 0 and day 60. The *Control* (contains 20% PF). The abbreviations 1, 2 and 3 refer to *FR1* (10% FR), *FR2* (20% FR); *FR3* (30% FR), respectively.

4.5 Discussion

Processed meat products typically contain high levels of fat which hydrolyse to form desirable flavours and aromas, however, pungent off-flavours (rancidity) can form if the product is not produced or stored correctly. At day 0 and day 60, no rancid aroma or flavour attributes were detected which indicated that the fatty acids (SFA, MUFA and PUFA) were not oxidised during storage. The reduction of fat can increase other aroma and flavour intensities due to the lack of fat, however, when present fat usually forms an oral coating which releases aromas at a slower rate (De Hoog *et al*., 2011). Mora-Gallego *et al*. (2013) noted that fat reduced non-acid fermented sausages had higher "piquantness" scores than high fat sausages; this could be due to the solubility of pepper components in fat which release slower during chewing.

At day 0 and day 60, the *FR* treatments were higher ($P \leq 0.05$) in "Meat spice_F" compared the *Control*. Gómez & Lorenzo (2013) noted that low-fat chorizo sausages had higher scores for spice odour. Therefore, it can be postulated that due to the lack of fat the "Meat spice_F" became more pronounced in the *FR* treatments, and more specifically *FR1*.

A Bitter taste occurred on after 60 days storage in all three *FR* treatments and the bitterness increased as the fat replacer percentage increased. The trend indicated that the fat replacer itself could be contributing or facilitating the Bitter taste. Free amino acids, as well as peptides with a specific

sequence are linked to bitterness and the hydrophobicity of the amino acids or peptides can increase a product's bitterness (Toldra, 1998; Raksakulthai & Haard, 2003). In the present study, the protease enzymes could have broken down the gelatine in the fat replacer gel structure to release proline which could have been associated with the low levels of bitterness (Table 4.4) (Raksakulthai & Haard, 2003). The development of the Bitter taste may be of some concern, especially in *FR3* where it was more pronounced. It is thus important to ascertain the actual source of this Bitter taste.

Although the flavour of the product is important; the texture can have a greater impact on the success of the product (Font-i-Furnols & Guerrero, 2014). In terms of texture, Crumbly was associated with crumbly, old dry bread which would imply that the lipid-protein and protein-protein interactions were negatively affected. At day 0 and day 60, all the *FR* treatments scored higher ($P \leq 0.05$) for crumbliness compared to the *Control*. Furthermore, *FR3* scored the highest and differed ($P \leq 0.05$) from the other three treatments. At day 60, Crumbly texture was strongly correlated ($P \leq 0.05$; $r = 0.923$) with Gelatinous, thus one can assume that the fat replacer had an effect on the efficacy of the meat emulsion. Mittal & Blaisdell (1983) noted that moisture loss was decreased as the fat-to-protein ratio increased due to the fat's hydrophobic properties resisting moisture diffusion. Mittal *et al.* (1983) further noted that moisture diffusivity could increase as the fat-to-protein ratio decreased with an increase in product temperature and moisture concentration. The fat replacer is a rigid gel that resists manipulation affecting its ability to fill voids in the protein matrix. In addition, the fat replacer can start to aggregate as the percentage increases resulting in the formation of capillaries, the latter could increase moisture loss. Furthermore, Mora-Gallego *et al*. (2013) found that the addition of sunflower oil (as a fat replacer) decreased the binding of the meat particles which affected the crumbliness of the final processed meat product. Similarly, the fat replacer gel may have influenced the binding due to its weak binding capability to the meat proteins and assisted in protein aggregation and thus a so-called Crumbly texture.

With the reduction of animal fat an increased number of protein-protein interactions could occur which can alter the product's chewiness. At day 0 and day 60 for all the treatments, Chewiness was found to have a reasonably good correlation ($P \leq 0.05$; $r = 0.718$, 0.500, respectively) with "Inst. Hardness." The reason for this is that fat provides lubricity to processed meat products which aids in chewing (Gómez & Lorenzo, 2013). Youssef & Barbut (2009) noted that a decrease in fat increased protein-protein interactions which increase the strength of the protein matrix. These interactions increase the mastication force required to chew the reduced-fat processed meat products. In the present study, *FR1* scored the highest for Chewiness and "Inst. Hardness", however, due to the fat replacers' insufficient binding potential to the meat proteins, it was observed that as the fat replacer percentage increased, the Chewiness decreased.

