THE EFFECT OF DIETARY OMEGA-3 FATTY ACIDS WITH SPECIFIC REFERENCE TO *ECHIUM* SEED OIL ON PORK QUALITY

By

BARBARA ELIZABETH VAN WYNGAARD (*nee* LIEBENBERG)

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Promoter: Prof. A. Hugo
Co-promoters: Dr. P.E. Strydom
Prof C.H. Pohl-Albertyn
Dr F.H. de Witt
Dr A. Kanengoni

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DECLARATION

DECLARATION

I, Barbara Elizabeth van Wyngaard, declare that the Doctoral Degree research thesis or interrelated, publishable manuscripts / published articles that I herewith submit for the Doctoral Degree qualification at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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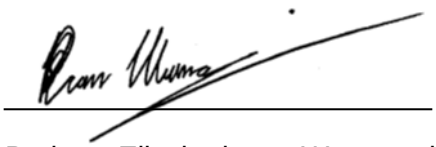

Barbara Elizabeth van Wyngaard

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GLOSSARY OF ABBREVIATIONS

*a**	Colour coordinate – redness value
@	At
ADG	Average daily gain
ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oils Chemist's Society
ARC	Agricultural Research Council- Irene
ASTM	American Society of Testing Materials
a_w	Water-holding capacity
*b**	Colour coordinate – yellowness value
BFAP	Bureau of Food and Agriculture Policy
BHT	Butylated hydroxytoluene
BSE	Bovine Spongiform Encephalopathy
c	*Cis*
CF	Crude fibre
cm	Centimeter
°C	Degrees Celsius
CP	Crude protein
CVD	Cardiovascular disease
d	Day
DAFF	Department of Agriculture, Forestry and Fisheries
DBI	Double bond index
Δ	Delta
DE	Digestible energy
DFD	Dark, firm and dry
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
EDTA	Ethylene diamino tetra-acetic acid
EFC	Extractable fat content
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester/s

Individual FAME:

Abbreviation name	*Common name*	*Complete formula*	*Systematic (IUPAC)*
C14:0	Myristic	C14:0	Tetradecanoic
C14:1	Myristoleic	C14:1*c*9	Tetradecanoic acid
C15:0	Pentadecylic	C15:0	Pentadecanoic
C16:0	Palmitic	C16:0	Hexadecanoic
C16:1	Palmitoleic	C16:1*c*9	*cis*-9-Hexadecenoic
C17:0	Margaric	C17:0	Heptadecanoic
C17:1	Heptadecenoic	C17:1*c*10	*cis*-10-Heptadecenoic
C18:0	Stearic	C18:0	Octadecanoic
C18:1*c*7	Vaccenic	C18:1*c*7	*cis*-7-Octadecenoic
C18:1*c*9	Oleic	C18:1*c*9	*cis*-9-Octadecenoic
C18:1*t*9	Elaidic	C18:1*t*9	*trans*-9-Octadecenoic
C18:2*c*9,12	Linoleic	C18:2*c*9,12(*n*-6)	*cis*-9,12-

C18:2*c*9*t*11	CLA	C18:2*c*9*t*11	Octadecadienoic (9*c*,11*t*)-octadeca-9,11-dienoic acid
C18:3*n*-3	α-Linolenic	C18:3*c*9,12,15(*n*-3)	*cis*-9,12,15-Octadecatrienoic
C18:4	Stearidonic acid	C18:4c6,9,12,15(n-3)	*cis-6,9,12,15-octadecatetraenoic acid*
C20:0	Arachidic	C20:0	Eicosanoic
C20:3*n*-6	Eicosatrienoic	C20:3*c*8,11,14(*n*-6)	*cis*-8,11,14-Eicosatrienoic
C20:4	Arachidonic	C20:4*c*5,8,11,14(*n*-6)	*cis*-5,8,11,14-Eicosatetraenoic
C20:5	Eicosapentaenoic	C20:5c5,8,11,14,17(n-3)	*cis-5,8,11,14,17-Eicosapentanoic*
C22:0	Behenic	C22:0	Docosanoic
C22:5	Docosapentaenoic	C22:5*c*7,10,13,16,19(*n*-3)	*cis*-4,7,10,13,16-Docosapentaenoic
C22:6	Docosahexaenoic	C22:6c4,7,10,13,16,19(n-3)	*cis-4,7,10,13,16,19-Docosahexanoic*

FCR	Feed conversion ratio
FFA	Free fatty acids
FFDM	Fat free dry matter
FHM	Fat hardness measurement
FS	Fat score
g	Gram
GC	Gas chromatograph
h	Hour
H_2O	Water
Hz	Hertz
i.e.	That is
IMF	Intramuscular fat
IV	Iodine value
KCl	Potassium chloride
kg	Kilogram
kN	Kilonewton
L	Litre
*L**	Colour coordinate – lightness value
LMC	Lean meat content
µl	Microlitre
µmol/g	Micromole per gram
µM	Micromolar
mg	Milligram
mM	Millimolar
m^2	Square meter
mm	Millimeter
ml	Milliliter
mmol/kg	Millimole per kilogram
Mb	Myoglobin
MFL	Myofibrillar fragment length/s
$MgCl_2$	Magnesium chloride

MSG	Monosodium glutamate
MUFA	Monounsaturated fatty acid/s
N	Normal
N_2	Nitrogen
NaCl	Sodium chloride (salt)
NaN_3	Sodium azide
NaOH	Sodium hydroxide
n-3	Omega-3 fatty acid/s
n-6	Omega-6 fatty acid/s
NBT	Nitroblue tetrasolium
ND	Not detected
NDF	Neutral Detergent Fibre
NIRS	Near infrared spectroscopy
NS	Not significant
P	Significance level
%	Percentage
PCA	Principle component analysis
pH_{45min}	pH value 45 minutes post mortem
$pH_{24hours}$	pH value 24 hours post mortem
ppm	Part per million (mg/kg)
PSE	Pale, soft and exudative
PUFA	Polyunsaturated fatty acid/s
PV	Peroxide value
PVC	Polyvinyl chloride
RDA	Recommended daily allowance
rH	Relative humidity
rpm	Revolutions per minute
S	Chroma
SI	Saturation index
SAMIC	South African Meat Industry Company
SFA	Saturated fatty acid/s
t	*trans*
TBA	2-Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
UFA	Unsaturated fatty acid/s
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
V	Volts
VIA	Video image analyses
WBS	Warner Bratzler shear force
WHC	Water holding capacity
w/w	weight per weight
<	Less than
>	More than
%	Percentage

CHAPTER 1

INTRODUCTION

During the last four decades consumers have become more health conscious and are presently more informed on diet and nutritional concerns than ever before (Michaelidou & Hassan, 2008). In the past consumers questioned the healthiness of pork as they believed it contained an excess of fat, saturated fatty acids and cholesterol (Hernández, Navarro, & Toldrá, 1998). The response of pig industries worldwide was to reduce the fat content by adopting new feeding and breeding strategies (Dugan, Vahmani, Turner, Mapiye, Juárez, Prieto, Beaulieu, Zijlstra, Patience & Aalhus, 2015). This led to pork with intramuscular (marbling) fat of as little as 0.8%-1%. A study by Fortin, Robertson and Tong (2005) revealed that the level of intramuscular fat needs to be at least 1.5% to ensure palatability and a pleasant eating experience.

Pork is the most consumed meat across the globe accounting for 38% of the meat production and over 36% of the world's meat intake (FAO, 2015). In pigs, being monogastric animals, the fatty acid (FA) composition of the fat tissue triglycerides mirrors the FA composition of dietary fat (Rhee, Davidson, Cross, & Ziprin, 1990). It is therefore possible to improve the image of pork among consumers by using dietary manipulation to design pigs with a healthier FA profile.

The first recommendation to consumers to reduce the intake of dietary fat, cholesterol and saturated fatty acids was during the late 1950's. These recommendations were made to prevent cardiovascular disease (CVD) by the American Heart Association (Lichtenstein, Apple & Brands, 2006). Worldwide cardiovascular diseases are one of the largest sources of morbidity and mortality (Mitka, 2004).

A number of prominent international bodies such as the World Health Organisation (WHO) and The International Society for Study of Fatty Acid and Lipids (ISSFAL), recommend the consumption of long chain (LC) (≥C20) n−3 polyunsaturated fatty acids (PUFA) to reduce CVD risk. In most cases these recommendations specifically advise consumption of LC n−3 PUFA, eicosapentaenoic acid (EPA, C20:5n−3) and docosahexaenoic acid (DHA, C22:6n−3) (Kitessa & Young, 2009). The American Heart Association recommends an average daily intake of about 500 mg EPA and DHA to reduce the risk of CVD (Gebauer, Psota, Harris, & Kris-Etherton, 2006).

There is a trend in developing nations that the rate of meat consumption increases parallel with increase in wealth (Myers & Kent, 2003). It is estimated that between 1997 and 2020 developing countries will increase their demand for meat by 92% due to the increase in population (Myers & Kent, 2003). It is therefore important to find ways to increase the n-3 LC-PUFA content of meat to help reduce the occurrence of chronic diseases (Kitessa & Young, 2009). Meat can then also be marketed as a nutraceutical as it will have added health benefits.

For a long time the inclusion of marine sources such as fishmeal in pig diets was an efficient method of increasing the n-3 fatty acid content of pork (Kitessa, Gulati, Ashes, Scott, & Fleck, 2001; Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003; Rymer & Givens, 2005). However, the use of seafood products and by-products in livestock feed is not a sustainable strategy (Kitessa & Young 2009). Worm et al. (2006) predicted that seafood resources would be depleted by 2050. Even though not everyone is in agreement with this extreme prediction, the increase in demand for human consumption and aquatic feed has cast doubt on the sustainability of captured fisheries (FAO, 2006). In anticipation of this future scenario of a discrepancy in supply and demand of n-3 LC-PUFA, some authors proposed the use of plant biotechnology to produce n-3 LC-PUFA in land plants (Ursin, 2003; Damude & Kinney, 2007).

It has been proposed that existing vegetable oils offer some scope for enriching meat with n-3 PUFA as most of them contain α-linolenic acid (ALA; 18 : 3n-3) (Kitessa & Young, 2009). The inclusion of linseed oil as a source of the precursor fatty acid α-linolenic acid (ALA, C18:3n3) in the maternal diet has been studied in pigs (Bazinet, McMillan, & Cunnane, 2003). Although some results indicate an increase in the DHA concentration in offspring, the conversion of ALA to eicosapentanoic acid (EPA, C20:5n-3) and DHA is rather low (Tanghe, Millet, & De Smet., 2013). It was estimated that up to 60% of dietary ALA in man is oxidised to carbon dioxide (Brenna, 2002), which left very little to be stored as ALA or conversion to EPA and DHA. Hence, there seems to be very limited opportunity for using ALA-containing oils to influence EPA and DHA levels in tissues.

A plant with potential as omega-3 source is *Echium plantagineum*. It grows wild in the Western Cape, South Africa (Sharma & Esler, 2008) and is a rich source of stearidonic acid (SDA; C18:4 c6,9,12,15) an omega-3 fatty acid (Tanghe et al., 2013). Stearidonic acid lies in a more advanced position than linolenic acid in the omega-3 biosynthetic pathway (Kitessa & Young, 2009). Therefore the belief is that *Echium* oil will bypass the Δ6-desaturase and be more capable than linseed oil to increase the sought after eicosapentaenoic (EPA) and DHA omega-3 fatty acids in pork (Tanghe et al., 2013). A critical review by Whelan (2009), compared the biological activities of SDA with other dietary n−3 PUFA, and concluded that “SDA could become a prominent surrogate for EPA in the commercial development of foods fortified with n−3 PUFA”. It is therefore very important that this potential source of omega-3 fatty acids must be evaluated as an omega-3 source for pigs.

Research problem and objective

Cardiovascular diseases are one of the leading causes of morbidity and mortality worldwide (WHO, 2015). It is widely accepted that dietary long chain n-3 PUFA play a significant role in minimizing the risk of cardiovascular disease (Ruxton, Reed, Simpson, & Millington, 2007). Being a monogastric animal, dietary fatty acids are deposited unchanged in intramuscular and subcutaneous fat of pigs (Rhee et al., 1990). Up till now the marine sources such as fishmeal were

an efficient method of increasing the omega-3 fatty acid content of pork (Moran, Morlacchini, Keegan, & Fusconi, 2018). The use of seafood products and by-products in livestock feed will not be a sustainable strategy in the near future due to overexploitation (Moran, et al., 2018).