Sensory texture analysis is a viable tool for indicating textural differences. The use of sensory texture analysis and instrumental texture analysis, in conjunction, can provide a further indication on textural flaws. The definitions for the physical parameters tested are as follows: Hardness (N) = the maximum force required to compress the sample, cohesiveness (A_1/A_2) = A_1 total energy of first compression and A_2 total energy of second compression, is the extent to which the sample could be deformed prior to rupture and gumminess (N/cm^2) = the force required to disintegrate a semisolid meat sample for swallowing (Mendoza *et al.*, 2001). Furthermore, the microstructure of a meat emulsion can be affected by factors such as: type of meat and fat; hardness and melting point of fats; levels of moisture, fat and salt; processing; increased protein-protein interactions; and lastly cooking and freezing (Lee, 1985; Wu *et al*., 2009; Youssef & Barbut, 2009). Fat stabilisation has a vital role in meat emulsion stability as the fat globule membrane interacts with the protein matrix via disulphide bonds and fills voids present in the emulsion (Wu *et al*., 2009; Zhang *et al*., 2013). Mittal & Blaisdell (1983) noted that moisture loss was inversely proportional to the fat-to-protein ratio and that the hydrophobic properties of the fat resisted moisture diffusion.

FR1, which had a low fat content (Chapter 3) and the lowest fat replacer present, illustrated the highest "Instr. Hardness" when compared to *FR2* and *FR3* (Figure 4.13). In other studies, a Bologna-style sausage that was partially substituted with pork skin, water and an amorphous cellulose mixture recorded increased hardness values due to the higher levels of fat replacer present (De Oliveira Faria *et al*., 2015). When pork fat, in a chorizo sausage, was partially substituted with konjac gel as fat replacer, the hardness increased (Ruiz-Capillas *et al*., 2012). Furthermore, Ruiz-Capillas *et al*. (2013) noted that meat batters containing polysaccharides (alginate and dextrin/ inulin) with olive oil exhibited higher hardness values than the pork fat control. In addition, higher hardness values were obtained for low-fat comminuted sausages, as well as chorizo sausages with higher protein levels (Yoo *et al*., 2007; Youssef & Barbut, 2009). Beriain *et al*. (2011) noted that chorizos containing emulsified olive oil with 3 and 10% inulin obtained higher hardness values. Youssef & Barbut (2011) also noted an increase in hardness as the meat protein level increased, as well as when the animal fat was substituted with soy or whey proteins. Beriain *et al*. (2011) found the lowest cohesiveness values in chorizos where 50% of the pork back fat was substituted with emulsified olive oil and 10% inulin. Youssef & Barbut (2009) noted that the hardness, cohesiveness and gumminess values increased as the meat protein content increased. When a meat batter is cooked, the salt soluble proteins form a gel matrix that encases the fat particles, however, as the fat content increases the meat particles decrease leading to the formation of a weaker protein gel matrix (Allais, 2010). In the present study, a trend was observed when using increasing amounts of the fat replacer in the cabanossi; the hardness, gumminess and cohesiveness all decreased significantly as the fat replacer levels increased (Figures

4.13 – 4.17). According to previous studies this trend was observed for other sausages containing a fat replacer and is in accordance with previous literature.

4.6 Conclusions

The aim of the study was to determine the descriptive sensory profile and instrumental texture profile of the *Control* and all three *FR* treatments, as well as compare the different profiles of all the treatments. The *Control*'s sensory profile was significantly different in appearance, aroma, flavour and texture when compared to all three *FR* treatments. The *Control* was thus discernible from the *FR* treatments however, *FR1* and *FR2* were very similar in their sensory profiles indicating that 10/ 20% fat replacement resulted in a similar sensory profile. *FR1* scored the highest in hardness in terms of the instrumental texture analysis and was not significantly different to the *Control*. The increased hardness can be explained; *FR1* contained a larger amount of meat, approximately 10% more, and therefore the increased number of protein-protein interactions could potentially increase the hardness. *FR3* performed the worst out of all the treatments due to a high amount of fat replacer (30%). The fat replacer was not able to mimic the animal fat properties, as indicated by the sensory and texture analysis results. Furthermore the fat replacer had a different taste to traditional animal fat which affected the flavour profile. The poor binding ability of the fat replacer inhibited effective binding of the proteins which negatively impacted on the cabanossi's structure, ultimately resulting in low instrumental texture scores as the fat replacer percentage increased. Overall, it is recommended that the fat replacer not be used for complete substitution however, further investigation with partial substation could yield further positive results. The data collected in this study will expand and add value to the already existing knowledge on processed meat products.

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Chapter 5

The effect of a fat replacer on the microbial quality of blesbok (*Damaliscus pygargus Pphillipsi*) cabanossi containing pork fat and a fat replacer

5.1 Abstract

The microbial quality of blesbok cabanossi containing different levels of fat and meat were evaluated at 0 and after 60 day of storage. Six replications with a control and three treatments that only contained the fat replacer (animal protein and alginate gel), were tested at day 0 and day 60. The *Control* contained 20% pork fat (PF), Treatment 1 contained 10% fat replacer (*FR1*), Treatment 2 contained 20% (*FR2*) and Treatment 3 contained 30% (*FR3*). Coliform counts for day 0 and day 60 were <10 cfu.g^{-1}and the aerobic endospore forming bacteria count for all batches were low, indicating good manufacturing practices (GMP) that adhered to the South African Department of Health (DOH) food safety regulations. *Listeria monocytogenes* and *Salmonella* spp. were not detected. *Staphylococcus aureus* was only present in one treatment. However, the production and storage parameters do not support the growth or toxin production of *Staphylococcus aureus* therefore, the treatment may have been contaminated with *Staphylococcus* during subsequent storage. Based on various regulations and their guidelines for food safety from South Africa and several international specifications the product would be deemed shelf-stable and microbiologically safe for 60 days.

5.2 Introduction

All food products around the world, raw or processed, need to be microbiologically shelf-stable for a period of time. Manufacturers have an obligation to their consumers to provide a safe and good quality product for the shelf-life of the product. In South Africa, new meat products need to follow regulations that are enforced by the Department of Health (DOH) before they are approved for commercialisation (DOH, 2014). This allows manufacturers to comply with exporting legislation which enables international trading. Food safety systems such as Hazard Analysis and Critical Control Points (HACCP) improve Good Manufacturing Practices (GMP) in factories which lowers the risk of contamination for processed meat products (McClure, 2008). A large number of processed meat products require no further preparation and are ready-to-eat, for this reason products need to be microbiologically safe. One of the main methodologies employed to minimise microbial growth is heat treatment, however, if the initial heat treatment during production was ineffective, the pathogenic and spoilage bacteria will be able to proliferate in the processed meat product during storage. This would increase the likelihood of higher pathogenic bacteria numbers occurring in ready-to-eat foods (Stratakos & Koidis, 2015). Humans are susceptible to three common hazards; food-borne

intoxications, food-borne infections and food poisoning, in a variety of processed meat products (Fung, 2010). The main pathogenic microorganisms in ready-to-eat food products are *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli* and *Salmonella* spp. (Yu *et al*., 2016). Manufacturers have started to use hurdle technology which combines certain procedures during the manufacturing process making the environment in and around the product more challenging for the microorganisms to survive and proliferate. These include: heat treatment, cold storage, vacuum packing, drying, smoking, pH and curing of meat products. In many processed cured products nitrate and nitrite are added for colour stability, as well as its antimicrobial properties and are frequently used in combination with salt (Jackson *et al*., 2011; Velisek, 2014). Salt is an important additive in processed meat products as it is a taste enhancer, extracts myofibrillar proteins and lowers the processed meat product's water activity (a_w). The water molecules in the product have an affinity towards salt due to their polarity and once bound the water molecules are immobilized, thus lowering the a_w (Honikel, 2010). In processed meat products the majority of the bacteria can survive temperatures up to 45°C with some being able to grow at 50 - 60°C. Therefore, it has been recommended that a processed/cooked meat product reach a core temperature of 70°C which is sufficient in destroying the bacteria (Jay *et al*., 2005a; Boles, 2010). High temperature processing can be combined with smoking leading to the term hot smoking which can be done between 50 - 90°C. Wood smoked products contain compounds such as phenols, carboxylic acids and formaldehyde which give smoke an antimicrobial effect which help extend processed meat products' shelf-life (Sikorski & Kolakowski, 2010). Drying the product increases the prevention of bacteria growth by decreasing the moisture content and lowering the water activity of the processed meat product (Zukal & Incze, 2010). To prevent microbial growth on the finished cabanossi product, the product was stored at 4°C as microbes do not grow well at temperatures below 5 - 7°C. The product was vacuum packed to reduce the oxygen concentration and prevent contamination during storage (Walsh & Kerry, 2002; O'Sullivan & Kerry, 2010). These techniques also prevent the growth of aerobic bacteria, aerobic endospore forming bacteria and various coliforms that are associated with the deterioration of products during shelf-life (Feiner, 2006). Total viable count also known as aerobic plate count, gives an indication of the total bacterial population present in a product. The test determines the population of bacteria that are able to grow aerobically at mesophilic temperatures (35 - 55°C) (Feiner, 2006). The test may have limitations in identifying specific bacteria however, it is a useful test for food manufacturers as it can be an indicator of quality and safety concerning raw materials, processing conditions, handling of the product and storage conditions. The results obtained give insight regarding shelf-life and sensorial changes to the product that may occur over a period of time (Morton, 2001). *Listeria monocytogenes* is a bacterium that can grow in temperatures between 1 - 45°C, optimum being 30 - 37°C, and can survive in salt concentrations of up to 10% NaCl.