Other sources of omega-3 fatty acids for use in pig feeding must therefore be investigated. A plant with such potential is *Echium plantagineum*. It is a rich source of the C18:4c6,9,12,15 (stearidonic acid) omega 3 fatty acid (Tanghe et al., 2013). Stearidonic acid lies in a more advanced position than linolenic acid in the omega-3 biosynthetic pathway (Kitessa & Young, 2009). It is therefore possible that dietary *Echium* oil supplementation may also lead to higher levels of the sought after eicosapentaenoic (EPA) and docosahexaenoic (DHA) omega-3 fatty acids in pork (Tanghe et al., 2013). It is therefore very important that this potential source of omega-3 fatty acids must be evaluated as an omega-3 source for pigs.

The first aim of this study was to evaluate the potential of omega-3 containing oils with special reference to *Echium* oil in increasing the omega-3 fatty acid content of pork.

The following hypothesis was formulated:

> In pigs the dietary fatty acids are deposited unchanged into the fat tissue triglycerides (Rhee et al., 1990). It is therefore possible to manipulate the fatty acid profile of pork tissue to produce pigs with a healthier FA profile.
>
> The null hypothesis would be that all experimental diets will increase the n-3 content of pork tissue.

The second aim of the study was to determine the effect of the inclusion of different omega-3 containing oils, including *Echium* oil, in pig diets on animal performance, meat quality and eating quality.

The following hypothesis was formulated:

> Various authors (Van Oeckel, Casteels, Warnants, Van Damme, & Boucqué, 1996; Leskanich, Matthews, Warkup, Noble, & Hazzledine, 1997; Bryhni, Kjos, Ofstad, & Hunt, 2002; Nuernberg et al., 2005; Teye et al., 2006; Mitchaothai, et al., 2007; Haak, De Smet, Fremaut, Van Walleghem, & Reas, 2008; Valencia, O'Grady, Ansorena, Astiasarán, & Kerry, 2008) have found that adding different dietary oils to the feed of pigs had no effect on animal performance, meat quality or eating quality.
>
> The null hypothesis would therefore be that feeding different omega-3 containing oils will have no effect on animal performance, meat quality or eating quality.

The third aim of the study was to compare the technological properties and chemical stability of fat tissue obtained from animals fed different omega-3 containing oils, including *Echium* oil.

The following hypothesis was formulated:

Increasing the PUFA to improve the healthiness of pork is usually accompanied by the deterioration in the technological quality (Hugo & Roodt, 2007) as it results in softer fat. Pork with higher levels of PUFA are more susceptible to lipid oxidation (Monahan et al., 1992).

The null hypothesis would be that increasing the omega-3 content of pork will result in poor technological properties or chemical stability.

The fifth aim of the study was to compare the oxidative stability of processed meat products manufactured from meat obtained from animals fed diets with inclusions of *Echium* oil and other omega-3 containing oils with control animals.

The following hypothesis was formulated:

Fat rancidity is usually not a big problem in cured meat products, such as bacon, due to the anti-oxidative action of nitrite (Weiss, Gibis, Schuh, & Salminen, 2010). Smoking also has a preservative effect and protects products from oxidation (Pearson & Gillett, 1996). Fat-rich fermented meat products, such as salami, are more susceptible to oxidation, because it is more exposed to oxygen than raw meat.

The null hypothesis would be that processed meat products manufactured from meat obtained from animals fed *Echium* oil and other omega-3 rich oils will oxidise faster than products from the control animals.

The final aim of the study was to improve the international competitiveness of the South African pig industry by developing a product that can be internationally marketed as a nutraceutical.

The following hypothesis was formulated:

Kitessa and Young (2009) found that the thigh muscle from chickens fed *Echium* oil can be labelled as being a source of long chain n-3 PUFA.

The null hypothesis would be that feeding *Echium* oil to pigs will increase the levels of n-3 PUFA that it can be labelled as a source of n-3 PUFA.

CHAPTER 2

LITERATURE REVIEW

Meat is an important part of the Western food culture and is typically seen as the centre of a meal (Charles & Kerr, 1988; Lupton, 1996). Meat provides a wide range of valuable nutrients such as high biological value proteins, fat, vitamins and micronutrients niacin, vitamins B1, B2, B3, B6, B12, iron and zinc, which are essential for good health (Williamson, Foster, Stanner, & Buttriss, 2005). For this reason most vegetarians suffer from vitamin B12 and iron deficiencies (Sanders & Reddy, 1994; Leonhardt, Kreuzer, & Wenk, 1997; Ryan, 1997). The consumption of animal-derived foods differ among and within populations, therefore, the impact thereof on human health is also varies (FAO, 2009).

Illnesses associated with lifestyle have increased in the Western world and the relationship between diet and health have been established, especially the consumption of saturated fats in animal products with illness and weight gain. The consumption of meat and processed meat products' have been linked to the occurrence of adverse health conditions such as some types of cancers (Linseisen, Rohrmann, Norat, et al., 2004; Sato, Nakaya, Kuriyama, Nishimo, Tsubono, & Tsuji, 2006; Demeyer, Honikel, & De Smet, 2008) and diabetes (Schultze, Manson, Willet, & Hu, 2003). In response to numerous, and often contradictory, scientific reports relating specific foods to health outcomes (Balder, Goldbohm, & van den Brandt, 2005; Lüchtenborg et al., 2005; Norat et al., 2005; Larsson, Bergkvist, & Wolk, 2006; Sato et al., 2006; Chan, Wang, & Holley, 2007; Alexander, Cushing, Lowe, Sceurman, & Roberts, 2009), it was recommended that consumers "limit the intake of red meat and avoid processed meat" (World Cancer Research Fund, 2010; Boada, Henríquez-Hernández, & Luzardo, 2016; Crippa, Larsson, Discacciati, Wolk, & Orsini, 2018).

It is clear that meat consumption is in a period of chance and the future of meat will be influenced not only by health but also economic, environmental and ethical issues. However, eating meat is a biocultural activity and has co-evolved with human development (Leroy & Praet, 2015), it evokes strong emotional responses, unlike any other food. This could explain why the debate on the nutritional benefits versus the possible unfavourable health effects of meat consumption is often contradictory and irrational.

Whatever the future holds for meat consumption, it is important to know the factors that determine the nutritional value of meat and the impact on human health and disease. It is possible to manipulate several micronutrients in meat, which may allow increasing their supply in the food chain through meat consumption (Givens & Gibbs, 2006, 2008; Wood et al., 2008; Rooke, Flockhart, & Sparks, 2010; De Smet, 2012).

2.1. Factors affecting pork consumption

In present times pork consumption and meat consumption in general, are influenced by several factors. At consumer level, these include price, income, availability, perceived healthiness, eating enjoyment, changes in consumer tastes and preferences, linking 'stories' to food, safety, animal welfare and religion (Burton, Dorsett, & Young, 1996; Rickertsen, 1996; Steenkamp, 1997; McCarthy, O'Reilly, Cotter, & De Boer, 2004). In the meat sector, these factors include accidents, scandals, animal welfare, and product safety incidents that attract negative media attention and damaged the image of the sector image (Steenkamp, 1997; McCarthy et al., 2004). Research found negative information has a stronger impact than positive information on consumers' perceptions and food choices (Verbeke et al., 2008; Smed, 2012) because consumers consider the avoidance of possible harm to be more important than the chance of a possible benefit (Verbeke, 2005).

The price of meat and gross income were two of the main factors determining the purchase and consumption of meat, however, Bansback (1995) and Becker and co-workers (2000) noted that health, convenience and quality issues now have a more important influence on behaviour. Although pork occupies a very high share in the total meat consumption basket of many people, pork's image among consumers is not univocally positive. Consumers profiled pork as a cheap, convenient and everyday type of meat that is not suitable for special occasions, but they also perceived it as the least healthy and fattest meat compared to beef and poultry (Verbeke & Viaene, 1999; Verbeke, Viaene, & Guiot, 1999; Bryhni et al., 2002; Ngapo et al., 2002; Pereira & Vicente, 2013). Clearly, the advice from doctors and dieticians is incorporated into consumers' assessment for pork and thus influences consumption.

Consumers are aware of hazards such as antibiotic residues, Bovine Spongiform Encephalopathy (BSE), cholesterol, *Escherichia coli* and *Salmonella*, however, they do not fully understand what these hazards are, the threats they pose and how widespread they are. Many researchers (Cahill, 1996; McCarthy, 2000) have pointed to this lack of knowledge as one of the main reasons for the high levels of confusion amongst meat consumers. Recently it seems that health and nutrition are now becoming more important to the consumer than safety concerns (Verbeke, Frewer, Scholderer, & De Brabander, 2007; da Fonseca & Salay, 2008).

Consumers view high animal welfare standards at the production stage as an indicator that the resulting food is safe, healthy and of high quality (Fallon & Earley, 2008; Weddle-Schott, 2009). The development of more safety control and traceability systems and the provision of information to consumers have been important in improving perceptions of meat safety (Angulo & Gil, 2007; Verbeke, 2001). Consumers, however, have different ideas about food safety compared to experts, and there are significant differences within both these groups (Verbeke et al., 2007).

Religion is one of the main factors determining food avoidance, taboos, and special regulations in particular with respect to meat (Simoons, 1994). Several religions impose some food restrictions e.g. prohibition of pork and not ritually slaughtered meat in Judaism and Islam,

and pork and beef in Hinduism and Buddhism. With respect to food prescriptions in Islam, Muslims have to follow a set of dietary laws intended to advance their well-being, in addition to the five pillars of Islam. These dietary laws or prescriptions determine which foods are halaal (i.e. permitted) for Muslims. Prohibited is the consumption of alcohol, pork, blood, meat from dead (not slaughtered) animals and meat that has not been slaughtered according to Islamic rulings.

2.2. Global meat consumption

Global meat consumption has expanded significantly over the past decade, as growing population numbers, as well as growing income levels in developing countries, drive changes in food consumption patterns. Global pork consumption accounted for 37% of total meat consumed worldwide from 2014 to 2016, making it the most consumed protein (OECD, 2017). Pork consumption saw a steady increase from almost 90 million tons in 2000 to over 116 million tons in 2016 (OECD, 2017). Global pork consumption is dominated by China, who consumed almost 47% of the world's pork in 2016. Pork consumption per capita in China gradually increased between 1975 and 2012. In 2016, pork consumption reached 54.6 million tonnes in China, more than twice as much as in the European Union (EU (20.9 million tonnes) and more than five times as much as the United States (9.4 million tonnes) (OECD, 2017).

In much of the developing world, per capita meat consumption declined in 2016 as income growth slowed. This decline was evident in pork with worldwide consumption declining from 117.2 million tonnes in 2015 to 116.7 million tonnes in 2016. In China, consumption fell from 55.4 to 54.6 million tonnes from 2015 to 2016. With living standards improving, urban consumers prefer beef, lamb and poultry as they believe it to be higher in protein, have a lower fat content and therefore have a higher nutritional value compared with pork (Li, Zhao, & Chen, 2011). However, pork consumption is expected to increase again as urbanization and disposable income are growing in China (USDA, 2013).

In the EU, during the 1990s, there was a decrease in the availability of meat due to changing consumer taste and preference patterns, the occurrence of meat safety crises (Bovine Spongiform Encephalitis – (BSE) and dioxin contamination) together with the related negative media attention, and a lack of initial responsiveness by the meat sector (Verbeke & Viaene, 1999). This decrease was seen in all EU countries except Norway, Portugal and the Republic of Ireland (European Commission, 2002; Trichopoulou, Naska, & Costacou, 2002). Today pork is the most widely consumed meat in the EU. In 2016 the EU consumed 20.9 million tonnes of pork, making it the second largest consumer of pork worldwide (OECD, 2017).