Although *L. monocytogenes* is commonly found in dairy products, it has been found in processed meat products containing pork (Ryser & Donnelly, 2001). *Salmonella* spp. can grow at temperatures between 5 - 45°C, optimum 30 - 37°C, with both microbes surviving at a water activity as low as 0.92 (Feiner, 2006). The Foodstuffs, Cosmetics and Disinfectants Act and Regulations, 54/ 1972 (issue 26, 2013) of South Africa states that under regulations governing microbiological standards for foodstuffs and related matters pathogens such as *Salmonella* spp. in processed meat products must not be detected in 25 g and *L. monocytogenes* must be <10 cfu·g-1(Table 5.2).

The objective of the study was to analyse the different micro-organisms presently found in processed meat products after 0 and 60 days of storage, at 4°C.

5.3 Materials and methods

5.3.1 Manufacturing process

The cabanossi production, fat replacer and treatment recipes can be found in Chapter 3.

5.3.2 Agar and dilution preparation

Three cabanossi sausages from each treatment (*Control*, *FR1*, *FR2* and FR3) were selected from each replication (A & B and C & D and E & F) vacuum packed and stored at 4°C until the treatments were examined on day 0 and day 60. Cabanossi samples (25 g) were added to 225 mL of sterilised buffered peptone water (BPW) (Merck Biolab, South Africa) and thoroughly homogenised by stomaching the contents for 15 s. The samples were then further diluted (10^{-2}-10^{-4}). The Total Viable Count (TVC) was spread plated on Tryptic Soy Agar (TSA) (Merck Biolab, South Africa). The agar plates were then incubated at 37°C and the results recorded after 24-48 h (Garriga *et al*., 2004). For the endospore count, the dilution series was heated at 80°C for 10 min before it was spread plated on Tryptic Soy Agar (TSA) (Merck Biolab, South Africa) and incubated at 37°C and the results recorded after 24-48 h (Logan *et al*., 2000). The Coliform count was spread plated on Violet Red Bile Agar (VRBA) (Oxoid, Basingstoke) and the plates were incubated at 37°C and the results recorded by counting the number of pink to reddish colonies after 24-48 h (Feng *et al*., 2002; Garriga *et al*., 2004). The *S. aureus* count was spread plated on Mannitol Salt Agar (MSA) (Merck Biolab, South Africa). The plates were incubated at 37°C and the results were recorded after 24-48 h (Gorwitz *et al*., 2008).

5.3.3 *Listeria* detection

Listeria monocytogenes strains were detected using a two-step enrichment procedure followed by sub-culturing on Oxford agar (Oxoid CM856), which is based on the principle of esculin hydrylosis, and a new chromogenic agar, RAPID'L. mono agar (Biorad), which is based on phospholipase C

detection and the inability of *L. monocytogenes* to metabolise xylose. The protocol for the identification of *L. monocytogenes* from foods was a modification of the EN ISO 11290-1 and Sanofi protocols (Biorad). A food sample of 25 g was added to 225 mL of half strength Fraser broth (Oxoid CM895) with supplement SR156 (Oxoid). Samples were homogenized with a stomacher (Seward stomacher 400) for 30–60 s and then incubated at 30°C for 24 h. This served as the primary enrichment phase. From this primary enrichment, 0.1 mL was then inoculated into 10 mL of Fraser broth and incubated at 37°C for 24–42 h in a shaking incubator. This served as the secondary enrichment. The primary and secondary enrichments were sub-cultured on both Oxford and RAPID'L. mono (Biorad, France) agar plates after their respective incubation periods. Presumptive *L. monocytogenes* colonies were streaked on nutrient agar (Oxoid CM1) and incubated at 37°C for 18–24 h. The colonies on RAPID'L. mono and nutrient agar were then used as templates in PCR reactions. The same protocol was used when selected colonies from previously tested products were used as a substitute for the food sample. All food samples were evaluated in duplicate.

The DNA extraction procedure was based on a protocol previously described for the detection of *L. monocytogenes* in food products. A few colonies were re-suspended in 50 mL of 1´PCR buffer in a 2 mL micro-centrifuge tube with an interlocking cap. A solution of 2% Triton X (50 mL) was then added to this cell suspension and thoroughly mixed. This mixture was heated at 100°C for 10 min and then allowed to cool to room temperature. For PCR amplification, 5 mL of this crude cell lysate were used. PCR assays were performed in 50 mL reaction volumes. The primer pair consisting of primer A _5'-CAT TAG TGG AAA GAT GGA ATG -3'_ and primer B _5'- -GTA TCC TCC AGA GTG ATC GA -3'_ was used for the amplification of a 730 bp region of the *hly* gene. PCR was performed in the Perkin Elmer GenAmp PCR system 2400 thermal cycler. Amplification conditions were optimised to the thermal cycler and were as follows: 80°C for 10 min, an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, then a final extension at 72°C for 2 min. The amplified DNA was analysed by gel electrophoresis on a 1.2% agarose gel stained with ethidium bromide. A 100 bp ladder (Promega) was used as a reference marker. Tris-borate EDTA (0.5´) was used as the running buffer and the gel was viewed using UV transillumination at a wavelength of 254 nm (Gouws & Liedemann, 2005; Rip & Gouws, 2009).

5.3.4 *Salmonella* detection

Meat samples were diluted using 25 g of meat in 225 mL of sterilised buffered peptone water (BPW; MerckBiolab, South Africa). This was thoroughly homogenised. The samples containing the BPW were used for serial dilutions in which 1 mL of the dilution were transferred into 9 mL sterile quarter strength Ringers solution (Merck, South Africa). Pour plates were then prepared using Tryptic Soy

agar (TSA; Merck, South Africa). The plates were incubated at 37°C for 18-24 h and the results were. For Salmonella detection, the dilutions containing BPW and meat samples were then incubated at 37°C for 24 h. After this incubation period, 1 mL of this overnight culture was aseptically transferred to 9 mL of sterile Rappaport Vassiliadis (RV) broth (Oxoid, Basingstoke, UK) and incubated at 42°C for 18-24 h. A loopful of RV was then streaked on Xylose Lysine Deoxycholate (XLD) agar (Merck, South Africa). These plates were incubated at 37°C for 18-24 h. Genomic DNA extraction was done as prescribed earlier on presumptive positive *Salmonella* isolates, PCR primers specific for the genus *Salmonella*, ST11 and ST15, were used that produced a 429 bp band on a 1% agarose gel. *Salmonella typhimurium* (ATCC14028) was used as a positive control whereas water as well as *L. plantarum* (ATCC8014) was used as a second negative control (Gouws *et al.*, 1998).

5.4 Results

The quality indicating analyses were total viable count, coliform count and aerobic spore forming count. The total viable (TVC) count for the cabanossi's showed <10 cfu.g^{-1} growth for all at day 0, irrespective of the fat treatment (Table 5.1). The control for batches AB and CD showed microbial growth of 25 cfu.g^{-1} and 20 cfu.g^{-1}, respectively at day 60. *FR2* for batches CD and EF exhibited microbial growth of 25 cfu.g^{-1} and 65 cfu.g^{-1} respectively at day 60. The coliform count at day 0 and 60 exhibited growth of <10 cfu.g^{-1} for all fat treatments (Table 5.1). Aerobic endospore forming bacteria showed growth of <10 cfu.g^{-1} at day 0 for all fat treatments. Batch AB exhibited growth of 30 cfu.g^{-1} and 110 cfu.g^{-1} at day 60 for the *Control* and *FR1*, respectively (Table 5.1). Batch CD had growth of 125 cfu.g^{-1} at day 60 for *FR2*. Batch EF exhibited a growth of 75 cfu.g^{-1}, 45 cfu.g^{-1} and 45 cfu.g^{-1} at day 60 for the *Control*, *FR2* and *FR3*, respectively (Table 5.2). The pathogenic analyses were *Staphylococcus aureus*, *Salmonella* spp. and *L. monocytogenes*. *S. aureus* at day 0, had growth of <10 cfu.g^{-1} for all fat treatments (Table 5.1). Batch AB *FR2* exhibited the only growth at day 60 with 760 cfu.g^{-1}. All fat treatments for day 0 and 60 detected no *Salmonella* spp. and *L. monocytogenes.*