In Ireland pork accounts for 41% of meat consumed. In the early 1990s, bacon was very popular in Ireland but by the end of the 1990s consumption decreased due to changing lifestyles, eating patterns and health consciousness. However, the Irish pig meat sector remained stable as the demand for fresh pork and other value-added pig meat products increased. Therefore, Irish pork consumption levels have remained quite steady over recent years, with a slight increase from

38 kg per capita in 1995 to 38.3 kg per capita during 2001 (CSO, 1997, 2002). Ireland is a society that has a strong relationship to livestock farming and meat might still be seen as an important ingredient of a 'proper meal', and therefore, they have a more positive attitude towards these meats (McCarthy, et al., 2004).

Globally there is a huge transition in the pork industry. The per capita pork consumption is expected decline marginally over the next decade as consumption will reach saturation levels in most developed countries. Growth is sustained in Argentina, Brazil, Mexico and Uruguay, albeit at a generally slower rate than the past decade. Pork consumption has experienced rapid growth over the past few years in Latin America, driven by increased domestic production, improved quality, and favourable relative prices that have positioned pork as one of the favoured meats, along with poultry. Many countries with favourable economic conditions and expanding meat consumption do not traditionally consume high levels of pork relative to other meats, resulting in stagnant and even declining consumption on a per capita basis at the regional level, however, population expansion will still supports growth in total pork consumption in these regions (OECD, 2017).

2.3. South African pork consumption

The South African pork industry is relatively small in terms of the overall South African agricultural sector. It contributes around 2.1% to the primary agricultural sector (DAFF, 2017). Regarding total expenditure on all meat during 2003 (including chicken and game), 6.8% was spent on pork, while 40.5% was spent on beef, 34.4% on chicken, 17.2% on sheep meat and 1% on game (Taljaard, Jooste & Aafaha, 2006). Within the global context, South African pork production also remains very small; the South African pork industry contributes only 0.18% of total pork produced worldwide, rendering it an insignificant player in world markets, while at the same time making it vulnerable to changes in global pork markets.

In response to the increasing consumption and/or demand for pork products, pork production has been increasing over the past decade and so has the number of pigs slaughtered. In February 2017, a total of 200 504 pigs were slaughtered in South Africa (Scheltema & Delport, 2017). From 2008/09 to 2016/17, both the slaughtering trend and production trend have been increasing. During the past decade, more than 26 million pigs were slaughtered, yielding almost 2 million tons of pork meat. During 2013/14, South Africa was self-sufficient by producing 236 300 tons of pork. Consumption slightly decreased during the period of 2013/14 (Fig. 2.1). This may be due to price increases in this period, which made the pork meat relatively expensive compared to other white meat in South Africa (DAFF, 2017). In 2014/15 and 2015/16, the consumption again outstripped the production. Pork had to be imported to meet local demand. The per capita consumption has shown an increasing trend from 2009/10 (4.4 kg) to 2015/16 (4.8 kg) (except in 2013/14). The increased per capita consumption may be due to the increasingly urbanised consumers with increased per capita income (DAFF, 2017).

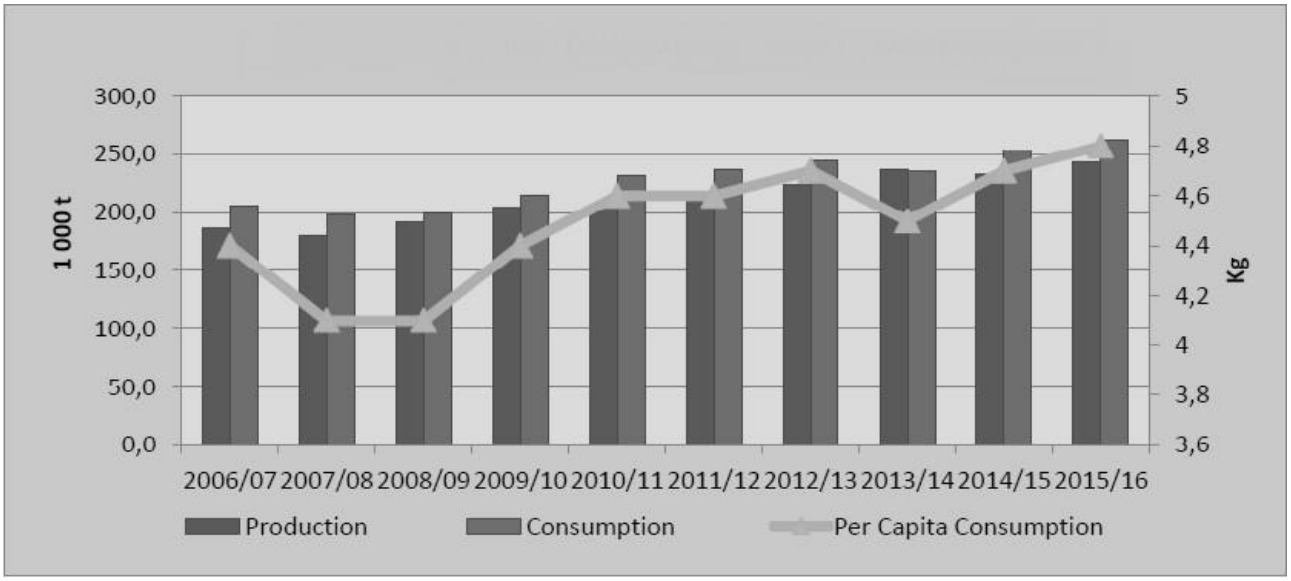

Figure 2.1: Pork production, consumption and per capita consumption from 2006/7 to 2015/16 (DAFF 2017)

South Africa is a net importer of pork products and given the role of imports in balancing the market, BFAP (2017) indicates that South Africa will remain a net importer of pork products over the next 10 years. The share of imports in domestic consumption is expected to decline from 11% to 7% over the next decade. However, pork production is projected to expand by 42% over the next 10 years (BFAP, 2017).

The local market for pork is split into the fresh meat market and the processed meat market, with 45% going to the fresh market and about 55% going to the processed meat market. The South African meat processing industry manufacture bacon, sausages, hams and other meat products. In South Africa there are 400 commercial pork producers and around 4000 non-commercial producers. The latter mainly produce pigs for domestic consumption and slaughter 350000 pigs annually. There are 46 registered pig abattoirs in South Africa that are responsible for the slaughtering of just more than the 2 million pigs annually (Eskort, 2017).

Fresh pork products include pork chops, roasts and gammons and are estimated to be worth more than R1.5 billion annually with volumes of around 120 000 tonnes. Polonies and vienna's hold a market share of 40% and 30%, respectively, with the balance, made up by bacon, sausages, russians, hams, spreads and meat rolls (Eskort, 2017).

Table 2.1 shows the preferences of households for pork products in the survey area by race and product preference. While white people consume the most pork products, the percentage of black people who consume high-value pork is close to that of whites. Asians consume the lowest amount of pork products, which could most probably be traced back to religious beliefs.

Table 2.1: Household preferences for pork products in Bloemfontein, Central South Africa (Oyewumi & Jooste, 2006)

Race	Fresh meat	Value-added product	Pre-prepared pork foods
Blacks	48.4%	70%	46.2%
Whites	76.9%	78%	57.1%
Coloureds	53.8%	48%	35%
Asians	25%	37.5%	25%

2.4. Pork quality

Fresh meat quality is difficult to define because it is a complex concept determined by consumer preferences. In the past emphasis in meat quality studies was on safety, sensory and shelf-life aspects of food products. Today the focus has shifted to nutritional value, well-being and health (Verbeke, Pérez-Cueto, de Barcellos, & Krystallis, 2010). Quality must be constantly measured, at all times maintained, opportunistically enhanced and always be evaluated in terms of consumer expectations and needs.

Quality characteristics are influenced by various factors such as muscle structure, chemical composition, chemical environment, the interaction of chemical constituents, post mortem changes in muscle tissues, stress and pre-slaughter effects, product handling, processing and storage, microbiological numbers and populations (Joo, Kim, Hwang & Ryu, 2013).

2.4.1. Consumer perspective on quality

For the consumer, some of the main quality cues of meat include appearance, eating quality, price and origin. These quality ques can be categorised based on major intrinsic and extrinsic cues. Intrinsic cues are the physiological characteristics of meat, (such as appearance- colour and visible fat) and extrinsic cues are everything else (price, origin and brand) (Joo & Kim, 2011). All these quality traits contribute to the consumer's expectation of high-quality meat unfortunately not all of these attributes can be evaluated by the consumer at the point of purchase.

Quality perception of meat has traditionally been largely based on intrinsic cues. However, consumers have not been very competent in interpreting quality from these cues (Bredahl, Grunert, & Fertin, 1998; Brunsø et al., 2005). For the average consumer meat of good quality has a desirable colour, firm texture, less drip, high marbling, moderate visible fat and a fresh meat odour, while discolouration, soft texture, large amount of drip, less marbling, excessive visible fat and abnormal meat odour are considered as poor quality traits for fresh meat. Of these, colour is probably the most important, as it is the first quality attribute for the consumer in the purchasing decision. A bright red colour is seen as an indication of freshness and wholesomeness and consumers will disfavour meat that does not meet their expectations, therefore, numerous commercial approaches have been used to meet consumer expectation (Hood & Mead, 1993) even though the colour of red meat is not well correlated with eating quality (Taylor, 1996).

Another intrinsic quality que that is closely related to colour is the water holding capacity (WHC) of meat. Poor WHC results in drip and purge loss that is accompanied by a loss of myoglobin (Mb) (Joo, Kauffman, Kim, & Kim, 1995).

Health conscious consumers prefer leaner meat with minimal visible fat (Fernandez, Monin, Talmant, Mourot, & Lebret, 1999; Brewer, Zhu, & McKeith, 2001; Resurreccion, 2004). Visible fat can easily be trimmed off the lean meat that is delivered to the consumers whereas, intermuscular fat cannot be removed. Therefore, intermuscular fat content has a great impact on consumer acceptability of meat commodities containing several muscles, such as pork chops or processed ham slices.

Because fresh meat is a largely unbranded product, few extrinsic cues are available. However, evidence suggests that intrinsic cues usually carry more weight in the formation of quality expectations by consumers than extrinsic ones (Steenkamp, 1989; Steenkamp & van Trijp, 1996; Verbeke et al., 1999). There is a widespread opinion, though, that the use of extrinsic cues for quality inference is and will be increasing (Bernués, Olaizola & Corcoran, 2003) due to the debate on food and health, the discussion about the advantages and disadvantages of eating red meat, and various meat scandals. Consumers attach more importance to issues related to health and safety, so interest in health and safety may fuel an increased use of extrinsic quality cues.

The final step in determining meat quality is at the point of consumption with eating quality traits such as tenderness, flavour, juiciness and absence of off-flavours (Bredahl, Grunert, & Fertin, 1998; Acebron & Dopico, 2000; Bryhni et al., 2002). Consumption frequency is influenced by the consumer's liking for pork (Bryhni et al., 2002). It is generally accepted that a higher level of marbling or intermuscular fat (IMF) has a positive influence on the sensory experience associated with eating pork (Bejerholm & Barton-Gade, 1986; Brewer et al., 2001).

A wealth of research has been done on improving and measuring meat quality. As most of these quality attributes are known, the meat industry should provide the consumer with meat that meets their expectations (Thompson, 2002).

2.4.2. The role of muscle structure in pork quality

The muscle fibres are characterised by their morphological traits and, contractile and metabolic properties (Lee, Joo, & Ryu, 2010). Morphology traits include the total number of fibres and cross-sectional area of fibres that are major determinant factors of muscle mass as well as meat quality. In addition, contractile and metabolic properties of muscle are differentiated by muscle fibre types, and thus fresh meat quality is strongly related to fibre type composition in muscle.

Skeletal muscle can be divided into four different muscle fibre types.: slow-oxidative (type I), fast oxido-glycolytic (type IIA), and fast glycolytic (type IIX and IIB) (Schiaffino & Reggiani, 1996). The proportions of these different muscle fibres can determine the muscle metabolic properties (Ozawa et al., 2000; Ryu & Kim, 2005). Consequently, post mortem muscle metabolism, which is a crucial factor to determine fresh meat quality, is affected by the total

number of fibres, cross-sectional area of fibres and fibre type composition (Kim, et al., 2013a; Ryu, Lee, Lee, & Kim, 2006). Muscle fibre characteristics influence appearance quality traits including meat colour, WHC, texture and marbling in meat and are, in turn, influenced by various factors including breed (Ryu et al., 2008), genetics (Larzul et al., 1997), gender (Ozawa et al., 2000), hormones (Rehfeldt, Fiedler, & Stickland, 2004), growth performance (Gondret, Lefaucheur, Juin, Louveau, & Lebret, 2006; Kim, et al., 2013b), diet (Jeong et al., 2012) and muscle location (Beermann et al., 1990; Hwang, Kim, Jeong, Hur, & Joo, 2010)

The influence of muscle fibre characteristics on post mortem aging is an important aspect of meat quality. The rate of aging is faster in type II muscles than in type I muscles (Totland, Kryvi, & Slinde, 1988). If type II fibres are predominant in muscle, post mortem glycolysis is rapid (Choi, Ryu, & Kim, 2007; Kim, et al., 2013a; Ryu & Kim, 2006). The calpain/calpastatin ratio is higher in type II muscles which could partly explain the faster rate of aging (Ouali & Talmant, 1990).

The Mb content and the rate of Mb oxidation are muscle-specific, and increasing the proportion of red muscle fibres (type I) is known to increase the Mb content and redness of meat (Kim et al., 2010). In contrast, the proportion of fast-twitch glycolytic (white) fibres in pork muscle are positively related to higher lightness and lower WHC (Kim, et al., 2013a).

Type I muscles contain more collagen, which plays an important role in binding muscle fibres and decreasing tenderness of meat (Kovanen, Suominen, & Heikkinen, 1984). The content of connective tissue including IMF also varies with muscle fibre characteristics (Klont, Brocks & Eikelenboom, 1998). There is a strong positive genetic correlation between the cross-sectional area of fibres and IMF content in porcine *longissimus* muscle (Larzul et al., 1997). Kim and co-workers (2013a,b) also reported that the proportion and size of type IIB fibres are positively related with IMF content in porcine longissimus muscle. Meat flavour and juiciness are strongly affected by IMF content in muscle (Maltin, Balcerzak, Tilley, & Delday 1998). A high proportion of type I fibres is associated with a high level of phospholipids which are an important determinant of cooked meat flavour (Hwang et al., 2010).

Drip loss an important quality characteristic in pork and originates from the spaces between muscle fibre bundles and the perimysial network, and the spaces between muscle fibres and the endomysial network (Offer & Cousins, 1992). These spaces appear during rigor development when muscle converts to meat. After slaughter, glycogen is converted to lactic acid that accumulates in the muscle. An increase of lactic acid in the warm muscle will result in protein denaturation which may alter the biophysical properties of meat i.e. water holding capacity of the proteins that will result in pale soft and exudative (PSE) meat. This is an especially prevalent problem for pork, which contains greater relative proportions of glycogen depleted fibres compared to beef or lamb.

2.4.3. The role of lipids in meat quality

Meat scientists have been studying carcass characteristics for many years and although the factors that influence the accumulation, distribution and composition of carcass fat in livestock have been extensively researched, the role, value and perceptions of animal fats in meat quality differ significantly in importance between producers, abattoirs, butchers, retailers and consumers (Webb & O'Neill 2008).

Even though fat is an unpopular component of meat for the health conscious consumer, fat and fatty acids (FA), both subcutaneous and IMF contribute to several aspects of meat quality and is important as part of the nutritional properties of meat. Various factors have an influence on both the quantity and the quality of lipids in animal products. Age (or weight), gender, genotype and castration have an influence on the number of lipids.

Eating quality (juiciness, tenderness and flavour) remain the most important aspect of meat quality. Juiciness is the most important sensory trait for pork as pork consumer rates juiciness higher than flavour and tenderness (Aaslyng et al., 2007). There is a strong positive correlation between juiciness and the WHC and IMF content of meat. IMF content affects juiciness by enhancing the WHC of meat, by lubricating the muscle fibres during cooking, by increasing the tenderness of meat, and thus the apparent sensation of juiciness, or by stimulating salivary flow during mastication (Luchak et al., 1998). The IMF contributes, not only to the juiciness of meat but also the flavour (Hocquette et al., 2010), and the human perception of juiciness is increased as the IMF content in meat increases (Jeremiah, Gibson, Aalhus, & Dugan, 2003). The location of IMF in the perimysial connective tissue between muscle fibre bundles may also be important in 'opening up' the structure of muscle, allowing it to be more easily broken down in the mouth (Wood, 1990). The quantity of IMF is affected by many factors including animal breed, slaughter weight (Park et al., 2002), feeding strategy (Du, Yin, & Zhu, 2010), and growth rate (Smith, Gill, Lunt & Brooks, 2009).

Wood (1984) defined good quality fat in pigs as firm and white and poor quality fat as soft, oily, wet, grey and floppy. The composition of subcutaneous fat changes as the tissue develops. It becomes more cohesive and does not separate easily within itself layer by layer. Fat tissue separation is unsightly in fresh pork and particularly in bacon or ham. Studies have shown that cohesiveness and firmness are closely related to water, collagen, stearic acid (C18:0) and linoleic acid (C18:2) fatty acid concentrations. Pork has a high concentration water in thin (i.e. underdeveloped) backfat and the amount of collagen is very high. Studies showed that C18:0 and C18:2 are particularly important contributors to fat tissue firmness. As fatty acid composition was changed for reasons of diet, genetics, sex or fatness, these two showed the highest correlations with firmness. The proposed levels should be less than 12% C18:0 (Lizardo, van Milgen, Mourot, Noblet, & Bonneau, 2002) and between 12% and 15% C18:2 (Houben & Krol, 1983). The ratio of C18:0:C18:2 was found to provide the best prediction of firmness (Wood et al., 1978) and is recommended to be less than 1.2 (Honkavaara, 1989)

Because IMF deposits mainly in the perimysium between muscle bundles, meat firmness is partially influenced by the IMF firmness which is affected by the composition of fatty acids and temperature as different fatty acids have different melting points. Fatty acids of meat have a melting point of between about 25 °C and 50 °C, with saturated fatty acids (SFA) melting at higher and polyunsaturated fatty acids (PUFA) at lower temperatures (Wood, 1984).

In a study by Wood and co-workers (1978) of pigs selected for lean content, the melting point of extracted lipid was also closely related to the concentrations of C18:0 and C18:2, with C18:0 showing the highest correlation. Cameron, Warriss, Porter, & Enser (1990) reported a positive correlation of fat firmness with C16:0 + C18:0 and C16:0/C18:2, while Lea, Swoboda, & Gatherum (1970) suggested that the monounsaturated fatty acid (MUFA) to SFA ratio (MUFA:SFA; C16:1 + C18:1/C16:0 + C18:0) may also be a measure of fat firmness and melting point, Changing the fatty acid composition of subcutaneous adipose tissue using different dietary oils also changes lipid melting point and fat firmness. For example, palm kernel oil produced firmer fat than soybean oil in the study by Teye et al. (2006b). When all the data were pooled, the proportions of lauric (C12:0) and myristic (C14:0) (high in pigs given palm kernel oil) were strongly correlated with fat quality parameters, as were C18:0 and C18:2. Groups of fat cells containing solidified fat with a high melting point appear whiter than when liquid fat with a lower melting point is present, so fat colour is another aspect of quality affected by fatty acids.

The fatty acid composition of meat also influence the flavour of the meat due to the production volatile odours and lipid oxidation products during cooking and the involvement of these with Maillard reaction products to form other volatiles, which contributes to odour and flavour. Early research showed that the fat tissues in meat were the source of the characteristic species flavour (Mottram, 1998). Pork lipids are relatively unsaturated, therefore further increasing the concentrations of PUFA might increase the formation of lipid oxidation products, leading to off-odours and flavours and colour changes. The Swiss guideline is one of the most restrictive and recommends a maximum of 12 g PUFA/kg feed (Perdrix & Stoll, 1995).

Using near-infrared spectroscopy (NIFS), the so-called fat score, is an at-line method to measure the amount of double bonds in backfat, and has been established for evaluation of fat quality in Swiss slaughter plants (Haüser, Seewer, & Gajcy, 1989) and batches exceeding the limit of 62 are punished with price reductions. The fat score, however, does not differentiate between poly- and monounsaturated fatty acids (MUFAs). MUFAs, like PUFAs, impair consistency of pig adipose tissue (Shackelford, Reagan, Haydon & Miller, 1990). Lizardo and co-workers (2002) suggested that the maximum level of MUFA for good quality fat should be <57%. Other parameters include <59% unsaturated fatty acids (UFA) (Prabucki, 1991) and >41% SFA (Häuser & Prabucki, 1990). Double bond index (DBI) is another fatty acid related fat quality parameter. For good quality fat, a DBI of < 80 is required (Häuser & Prabucki, 1990). Barton-Gade (1983) indicated that iodine value (IV) is an indicator of soft fat and that a maximum IV of 70 would produce firm fat. Iodine value determination has the disadvantage that it is expensive and time-consuming (Andersen,

Borggaard, Nishida, & Rasmussen, 1999). Refraction index (RI) is another measurement of fat quality. The corresponding limit for RI in terms of fat quality is < 1.4598 (Hart, according to Houben & Krol, 1983). Refraction index measurement has the advantage that it is rapid, but fat still has to be extracted, which can be a lengthy process. During processing and retail storage meat with higher PUFA are more prone to oxidative breakdown. A standard test for lipid oxidative stability in foods is the thiobarbituric acid reacting substances (TBARS) test, which measures the oxidation product malondialdehyde. Values above 0.5 are considered critical since they indicate a level of lipid oxidation products, which produce a rancid odour and taste that can be detected by consumers (Tarladgis et al., 1960).

2.4.4. Lipids and technological quality

Due to consumer demand for leaner, healthier pork, the pork industry is making an effort to producing increasingly leaner pigs. However, this may have an adverse effect fat quality that can have a negative effect on further processing, and lipid stability. Soft pork fat can result from using the typical concentrate diets, which are high in fats and oils rich in PUFA, especially, C18:2. These pork production techniques to meet consumer demands for leaner meat with a reduced SFA content are in conflict with the optimal physical qualities of fat desired for further processing (Gatlin, See, Hansen, Sutton & Odle, 2002).

The components of technological meat quality influenced by fatty acids are fat tissue firmness (hardness), shelf life (lipid and pigment oxidation) and flavour. Variation in the structure of the molecule is also important (Enser, 1984). Pork fat, for the use in processed meat products, needs to be firm as opposed to soft, unappealing fat (Jaturasitha, Kreuzer, Lange, & Köhler, 1996). Therefore, there are even trends to use feeds rich in SFA, and especially medium-chain-length fatty acids (C12:0; C14:0). These SFA are considered adverse to human health. Such feeds (coconut oil, copra and palm kernel oil) are astonishingly efficient in creating a firm fat tissue in pork (Jaturasitha et al., 1996).

The tendency of UFA to oxidise is important in flavour development during cooking. The colour change is due to the oxidation of red oxymyoglobin to brown metmyoglobin, this reaction generally proceeds parallel to that of rancidity. Several studies have shown that lipid oxidation products can promote pigment oxidation and vice versa, although the strength of the relationship between these two aspects of shelf life is sometimes low (Renerre, 2000). Antioxidants, especially a-tocopherol (vitamin E) have been used to delay lipid and colour oxidation and to extend shelf life.

2.4.5. Lipids and health

To promote human health, a relatively low n-6:n-3 ratio from an adequate intake of n-3 fatty acids is recommended as n-3 PUFAs are essential for brain development, visual sight, and the immune system (Simopoulos, 2002). Scollan et al. (2006) recommended that the n-6:n-3 PUFA ratio be limited to 4:1. Ulbricht and Southgate (1991) suggested that the ratio of PUFAs to SFAs (P:S)

should be at least 0.4 and the atherogenic index lower than 0.5. The atherogenic index and thrombogenicity index indicate the global dietetic quality of lipids and their potential effects on the development of coronary disease (Jankowska, Zakes, Zmijewski & Szczepkowski, 2010).