5.5 Discussion

According to South African regulation the total viable count must be <2x10^{5} cfu.g^{-1} present in a processed meat product to comply with government guidelines (DOH, 2014). Therefore, all treatments stored for a period of 60 days at 4°C were in accordance with South African (SA) regulations governing microbiological standards for foodstuffs and related matters and can be deemed shelf-stable for up to 60 days, at 4°C (Table 5.2).

Coliforms grow at temperatures between 5 - 45°C (Jay *et al.*, 2005b). The regulations state that the coliform count should be <200 cfu.g^{-1} in a processed meat product (DOH, 2014). All batches stored for a period of 60 days at 4°C were in accordance with regulations governing microbiological standards for foodstuffs and related matters and were also below the internationally acceptable levels of 10^2 - $<10^4$ cfu.g^{-1} (Table 5.2) (European Union, 1993; Jay *et al.*, 2005b). Therefore, the product can be deemed shelf-stable. If the manufacturing can consistently provide good GMP's, it can be postulated that the shelf-life of blesbok cabanossi containing a fat replacer will have a shelf-life exceeding 2 months when kept at 4°C although the length of the longer period would need to be verified.

Bacillus cereus, a spore forming bacteria producing enterotoxins can be associated with processed foods such as cooked meats. It does not pose a serious health threat to humans unless it grows to a population of $\geq 10^5$ *Bacillus cereus* cfu.g^{-1} at a temperature of 25 - 35°C. Refrigerated products have been known to contain various *Bacillus* strains (Bennet & Belay, 2001; Fritze & Pukall, 2012; Ding *et al.*, 2013). Even with hurdle technology aerobic endospore forming bacteria are difficult to destroy (Table 5.2) along with their spores. Heat resistant bacteria and spores can be destroyed at very high temperatures however, new alternatives to thermal processing are being developed such as High Pressure Processing or UV-based treatment to eliminate these heat resistant bacteria (Tokarskyy & Marshall, 2010; Aguirre *et al.*, 2015). The regulations state that for *Bacillus cereus* there should be <100 cfu.g^{-1} in a processed meat product (DOH, 2014). Due to the nature of the product a total aerobic endospore forming count analysis was performed. Two treatments from separate batches showed values slightly exceeding the recommended guidelines for *Bacillus cereus* but are well within international specifications regarding this specific organism (Table 5.2) (ANZFA, 2001). The aerobic endospore forming count was just outside of specification with regards to DOH (2014), however, a comparison to international regulations (ANZFA, 2001) indicates that the product was satisfactory and could be consumed. Two different credible regulations can justify why products are safe to consume if out of specification. All the treatments were in accordance with SA regulations governing microbiological standards for foodstuffs and related matters (DOH, 2014).

Staphylococcus aureus has the potential to grow in contaminated food products and produce enterotoxins which are hazardous to human health. *S. aureus* can grow at temperatures as low as 7°C however, the toxin can only be produced at temperatures between 10 - 46°C, with an optimum 40 - 45°C, and levels of $\geq 10^5$ *Staphylococcus aureus* cfu.g^{-1} (Lancette & Bennet, 2001; Jay *et al.*, 2005c). The growth of staphylococcal bacteria occurs more frequently after the food items undergo processing or heat treatments as competing micro-organism are eliminated. Mannitol Salt Agar (MSA) was used for the isolation of staphylococcal species as the 10% sodium chloride (NaCl) slows the growth of other micro-organisms. The regulations state that there should be <100 cfu.g^{-1} present in processed

meat products (DOH, 2014). Internationally, satisfactory levels are <20, acceptable levels are 20 - <100 cfu.g^{-1} and unsatisfactory levels are 100 - <10^4 cfu.g^{-1} (Table 5.2) (European Union, 1993). The NSW Food Authority constructed a table consisting of microbiological guidelines based on the UK Food Standards Agency and Food Standards Australia New Zealand (FSANZ) where the acceptable level for coagulase positive staphylococci are 10^2 - <10^3 cfu.g^{-1} (ANZFA, 2001). With regards to the above mentioned results, the TVC and coliform counts were both low indicating the GMP's and parameters do not support the growth of *S. aureus*. AB *FR2* had to be contaminated after processing either by handling or an unsanitary surface. The temperature at which the cabanossi was stored inhibits further growth thus eliminating the risk of toxin production. Therefore, the product was in accordance with SA regulations governing microbiological standards for foodstuffs and related matters and can be deemed safe to consume. The treatments for day 0 and 60 detected no *Salmonella* spp. and no *L. monocytogenes* thus, the processed meat product was in accordance with SA regulations (DOH, 2014).