The nutritional quality of pork is a significant factor affecting the consumer's health, and this is particularly relevant to consumers worldwide where pork is a primary source of meat. Pork is a rich source of proteins, possessing high biological value and a number of bioactive molecules, including taurine, B vitamins and minerals. However, pork also contains high levels of lipids, which have been a topic of discussion for meat consumers due to their associated health implications. Relationships between dietary fat intake and incidence of various lifestyle disorders, including cardiovascular diseases, are well established and several health agencies have specific guidelines in this regard (Dugan et al., 2015; Troy, Tiwari, & Joo, 2016). It is recommended that the total fat should contribute to less than 15–30% of total energy intake, including precise recommendations concerning SFA, n-6 PUFAs, n-3 PUFAs, trans fatty acids and cholesterol (Hocquette et al., 2010).

Lipid content of pork generally varies from 4 to 15% on a fresh basis, depending on several factors, including; geographical origin, genotype, feeding regime and meat cut (Wood et al., 2008: Park et al., 2012). In the last decade, specific strategies for increasing the level of fatty acids with beneficial health effects, while reducing the content of SFA, have been a subject of active research. Some research has focused on reducing the cholesterol content in meat by dietary modifications; curiously, reducing the fat content of meat can actually increase the cholesterol levels in lean meat (Parra et al., 2010). Nowadays consumers choose low-fat and low-cholesterol products. There is a strong belief in society that cholesterol is responsible for many diseases in humans (Kasprzyk, Tyra & Babicz, 2015).

Although excessive intake of SFAs has been linked as one of the causes for cancer and coronary heart disease (Webb & O'Neill, 2008), not all SFA express the same behaviour as regards the increase in serum cholesterol. Stearic acid is considered a neutral fatty acid, on the other hand, C12:0, C14:0 and palmitic (C16:0) acids increased plasma cholesterol concentration (Ulbricht & Southgate, 1991). Furthermore, C14:0 was considered to have the most harmful cardiovascular effect on humans, the effect being almost four times that of C12:0 and C16:0 (Hegsted, Gotsis, Stare, & Worcester, 1959). Palmitic and stearic acids are the predominant SFA in animal fat.

2.5. Fat deposition in the pig

The deposition of adipose tissue results from the maintenance of a fine balance between the related processes of energy intake and energy expenditure, energy storage and energy mobilisation. The regulation of the pathways of lipogenesis and lipolysis is well documented (Mersman, 1991; Jenkins, 1993).

Porcine carcass fat is deposited in four depots with different anatomical locations namely: visceral (internal fat, accounting for 5% of total body fat), subcutaneous (60 – 70% of total body

fat), intermuscular (between muscle, 20 – 35% of total body fat) and intramuscular (within muscles, 2 – 4% of total body fat) (Monziols, Bonneau, Davenel, & Kouba, 2007; Joo et al., 2013). Some authors have suggested that not all adipose tissues are similar but each shows specific development and metabolism (Budd, Atkinson, Buttery, Salter, & Wiseman, 1994; Mourot, Kouba, & Peiniau, 1995; Mourot, Kouba, & Bonneau, 1996). The formation of fat (adipogenesis) occurs the earliest in the visceral fat deposit. Several studies have established that the total lipid content of internal adipose tissues such as flare fat is very high (Wood, Buxton, Whittington & Enser, 1986; Gandemer, Viau, Caritez, & Legault, 1992; Kouba, Hermier, & Le Dividich, 1999, 2001). Adipogenis in the subcutaneous and intermuscular deposits closely follows that of visceral fat and the formation of fat in the intramuscular depots occur last (Hausman et al., 2009). In a study by Monziols and co-workers (2007), they found that the total lipid contents were lower in intermuscular than in subcutaneous adipose tissues (range 57.5–61.5%, and 63.1– 67.8%, respectively). Adipogenesis in skeletal muscle can be affected by genetic, nutritional and environmental factors (Du & Dodson, 2011).

In pigs, the effect of dietary fat on fat deposition does not always result in an increase of fat deposition, due to the negative effect on *de novo* lipogenesis in the adipose tissue. Fat deposition increases only when the ambient temperature is thermoneutral or hot, due to a lower heat production with fat than with carbohydrate. Low temperatures increase fat deposition as a proportion of energy deposited, but this effect is reduced by dietary fat (Stahly, 1984).

2.6. Fat composition of the pig

2.6.1. Fatty acid composition of adipose tissue and muscle in pigs

Considerable anatomical variation in fatty acid composition in the pig has been reported (Sink, Watkins, Ziegler, & Miller, 1964; Barber et al., 2000). It has been known for a long time that the unsaturation degree of fat deposits in the pig follows a negative gradient from outside to inside (Dean & Hilditch, 1933). Monziols, Bonneau, Davenel and Kouba (2005), among others, confirmed this feature and found that the degree of unsaturation of intermuscular adipose tissue is lower than that of subcutaneous adipose tissue, but is higher than that of visceral fat. The lower degree of unsaturation of intermuscular adipose tissue compared to subcutaneous adipose tissue is due to a lower concentration of both mono- and poly-unsaturated fatty acids (Duran-Montgé et al., 2008).

The reason for preferential deposition of PUFAs (particularly C18:2) in the outer layer are not fully understood but according to Bee, Gebert & Messikommer (2002), lipid metabolism is lower in the outer than in the inner layer of subcutaneous adipose tissue. The inner layer exhibits larger de novo lipogenesis, with the result that PUFAs (particularly C18:2) of feed origin are diluted with more endogenous fatty acids than in the outer layer (Koch et al., 1968; Christie, Jenkinson & Moore, 1972). Oleic acid (C18:1), the most abundant MUFA in pigs, is synthesized by Δ9-desaturase (stearoyl- CoA-desaturase) (Kouba, Mourot, & Peiniau, 1997). The activity of this enzyme could be different in the various adipose tissues. A study indeed showed that the activity

of ∆9-desaturase was higher in the subcutaneous adipose tissue than in visceral fat, which could explain, at least partly, the difference in the content of MUFAs (Thompson & Allen, 1969).

Adipose tissue has much higher fatty acid content than muscle but the fatty acid composition of the two tissues is broadly similar. Muscle contains significant proportions of long chain (LC) (C20-22) PUFAs, which are formed from C18:2 and C18:3 by the action of ∆5 and ∆6 desaturase and elongase enzymes. Important products are arachidonic acid (C20:4) and eicosapentaenoic acid (EPA, C20:5) which have various metabolic roles, including eicosanoid production. Greater incorporation of C18:2 into pig muscle fatty acids produces higher levels of C20:4 by synthesis and the net result is a higher ratio of n-6: n-3 PUFA compared with ruminants (Wood et al., 2008).

2.6.2. Fatty acid composition of triacylglycerol and phospholipid fractions

Muscle lipids are composed of polar lipids, mainly phospholipids, and neutral lipids consisting mainly of triacylglycerols. Phospholipids are located in the cell membranes and triacylglycerols in the adipocytes that are located along the muscle fibres and in the interfascicular area. A small amount of triacylglycerols is also present as cytosolic droplets in the muscle fibres (Gandemer, 1999).

The content of phospholipids in the muscle is relatively independent of the total fat content and varies between 0.2 and 1% of muscle weight. However, the content of muscle triacylglycerols is strongly related to the total fat content and varies from 0.2% to more than 5% (Sinclair & O'Dea, 1990; Fernandez, Monin, Talmant, Mourot & Lebret, 1999: Gandemer, 1999).

The major lipid class in adipose tissue (>90%) is triacylglycerol. Triglycerides are storage lipids and are composed of three fatty acids esterified to glycerol and richer in SFA, whereas phospholipids are functional lipids prevalent in cell membranes and as such contain more PUFA than triglycerides (Mapiye et al., 2012).

In general, the two main PUFAs, linoleic (LA, C18:2) and ALA (C18:3), which form a substantial part of the membrane lipids, but are also form part of the storage lipids, cannot be synthesized in the body and thus are essential and have to be provided by the animal's diet (Nakamura & Nara, 2004). In muscle, a significant proportion is phospholipid, which has a much higher PUFA content in order to perform its function as a constituent of cellular membranes. Oleic acid (C18:1), the major fatty acid in meat, is much more predominant in triacylglycerols. This fatty acid is formed from stearic acid (C18:0) by the enzyme stearoyl CoA desaturase, a major lipogenic enzyme. On the other hand, C18:2 are at much higher proportions in phospholipid than in triacylglycerol. The proportion of C18:3 are slightly higher in triacylglycerol than in phospholipid in pigs (Wood et al., 2008). Long chain n-3 and n-6 PUFA are mainly found in phospholipid but are detected in muscle triacylglycerol and adipose tissue (Enser et al., 2000; Cooper et al., 2004).

2.6.3. Effect of fat content on fatty acid composition

Over the years one of the biggest goals for pig breeders were to produces leaner pigs to meet the demand of consumers. Reducing the fat content and increasing the lean muscle results in a smaller backfat thickness and increased concentrations of PUFA in fat (Wood & Enser, 1997). The fat from lean pigs contains more C18:2 (Girard, Bout & Salort, 1988)and there is a strong correlation between the amount of fat, lean meat and the proportion of C18:2 in the backfat of pigs. These changes will improve the nutritional value of pork and meet the consumers demand, however this will result in fat with poor technological properties and higher levels of lipid oxidation.

From early life to time of slaughter, the fat content increase and the fatty acid composition of the adipose tissue changes. The most prominent changes in fatty acids are increase in both C18:0 and C18:1 and a decrease in C18:2. A possible explanation for this is the role of *de novo* tissue synthesis of saturated and MUFA as well as the relatively decline in the role for the direct incorporation of C18:2 from the diet (Kouba, Enser, Whittington, Nute, & Wood, 2003; Wood, et al., 2008). When feeding pigs a control diet for 20, 40 and 60 days Kouba and co-workers (2003) found an increase in the levels of C18:0 (10% to 13% of total FA), C18:1 (38% to 42% of total FA) and a decrease in C18:2 (19% to 11% of total FA).

The inverse relationship between the proportions of C18:2 in subcutaneous adipose tissue and the amount of fat or backfat thickness have been observed in several studies in pigs. Wood et al., (1978) found a strong correlation (r = 0.3) between the levels of C18:2 in the inner layer of subcutaneous fat and the loin fat thickness in a line selected for fast growth and low fat thickness compared to a control line. The concentrations of C18:2 were 9.3% in the control line and 10.7% in the selection line. When measuring the backfat thickness and concentration of C18:2 in subcutaneous fat, Wood, Enser, Whittington, Moncrieff and Kempster (1989) found that as the backfat thickness increased from 8mm to 12mm to 16mm the levels of C18:2 decreased from 14.9% to 12.4% to 10.6%. The *de novo* lipogenesis is lower in leaner pigs resulting in less endogenous FA and less dilution of exogenous C18:2.

In the past most of the fatty acid work was carried out on the adipose tissue, however, visible fat can be removed. Nowadays the fatty acid composition of muscle is getting more attention as it has more significance as food and contains more LC n-6 and n-3 PUFA. The overall fat content of the animal and muscle have an important impact on proportionate fatty acid composition because of the different fatty acid compositions of neutral lipid and phospholipid.

2.6.4. The effect of gender on fatty acid composition

The fatness of the pig is strongly influenced by gender as boars have substantially less total backfat, perirenal fat, intermuscular and intramuscular fat depots than gilts (Martin, Fredeen, Weis & Carson, 1972). However, at the same weight of total side fat, boars have more intermuscular and less subcutaneous fat than females (Fortin, Wood & Whelehan, 1987), also carcasses from boars are markedly leaner than those from barrows (Martin et al., 1972; Desmoulin, Bonneau &

Bourdon, 1974; Knudson, Hogberg, Merkel, Allen & Magee, 1985; Wood et al., 1986). This increase in fat deposition in castrates is mainly due to stronger hypertrophy of adipocytes (Lee, Kauffman & Grummer, 1973). Eggert, Grant and Schinckel (2007) showed that lipid content of both subcutaneous and intermuscular adipose tissues was higher in barrows than in gilts. Therefore, in slaughter pigs, the number of fatness increases in the order of, boars > gilts > barrows (Enser, 1991; De Smet, Raes, & Demeyer, 2004).

As discussed earlier, the amount of fat has an impact on the fatty acid composition of the pig. Therefore as the fatness increases from barrow to gilt to boar, the amount of C18:2 and PUFA decreases and the SFA increases (Nürnberg & Ender, 1989). Muscle phospholipid fatty acid composition does not seem to differ between gilts and barrows, but higher PUFA concentrations have repeatedly been found in total lipid or triacylglycerols for gilts, even after correction for differences in fat content (Cameron & Enser 1991; Hartmann, Otten, Kratzmair, Seewald, Iaizzo & Eichinger, 1992; Warnants, Oeckel & Boucqué, 1999). Interestingly, Wood and co-workers (1989) found that the concentration of PUFA tend to be higher in subcutaneous adipose tissue from boars than gilts due to their thinner backfat. However, even at the same backfat thickness, there was a higher proportion of C18:2 and a lower proportion of C18:1 in subcutaneous adipose tissue from boars. At the same fat thickness as gilts, subcutaneous adipose tissue from boars contained a higher proportion of water and a lower proportion of lipid, signifying a less mature tissue. These results help explain why fat quality tends to be lower in boars than barrows and gilts.

2.6.5. The effect of age and slaughter weight on fatty acid composition

The body fat content of new-born pigs is only about 2% (Le Dividich et al., 1991), and the amount of adipose tissue increases rapidly with age (Mersmann, Allen, Steffen, Brown, & Danielson, 1976; Schinckel, Mahan, Wiseman, & Einstein, 2008), and exhibits a very large enrichment in lipids in the few first weeks of life whereas the augmentation of lipid content is much weaker afterwards (Moody, Enser, Restall, & Lister, 1978).

The effect of age on fatty acid profiles is also related to body fatness (Robelin, 1986; Huerta-Leidenz et al., 1996). The rapid growth in both the backfat layers of the pigs (100–180 days of age) is followed by a phase when adipocyte growth is minimal (180–220 days of age) (Nürnberg & Wegner, 1990).

During growth, the proportion of energy available for fat deposition in pigs increase so that the rate of *de novo* fatty acid synthesis is increased (Enser, 1991). The result of synthesis is mainly palmitic and stearic acid. The accumulation of saturated acids in adipose tissues increases also with age and growth of animals. The relative percentage of unsaturated fatty acids decreased with growth up to 180 days of age. There are no changes in fatty acid composition after 180 days of age (Nürnberg, Wegner, & Ender, 1998).

2.6.6. The effect of genotype on fatty acid composition

Breeding for leaner carcasses has led to a decrease in total fat from 35 - 45% to less than 20% today for a commercial pig (De Smet et al., 2004). But selective breeding results not only in changes of total fat content but also in changes in fat distribution between different depots, which allows producing animals with lower subcutaneous fat without decreasing intramuscular fat which is very important for meat organoleptic qualities (Cameron & Enser, 1991).

The IMF content and the fatty acid composition of muscle in pigs appear to be highly heritable (Cameron & Enser, 1991; Sellier & Monin, 1994).

The concentrations of PUFA are higher in breeds or genetic types with lower levels of IMF. One example is the Duroc breed that has high levels of IMF content and therefore the IMF also has lower concentrations of PUFA and higher levels of SUFA and MUFA compares to Landrace pigs (Cameron & Enser, 1991). Pietrain pig have thinner backfat-, IMF- and intramuscular fat thickness compared the the Scwerfurter pigs, this resulted in higher concentrations of PUFA and lower SFA in fat tissue (Nürnberg, 1995; Hauser, Mourot, De Clercq, Genart & Remacle, 1997).

When traditional breeds (Berkshire and Tamworth) were compared to modern breeds (Duroc, Large White) on a standard concentrated diet for 12 weeks, the modern breeds grew faster and were heavier and leaner than the traditional breeds (Wood et al., 2004). The amount of phospholipid in the loin was similar between the breeds but the amounts of neutral lipid and total lipid was higher in Berkshire and Duroc than in Large White and Tamworth.

2.6.7. The effect of diet on fatty acid composition

In pigs, being a monogastric species, dietary fatty acids are absorbed unchanged from the intestines and incorporated into adipose tissue and muscle (Rhee, Davidson, Cross, & Ziprin, 1990). Therefore, the fatty acid composition and quality of adipose tissues are directly influenced by the dietary fatty acid composition (Bee & Wenk, 1994; Warnants et al., 1999). This involves the source and content of dietary fat and the duration and time of feeding. Since the recommendation that consumers should limit their intake of SFA there has been an increased interest in increasing the PUFA content of meat. Due to successful breeding for a high lean meat content of carcasses and a reduced amount of adipose tissue (Scheper, 1982), the proportion of PUFA is generally rather high in modern pigs.

Several studies, some as early as 1926, have examined the effect of the inclusion of PUFA containing oils in pig diets on its concentration in pork tissue. Linoleic acid (LA; C18:2) and α-linolenic acid (ALA; C18:3) are essential fatty acids and cannot be synthesized and tissue concentrations respond quickly to elevated dietary concentrations (Araujo de Vizcarrondo, Carillo de Padillo, & Martín, 1998). Saturated and MUFA, on the other hand, are synthesized and their concentrations are less readily influenced by diet. Astonishing results have been achieved by using diets with high levels of LA, which is a commonly found in grains and oilseeds. In general, the proportion of C18:2 in tissues increase linearly as the dietary intake increases (Wood, 1984).

In a study by Teye et al. (2006a) pigs were fed concentrated diets of 2.8 % palm kernel oil (high in C12:0, C14:0 and C18:0); 2.8 % palm oil (high in C16:0 and C16:1); and 2.8 % soybean oil (high in C18:2). They found that the concentration of C18:2 in the adipose and muscle tissue was impacted the most by the dietary treatments and to a lesser extent C12:0 and C14:0. There was however very little effect on the concentrations of C16 and C18 saturated and MUFA. Dietary soya bean oil had the highest impact the levels of C18:2 in the adipose tissue and the levels were lower and the dietary effect smaller. These results are explained by the fact that C12:0 and C14:0 are mainly derived from the diet and C18:2 is entirely derived from the diet. Whereas, the C16 and C18 SFA and MUFA are mainly the products of synthesis in the animal and interconversions between them limit the impact of dietary additions (Wood et al., 2008).

Unfortunately, PUFA incorporation is restricted by the increased susceptibility to oxidation (Monahan, Buckley, Morrissey, Lynch, & Gray, 1992; Flachowsky, Schöne, Schaarmann, Lübbe, & Böhme, 1997) and an impaired consistency of adipose tissue (Whittington, Prescott, Wood, & Enser, 1986). Apart from microbial spoilage, lipid oxidation is the primary process by which sensory quality declines in muscle foods (Gray, Gomaa, & Buckley, 1996). Precooked and restructured meat products are susceptible to lipid oxidation. Therefore, various guidelines recommend limiting the PUFA concentrations in diets for growing-finishing pigs (e.g. Warnants et al., 1996).

2.7. Different dietary omega-3 and 6 fatty acid sources

Plants are the main source of n−3 PUFA (Scollan et al., 2006). Plants and seeds, and the vegetable oils produced from them are important sources of feeds rich in PUFA. Both C18:3 and C18:2 are considered essential fatty acids (Guil-Guerrero, 2007). C18:2 is abundant in a number of vegetable oils at about 20 times the concentration as found in meat. C18:3 and C18:2 are the precursors of two families of PUFA. The first is the n-6 family which includes the above-mentioned LA, γ-linolenic acid (GLA, C18:3n-6), dihomo GLA (DGLA, C20:3n- 6), and arachidonic acid (AA, C20:4n-6) (Charles, Harper, Terry & Jacobson, 2001).

2.7.1. Linoleic acid

The effects of dietary oils containing a high proportion of C18:2 on the fatty acid composition and quality of pork have been examined in several papers. Examples of LA rich oils are soya, peanut, maize and sunflower. Hartman, Costello, Libal, and Walhlstrom (1985) as well as West and Myer (1987) found that the concentrations of C18:2 in pig tissue can easily be raised from basal levels (10–15%) to over 30% of fatty acids. This can be achieved in only 6 weeks by feeding pigs full fat soya oil from 30kg live weight (Warnants, Van Oeckel, & Boucque, 1999). The impact of LA rich oils on meat quality is not well known. Some authors found no effect on the flavour or colour of pork chops with raised C18:2 concentrations (Hartman et al., 1985; West & Myer 1987). On the other hand, Larick, Turner, Schoenherr, Coffey, and Pilkington (1992) showed that muscles with

raised levels of C18:2 oxidised rapidly when heated, producing various volatile compounds, including the aldehydes pentanal and hexanal. Wood (1984) found that feeding high levels of linoleic acid (C18:2) resulted in less firm fat, but Rhee, Ziprin, Ordonez and Bohac (1988) and Miller, Shackelford, Hayden, & Reagan, (1990) found no clear influence on various characteristics of meat quality.

2.7.2. α-Linolenic acid

Supplementing pig diets with C18:3 (ALA) have been examined by several workers. The motivation for this research is the high n-6:n-3 fatty acid ratio in pork and the need to reduce this for human nutritional reasons. ALA is particularly abundant in the lipids of leafy plant tissues (Decker, Faustman, & Lope-Bote, 2000), making grass a good source, even though it is low in lipid content. Certain vegetable oils like linseed (flaxseed) oil, safflower oil, camelina oil and rapeseed oil are rich in ALA as well.

Enser and co-workers (2000) and Sheard and co-workers (2000) showed that the n-6:n-3 ratio in pork could be reduced close to the target level of less than 4, by feeding crushed whole linseed. They showed that increasing the C18:3 content in the lipids of the *longissimus* muscle from 1% (control) to 1.6% (linseed), lowered the n-6:n-3 ratio to five (compared with 9 in controls). In a review, Mourot and Hermier (2001) compared the effect of tallow, rapeseed and linseed oil on the fatty acid composition of muscles of pigs. Among the three diets, the linseed oil supplemented diet led to the highest n-3 PUFA content in pig muscles, with the lowest n-6:n-3 ratios.

Adverse effects on odours and flavours have been detected in meat with raised C18:3 levels, especially when oxidative stress is increased by preparation treatment (e.g. salt injection, comminution, freezing and reheating) (Nüernberg, et al., 2005; Musella, Cannata, Rossi, Mourot, Baldini & Corino 2009). In other papers, no effects on meat quality parameters were observed (Enser et al., 2000; Sheard et al., 2000). The reason for these conflicting results lies in the level of C18:3 in both the fat and muscle. Levels of more than 3% have been shown to impact the flavour of cooked meat (Shackelford et al., 1990; Wood and Enser, 1997). Wood (1984) concluded that levels of above 15 g linoleic acid /100 g total fatty acids of backfat caused a critical reduction in lipid melting point. These levels occur when the typical pig diet contains more than 16 g linoleic acid/kg. This level has since been used as a threshold for formulating pig diets in the UK.

There is a belief that feeding diets rich in α-linolenic acid could increase the synthesis of the longer-chain PUFA, C20:5 (eicosapentaenoic acid, EPA) and C22:6 (docosahexaenoic acid, DHA). Guillevic, Kouba and Mourot (2009), found that feeding 4% extruded linseed increased the n-3 PUFA contents in chops (raw and cooked). However, DHA level was not affected by the linseed diet. The bioconversion of ALA to EPA and DHA is very low –approximately 6% for EPA and 3.8% for DHA (Guil-Guerrero, 2007). According to Whelan (2009), the only way to increase tissue levels of DHA is by dietary supplementation of preformed DHA.

2.7.3. Marine oil

The most effective way to increase tissue concentrations of EPA and DHA is to supplement the diet with fish oils, which are good sources of these fatty acids (Kouba, 2006). Concentrations of EPA and DHA were increased in both backfat and perirenal fat when a basal diet was supplemented with sardine oil at level of 60g oil/kg for 4 weeks (Irie & Sakimoto 1992). Morgan, Noble, Cocchi and McCartney (1992) increased the DHA concentrations from 0.06 to 0.45/100g by supplementing the diet with 9.5 g purified fish oil/kg from 25 to 70 kg. In both the aforementioned studies, the increase in EPA and DHA had no effect on the melting point of extracted lipids or consistency of the backfat. Processed meat products derived from marine oil supplemented pork has a healthier fatty acid profile and better fatty acid ratios compared to control products. In a study by Jaturasitha and co-workers (2006) vienna sausages derived from tuna oil supplemented pork had higher PUFA, n−6 fatty acids and n−3 fatty acids and lower n−6:n−3 ratios than sausages prepared from the meat of the control group. Bacon from pigs fed tuna oil had a greater total n−3 fatty acid proportion and a lower n−6:n−3 ratio than control bacon (Jaturasitha et al., 2002; Khiaosa-ard et al., 2011; Kayan et al., 2012). However, incorporating fish oils into the diets of monogastric animals, even at relatively low levels, can result in off-flavours and odours in the product (Hargis & Van Elswyk, 1993; Sheard, et al., 2000; Kouba, Benatmane, Blochet & Mourot, 2008). Øverland, Taugbøl, Haug, and Sundstøl (1996), found that feeding fish oil at levels of as low as 1% resulted in higher scores for ‘off odour’ and ‘off flavour’ in cooked fat samples of both fresh meat and after 6 months frozen storage.