Table 5.1 Bacteria count of all blesbok cabanossi replications and treatments for day 0 and day 60

Batches	Treatments	Bacteria found	Day 0 (cfu.g^{-1})	Day 60 (cfu.g^{-1})
AB	*Control*	Aerobic count	<10	25
		Aerobic endospore forming	<10	30
	FR1	Aerobic endospore forming	<10	110
	FR2	*S. aureus*	<10	760
CD	*Control*	Aerobic count	<10	20
	FR2	Aerobic count	<10	25
		Aerobic endospore forming	<10	125
EF	*Control*	Aerobic endospore forming	<10	75
	FR2	Aerobic count	<10	65
		Aerobic endospore forming	<10	45
	FR3	Aerobic endospore forming	<10	45

Control (contains 20% pork fat), *FR1* (contains 10% fat replacer), *FR3* (contains 20% fat replacer) and *FR3* (contains 30% fat replacer).

Table 5.2 The microbial guideline (cfu.g^{-1}) for ready-to-eat foods implemented by several international countries

	Criterion				
	***DOH, (Act No. 54 of 1972): Regulation 692 of 1997**	**Satisfactory**	**Acceptable**	**Unsatisfactory**	**Unacceptable/ potentially hazardous**
Aerobic plate count	$<2x10^5$	$<10^5$	10^5 - $<10^6$	$\geq 10^6$	N/A
Coliform count	<200	<100	10^2 - $<10^4$	$>10^4$	N/A
Bacillus cereus	<100	$<10^3$	10^3 - $<10^4$	10^4 - $<10^5$	$\geq 10^5$
S. aureus	<100	<20	20 - <100	100 - $<10^4$	$\geq 10^4$
***Salmonella* spp.**	Not detected in 25g	Not detected in 25g	-	-	Present in 25g
L. monocytogenes	<10	<20	20 - $<10^2$	N/A	$\geq 10^2$

N/A denotes not applicable. Food Safety Standard 3.2.2 of the Australia New Zealand Food Standards Code specifies food handling controls. EU Food Hygiene Directive (93/43/EEC: Section 11.6).

*Department of Health, DOH (2014), foodstuffs, cosmetics and disinfectants Act No. 54 of 1972. Regulations governing microbiological standards for foodstuffs and related matters (R.692 of 1997). http://www.health.gov.za.

5.5 Conclusions

To conclude, the total aerobic and coliform counts were well below the DOH (2014) regulations. The total viable and coliform counts both indicate good manufacturing practices therefore, the manufacturing process that was designed effectively eliminated micro-organisms that cause quality concerns for 60 days. One treatment was out of specification for *S. aureus* counts regarding South African and international regulations. Therefore, it was postulated that contamination had occurred post-production and it can therefore be concluded that the parameters can prevent the growth and toxin production of this organism for up to 60 days. The aim of the study was to produce amicrobial safe product that would have a shelf-life of 60 days. The fat replaced product containing canola oil obtained microbial specifications that were in accordance with DOH (2014) and achieved a bacterial safe shelf-life of 60 days.

5.6 References

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Chapter 6

General conclusions and recommendations

Processed meat products such as cabanossi contain high levels of animal fat (pork fat) that is considered unhealthy due to the saturated fatty acid content. It is well-known that animal fat contains high levels of SFA which can lead to CVD, that is why it is important to alter the fatty acid composition with a "healthier" alternative such as canola oil which contains high levels of MUFA's and PUFA's. Furthermore, fat replacers have been growing in popularity due to their ability to reduce the use on animal fats in processed meat products. Protein-based fat replacers are able to incorporate vegetable oils into cabanossi and benefit the diet of individuals. Therefore, a full study was conducted on the complete replacement of animal fat (pork fat, PF) with a canola oil containing protein-based hydrocolloid gel (*FR*) in a traditional processed/cooked meat product, cabanossi. The full study tested three levels of fat replacer containing canola oil: low inclusion (10% *FR1*), medium inclusion (20% *FR2*), high inclusion (30% *FR3*), and compared these to a *Control* sample containing a standard 20% PF.