According to the FAO of the United Nations, world captures fisheries production has declined since 1989, while the world aquaculture production has dramatically increased since 1970 (Vannuccini, 2004). The major commercial use of fish oil is for aquaculture, where 87% of the world’s fish oil is used in fish feed (FAO, 2008). Only 6% is used for human consumption, the rest is used in animal feed (6%), and industrial uses (1%) (Tacon, Hasan & Subasinghe 2006; FAO, 2008). Fish oil is a costly source of dietary energy relative to other feed ingredients. There is also the increasing concerns regarding the sustainability of the use of fish products in animal feeds (Worm et al., 2006), and whilst this is a contentious issue, it has been considered important to examine possible alternative sources of long chain n-3 PUFA (Matthews, Homer, Thies & Calder, 2000).

2.8. Stearidonic acid

Although the scientific community recognises the health promotion and disease prevention benefits of n-3 LCPUFA, convincing the consumer to consume fish products is challenging because of the negative aspects (presence of chemical contaminants, unattractive odour, oxidisability) associated with the consumption of fish and fish oils (Mahaffey, Clickner, & Jeffries, 2008; Bourdon et al., 2010). An alternative approach to increase the intakes of n-3 PUFA without changing the nutritional behaviour of consumers would be to fortify traditional food items such as meat and meat

products with n-3 PUFA. Pork is an important human food and it is now possible to improve its nutritional value with regard to human health by increasing its content of n-3 PUFA and balancing its n-6:n-3 ratio. Thus, stearidonic acid has been identified as a potential biologically active surrogate for n-3 LCPUFA, such as EPA, as it is believed that the conversion rate is more efficient.

2.8.1. Background to Stearidonic acid

Stearidonic acid [SDA; C18:4 (n-3)] is an n-3 long chain PUFA that is the first product during the conversion of ALA to EPA, DPA and DHA. Even though SDA is a highly unsaturated n-3 PUFA its unsaturation index is less than that of EPA and DHA. It possesses improved, stability characteristics, therefore enhancing its commercial value. Similarly, if its biological effects were to mimic those of its downstream cousins, EPA and DHA, SDA could become a valuable tool in meeting current recommended intakes for LC PUFA. SDA is generally not found in most terrestrial plant sources and is rarely found in commonly consumed vegetables, fruits, seeds, nuts, or commercial oils. However, plants from the *Boraginaceae, Grossulariaceae, Caryophyllaceae*, and *Primulaceae* families are unique because of their SDA contents. Dietary sources from plants of the *Boraginaceae* family are by far the most common (i.e. seed oils from *Echium* and borage).

Echium plantagineum (Boraginaceae) is an erect spring-flowering annual (occasionally biennial) plant growing on average 30–60 cm in height. Other names for *Echium plantagineum* include Patterson's Curse and purple viper's bugloss and it is native to south Europe (Grigulis et al., 2001). It reproduces by seed and produces an abundance of seed (≈10,000 seeds/ m^2) (Piggin, 1978). In South Africa it mostly grows in the Western Cape in tar road verges, dirt road verges, abandoned fields and natural areas. By weight, *Echium* oil is the richest commercially available plant source of SDA (3.5–9.0%) (Guil-Guerrero, Gomez-Mercado, Garcia-Maroto & Campra-Madrid, 2000; Guil-Guerrero, Gomez-Mercado, Rodriguez-Garcia, Campra-Madrid & Garcia-Maroto, 2001; Surette, Edens, Chilton & Tramposh, 2004)

2.8.2. Biosynthesis of Stearidonic acid

α-Linolenic acid is an essential FA from which all n-3 PUFA are derived (Figure 2.2). When C18:3 is consumed the first desaturation product is SDA and this conversion is via the ∆6 desaturase enzymes (Sprecher, 2000). However this step seems to be rate limiting. Therefore the conversion of ALA to the LC n-3 PUFA, EPA, DPA and DHA seem to be minimal (Pawlosky, Hibbeln, Novotny & Salem, 2001; Brenna, 2002; Pawlosky et al, 2003; Hussein et al., 2005). Surette and co-workers (2004) and Harris and co-workers (2007) found that consumption of SDA results in an increase of up to 5 times the initial value of EPA in plasma, neutrophil, heart, and erythrocyte phospholipids. SDA is 17–30% as effective as EPA in increasing the levels of EPA in red blood cells and in plasma phospholipids (James, Ursin & Cleland, 2003; Harris et al., 2008). Because SDA bypasses the late limiting ∆6 desaturase enzymes if is more effective than ALA in increasing the LC n-3 PUFA. The use of SDA to increase the levels of EPA, DPA and DHA in animal tissue will take

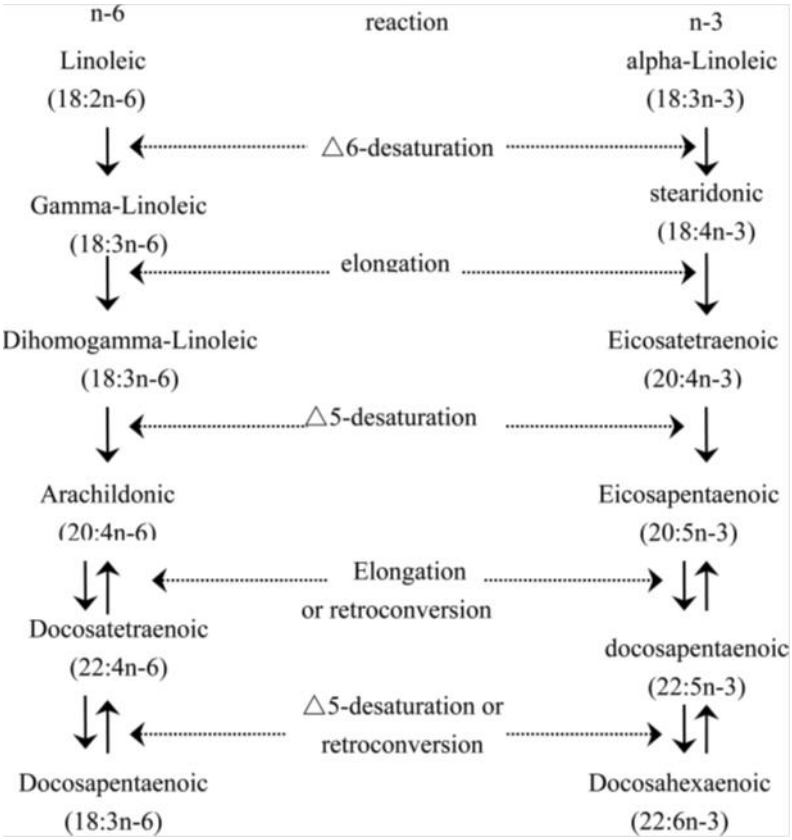

Figure 2.2: The n-6 and n-3 pathways for the synthesis of long chain PUFA in eukaryotes (Ma, Jiang & Lai, 2016)

some of the commercial pressures on fish oil, particularly if dietary SDA can be converted to EPA, DPA and DHA.

2.8.3. Previous research on stearidonic acid

A number of studies have been conducted on humans and various animals. To our knowledge, only one study has been done on pigs. In this study by Tanghe, Millet and De Smet (2013) *Echium* and linseed oils were evaluated as alternatives for fish oil in the diet of sows to increase the DHA levels of the offspring. Compared with the control diet (palm oil), *Echium* oil was able to increase the EPA and DPA concentrations in the sow plasma, colostrum, and piglet plasma. Furthermore, the DHA concentration was increased in the plasma of the newborn and weaned piglets, but not in the plasma and colostrum of the sows. However, the increases in EPA, DPA, and DHA concentrations of the sow plasma, and piglet plasma were equal for the linseed oil. Therefore, they found no difference between the two treatments to increase the LC n-3. Cleveland, Francis and Turchini (2012) also found no benefit in overall DHA production when *Echium* oil was fed to rainbow trout, compared with linseed oil. Similar results were found by Kitessa et al. (2012) in lamb. Miller, Nichols & Carter (2007) found that a plant oil containing 14% SDA can replace fish oil in the diet of salmon without any negative effects on fish growth, health or a reduction in concentration of specific key FA, especially, EPA and DHA

In contrast, Yamazaki et al. (1992) found that feeding rat a diet containing SDA increased the levels of EPA in both the plasma and liver compared to ALA. James and co-workers (2003) found the same in a human trail where SDA increased the concentrations of EPA and DPA in plasma, whereas ALA had no effect on these FA (James et al., 2003). *Echium* oil was also more effective than rapeseed oil in increasing the concentration of EPA in chicken meat (Kitessa & Young 2009). In all three studies, DHA concentrations were not affected. It is clear that none of the n-3 precursors (ALA, SDA or EPA) has an effect on the levels of therefore it seems that the only way to effectively increase DHA levels in tissue is through the consumption of preformed DHA (James et al., 2003; Surette et al., 2004; Hussein et al., 2005; Arterburn, Hall & Oken, 2006; Harris et al, 2008).

2.9. Conclusions

The long chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exert positive effects on human health. The World Health Organisation recommends the consumption of long chain PUFA to reduce CVD risk. Fish and fish oil are the best sources of these LC PUFAs. However, because the intake of fatty fish is low in Western societies, the consumption of LC n-3 PUFA from animal products may become more important. Enrichment of meat products with n-3 PUFA by dietary means could help bridge the gap between their recommended and actual intake.

In pigs, being monogastric animals, the fat tissue triglycerides can be changed by altering the FA composition of dietary fat. It is, therefore, possible to utilize dietary manipulation to design pigs with a healthier FA profile, which can improve the image of pork among consumers.

Supplementing pig diets with ALA was successful in lowering the n-6:n-3 ratio by increasing the total n-3 levels, however the conversion of ALA to longer chain EPA and DHA is rather low. Marine sources such as fish oil and fishmeal were an efficient method of increasing the n-3 fatty acid content of pork. However, using seafood products and by-products in livestock feed is not a sustainable strategy due to the increasing demand for human consumption and aquatic feed. Therefore, various authors suggest feeding pigs diets high in SDA. The hypothesis is that SDA will bypass the rate limiting $\Delta 6$ desaturase enzyme and will, therefore, be more successful in increasing the sought after EPA and DHA than ALA.

To date, only one study has been done on pork to determine the effect of SDA. However, the focus of the study was not on meat and eating quality but on reproduction. Therefore, it is very important that the effect of SDA be evaluated on meat and eating quality and the possible health benefits it can have for consumers.

CHAPTER 3

MATERIALS AND METHODS

3.1. Formulation and analyses of diets

The formulation and nutrient composition of the diets are shown in Tables 3.1, 3.2 and 3.3. Diets were formulated to be isocaloric and isonitrogenous. The BergaFat HPL-160 for the control diet was supplied by Pennville Nutrient Solutions (Pretoria, South Africa) and the *Echium* oil (15200 NEWmega™ *Echium* Oil REF) was imported from De Wit Specialit Oils (Texel, Holland) in two 25kg drums. All other oils were obtained from Energy Oil Feed in 25kg drums, (Wadeville, South Africa). The oil containers were transported to Pennville Animal Nutrient Solutions in Pretoria where the diets were mixed on two separate occasions. First a grower diet was mixed and fed to the pigs until an average live weight of 55kg, and then the finisher diet was mixed and fed to the pigs till slaughter. The difference in nutrient composition between the grower and finisher diets can be seen in Table 3.3. For both the grower and finisher diets the control diet contained 1% palm oil and experimental diets comprised of 1% soya oil, 1% linseed oil, 1% fish oil and 1% *Echium* oil (Table 3.1 and 3.2). These diets were specifically chosen for their very different fatty acid profiles (Table 3.4). Feed was packaged in 20 kg bags and stored in the dark, at room temperature, until used.