The study began analysing the proximate content (moisture, protein, fat and ash) of the *FR* treatments and *Control*. Due to the *FR* treatment's ability to bind water the moisture content was higher ($P \leq 0.05$) than that of the *Control*. The results showed that the good binding capacity of the fat replacer retained moisture better during processing than the *Control* containing pork fat. The fat content of the three *FR* treatments were lower ($P \leq 0.05$) than that of the *Control.* The addition of the canola oil had little effect on the overall fat content *of* the *FR* treatments as the cabanossi's fat content was reduced by ~20% across all three *FR* inclusion levels (10%, 20% and 30% fat replacer). The lipid oxidation did not differ ($P > 0.05$) at day 0 and day 60 between the *Control* and the three *FR* treatments. The fatty acids also did not differ ($P > 0.05$) at day 0 and day 60 which correlates well with the lipid oxidation results. With regard to the fatty acid ratios, *FR2* and *FR3* had a larger ($P \leq 0.05$) PUFA:SFA ratio than the *Control*. Furthermore, all three *FR* treatments had lower n-6:n-3 ratios compared to the *Control*. Based on the ratios, the *FR* treatments have a better fatty acid composition due to the added canola oil (high in MUFA's and PUFA's) in the fat replacer, potentially adding to the drive to increase the consumption of unsaturated fatty acids as part of a daily diet. The chemical profiles of the *FR* treatments were acceptable from a nutritional point of view.

When producing processed meat products consumer acceptability is also governed by a product's overall sensory quality, i.e. aroma, flavour and texture. The results showed that the traditional cabanossi, *Control*, scored high in attributes such as smoky aroma and flavour, fatty aroma and flavour, cabanossi flavour, chewiness and juiciness. These sensory attributes are considered to be typical of traditional cabanossi. The sensory profile of the three *FR* treatments are definitely not

"traditional" when considering the typical sensory profile of cabanossi with the *FR* treatments scoring high in saltiness, bitter taste, crumbliness and gelatinous which are not commonly referred to as favourable attributes in processed meat products. The results thus showed that the fat replacer had a negative influence on the cabanossi's overall sensory profile. The instrumental texture analysis correlated with sensory hardness, substantiating, but also adding to the results of the DSA analysis. *FR1* contained the most meat which means that the increased protein-protein interactions increased the hardness. Although the *Control* contained animal fat and less meat than *FR1*, these two treatments did not differ ($P > 0.05$) in hardness which showed the influence animal fat has on the protein matrix of a processed meat product.

The *FR* treatments had a high moisture content which, if available, positively influences the growth of micro-organisms. For this reason the effect of the fat replacer on microbial quality was evaluated. The *Control* and all three *FR* treatments had a stable shelf-life for 60 days and were microbiologically safe, adhering to the South African Department of Health's microbiological regulations (DOH, 2014). The results further support the postulation that the extra moisture in all three *FR* treatments is bound by the fat replacer.

It can be concluded that the fat replacer in the form of canola oil containing protein-based hydrocolloid gel had an effect on the chemical, physical, microbiological and sensory characteristics of a blesbok cabanossi. The fat replacer had a positive effect on the chemical characteristics by reducing the fat content and positively altering the fatty acid profile of the game characteristics. The microbiological characteristics evaluated indicated that the manufacturing procedure effectively produced a fat replaced game cabanossi that was microbiologically safe for 60 days, at 4°C. The sensory and texture profiling were negatively affected by the fat replacer. Negative attributes such as bitter taste and gelatinous are unfavourable flavour and textural attributes in processed meat products. Therefore, for further research it would be recommended to formulate a partial substitution of pork fat with the canola oil containing protein-based hydrocolloid gel (*FR*) which would improve the sensory and texture profile; animal fat provides important properties which would improve the binding and moisture retention. The sensory attributes from the partially substituted treatments would include higher fatty aroma, flavour and fattiness scores which contribute to a better cabanossi flavour.

MDA is a liable compound overtime therefore, in future research projects such as this it is recommended that the lipid oxidation time intervals be reduced which would improve the profiling of the products lipid oxidation curve. Lastly, it is also recommended that future to re-visit the descriptive sensory analysis also at 14 and 30 days to identify if or when, the bitter taste starts to develop. The bitter taste development would reduce the shelf-life with regards to quality.

6.1 References

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