Proximate analysis on feed was performed by Feed First Laboratories. Analysis included moisture, dry matter, crude protein, fat, fibre, ash and minerals (Ca, P, Mg, K, Na, Fe, Mn, Zn, and Cu). Tests on feed samples were performed on samples that were collected on the day of mixing. Lipids from feed samples were extracted with diethyl ether, using the Soxhlet extraction method (AOAC, 2005; method nr. 2003.06). The feed and oils were chemically analysed for peroxide value (PV; AOAC, 2005; method nr. 965.33), free fatty acid value and ρ-anisidine value (Hamilton, Hamilton, & Sewell, 1992).

3.2. Digestibility study

3.2.1. Animals

Twenty Large White x Landrace pigs from the Agricultural Research Council (ARC) breeding stock were selected at an average weight of 38 ± 0.4 kg, and divided randomly into five groups of four pigs each, balanced on gender. Groups were then assigned randomly to each of the five dietary treatments that consisted of a control diet containing 1 % Bergafat HPL-106 (Palm oil) and four experimental diets containing 1 % soya oil, 1 % linseed oil, 1 % fish oil, and 1 % *Echium* oil. The pigs were housed individually in 1.0 x 0.9 m^2 pens with solid concrete floors in a house maintained at a temperature range of 20 - 24°C. The housing facility was cleaned and disinfected using Virkon® (LANXESS (PTY) LTD, Modderfontein,

Table 3.1: Composition (%) of grower diet on an air dry basis as based on different n-3 sources.

Ingredient	Control As Is %	Soya oil As Is %	Linseed oil As Is %	Fish oil As Is %	*Echium* oil As Is %
Yellow Maize 8.0%	63.98	63.98	63.98	63.98	63.98
Soya oil cake 47%	18.50	18.50	18.50	18.50	18.50
Bran 15%	7.00	7.00	7.00	7.00	7.00
Sunflower oil cake 36%	6.00	6.00	6.00	6.00	6.00
BergaFat HPL-106[1]	1.000				
Soya Oil		1.000			
Linseed Oil			1.000		
Fish Oil				1.000	
Echium Oil					1.000
Limestone	1.050	1.050	1.050	1.050	1.050
Monocalcium Phosphate	0.650	0.650	0.650	0.650	0.650
Salt	0.500	0.500	0.500	0.500	0.500
Lysine HCL	0.350	0.350	0.350	0.350	0.350
Pig Grower Vitamin & Mineral Premix[2]	0.280	0.280	0.280	0.280	0.280
Oxytetracycline hydrochloride 200 mg	0.200	0.200	0.200	0.200	0.200
Mould Inhibitor[3]	0.150	0.150	0.150	0.150	0.150
Toxin Binder[4]	0.100	0.100	0.100	0.100	0.100
Methionine (MHA)	0.080	0.080	0.080	0.080	0.080
L Threonine	0.070	0.070	0.070	0.070	0.070
Lecithin 60%		0.060	0.060	0.060	0.060
Tylan 100[5]	0.030	0.030	0.030	0.030	0.030
Ammoxicillin	0.020	0.020	0.020	0.020	0.020
Antioxidant[6]	0.020	0.020	0.020	0.020	0.020
Phytase[7]	0.015	0.015	0.015	0.015	0.015
Xylanase[8]	0.010	0.010	0.010	0.010	0.010
Total	**100.003**	**100.063**	**100.063**	**100.063**	**100.063**

[1]Berg + Schmidt, Germany; [2]Standard Vitamin Mineral Premix (Pennville Pty LTD); [3]MycoCurb Dry (Kemin Industries); [4]Freetox (Nutrex, Belgium); [5]Elanco Animal Health; [6]Antyox Vit Dry (Kemin Industries); [7]Quantum Blue 500G (AB Vista enzymes); [8]Econase XT (AB Vista Enzymes)

Edenvale, South Africa) before the commencement of the trial. Pigs were allowed to adapt to their environment for a period of 7 days before commencement of a 5 day faecal collection period. The pigs were fed the experimental diets at a rate of two times the maintenance rate. The feed offered to each pig was weighed and any feed remaining in the feed through the next day was also weighed to estimate feed intake. Water was provided *ad libitum* from low pressure nipple drinkers. The total collection method was used to estimate nutrient digestibility. Faeces were collected three times a day at 08:00, 12:00 and at 16:00. All the faeces accumulated for each pen per day were collected, weighed on a Mettler Toledo scale (Microsep (PTY) Ltd; Bramley, South Africa) and recorded for the five day period. Fresh

Table 3. 2: Composition (%) of finisher diet on an air dry basis as based on different n-3 sources.

Ingredient	Control As Is %	Soya oil As Is %	Linseed oil As Is %	Fish oil As Is %	*Echium* oil As Is %
Yellow Maize 8.0%	64.440	64.440	64.440	64.440	64.440
Soya oil cake 47%	12.509	12.509	12.509	12.509	12.509
Bran 15%	11.508	11.508	11.508	11.508	11.508
Sunflower oil cake 36%	8.006	8.006	8.006	8.006	8.006
BergaFat HPL-106[1]	1.000				
Soya Oil		1.000			
Linseed Oil			1.000		
Fish Oil				1.000	
Echium Oil					1.000
Limestone	0.891	0.891	0.891	0.891	0.891
Salt	0.500	0.500	0.500	0.500	0.500
Monocalcium Phosphate	0.330	0.330	0.330	0.330	0.330
Pig Finisher Vitamin & Mineral Premix[2]	0.250	0.250	0.250	0.250	0.250
Lysine HCL	0.235	0.235	0.235	0.235	0.235
Mould Inhibitor[3]	0.150	0.150	0.150	0.150	0.150
Toxin Binder[4]	0.100	0.100	0.100	0.100	0.100
Lecithin 60%		0.060	0.060	0.060	0.060
Ammoxicillin	0.020	0.020	0.020	0.020	0.020
Tylan 100[5]	0.020	0.020	0.020	0.020	0.020
Antioxidant[6]	.020	0.020	0.020	0.020	0.020
Phytase[7]	0.015	0.015	0.015	0.015	0.015
Xylanase[8]	0.010	0.010	0.010	0.010	0.010
Total	**100.003**	**100.063**	**100.063**	**100.063**	**100.063**

[1]Berg + Schmidt, Germany; [2]Standard Vitamin Mineral Premix (Pennville Pty LTD); [3]MycoCurb Dry (Kemin Industries); [4]Freetox (Nutrex, Belgium); [5]Elanco Animal Health; [6]Antyox Vit Dry (Kemin Industries); [7]Quantum Blue 500G (AB Vista enzymes); [8]Econase XT (AB Vista Enzymes)

Table 3.3: Formulated nutrient composition of the experimental diet on an air dry basis.

Nutrient	Units	Grower Diet	Finisher Diet
DE[1] Swine	MJ/kg	14.04	13.85
Crude Protein	g/kg	180.82	165.23
Lysine	g/kg	11.19	9.08
Methionine	g/kg	3.69	2.89
T.S.A.A.[2]	g/kg	7.07	6.13
Fat	g/kg	33.60	34.80
Acid detergent fibre	g/kg	43.04	49.23
Calcium	g/kg	8.07	6.77
Sodium	g/kg	2.55	2.57
Total Phosphorus	g/kg	5.47	5.00

[1]Digestible Energy; [2]Total sulphur amino acids

Table 3.4: Fatty acid composition of the different fat sources used in the five experimental diets.

	Bergafat HPL 106[1] (n=6)	**Soya oil** (n=6)	**Linseed oil** (n=6)	**Fish oil** (n=6)	***Echium* oil** (n=6)
C12:0	0.02 ± 0.01	ND	ND	0.02 ± 0.02	ND
C14:0	0.44 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	12.55 ± 0.30	ND
C14:1c9	ND	ND	ND	0.04 ± 0.02	ND
C15:0	0.05 ± 0.01	ND	ND	0.37 ± 0.01	ND
C16:0	87.62 ± 0.36	10.41 ± 0.13	6.90 ± 0.09	15.77 ± 0.32	6.72 ± 0.12
C16:1c9	0.23 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	14.75 ± 0.28	0.07 ± 0.01
C17:0	0.12 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.30 ± 0.02	0.07 ± 0.01
C17:1c10	ND	ND	ND	0.46 ± 0.71	ND
C18:0	0.43 ± 0.01	4.94 ± 0.03	3.70 ± 0.04	3.00 ± 0.05	3.34 ± 0.06
C18:1t9	0.01 ± 0.01	ND	0.01 ± 0.01	0.87 ± 0.01	ND
C18:1c9	7.08 ± 0.16	21.37 ± 0.08	19.10 ± 0.04	6.33 ± 0.09	14.06 ± 0.86
C18:2c9,12(n-6)	3.77 ± 0.12	54.69 ± 0.05	25.44 ± 0.05	2.52 ± 0.02	15.14 ± 0.21
C18:3c6,9,12(n-6)	ND	ND	ND	0.23 ± 0.01	11.57 ± 0.14
C18:3c9,12,15(n-3)	0.22 ± 0.01	7.55 ± 0.06	44.28 ± 0.13	0.77 ± 0.01	33.95 ± 0.86
C18:4c6,9,12,15(n-3)	ND	ND	ND	4.86 ± 0.04	14.44 ± 0.28
C20:0	ND	0.37 ± 0.01	0.15 ± 0.01	0.54 ± 0.01	0.07 ± 0.01
C20:1c11	ND	ND	ND	0.50 ± 0.01	0.15 ± 0.24
C20:2c11,14(n-6)	0.01 ± 0.02	ND	ND	0.14 ± 0.01	0.01 ± 0.02
C20:3c11,14,17(n-3)	ND	ND	ND	0.06 ± 0.01	0.27 ± 0.03
C20:3c8,11,14(n-6)	ND	0.40 ± 0.01	0.26 ± 0.01	0.09 ± 0.01	0.01 ± 0.01
C20:4c5,8,11,14(n-6)	ND	ND	ND	0.76 ± 0.01	ND
C20:5c5,8,11,14,17(n-3)	ND	ND	ND	25.24 ± 0.28	ND
C21:0	ND	ND	ND	ND	0.03 ± 0.01
C22:1c13	ND	ND	ND	0.13 ± 0.01	ND
C22:2c13,16(n-6)	ND	ND	ND	0.05 ± 0.01	ND
C22:5c7,10,13,16,19(n-3)	ND	ND	ND	2.59 ± 0.05	ND
C22:6c4,7,10,13,16,19(n-3)	ND	ND	ND	6.89 ± 0.16	ND
C23:0	ND	0.01 ± 0.01	ND	ND	ND
C24:0	ND	0.11 ± 0.01	0.08 ± 0.01	ND	ND
C24:1c15	ND	ND	ND	0.17 ± 0.01	0.09 ± 0.01

[1]Berg + Schmidt, Germany
ND = Not detected

uncontaminated faecal samples were pooled per pig and bagged in a zip lock plastic bag for the duration of the digestibility trial for laboratory analyses. The samples were frozen immediately at -20ºC after collection. At the end of the fifth day all samples were homogenously mixed, oven dried in a LABOTEC® oven dryer (Midrand Labotec Park, Randjespark, Midrand, South Africa) at a temperature of 70°C for 48 hours and they were milled on in a Wiley mill (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, Phil, USA) fitted with a 1-mm sieve

3.2.2. Chemical analyses of faecal matter

The representative feed and faecal samples for each treatment that had been ground through a 1-mm sieve were used for analysis. Dry matter (DM) and Crude protein (CP) were determined according to the procedures of the Association of Official Analytic Chemists (AOAC, 1995). The Kjeldahl method was used to determine the nitrogen content in samples and then the nitrogen content was multiplied by 6.25 for conversion into CP. Acid detergent fibre (ADF) were analysed by the methods of Van Soest (1963a & b). Calcium and phosphorus were determined using AOAC Methods 927.02 and 964.06 respectively. Digestibility was calculated using the following formula:

$$\text{Digestibility coefficient} = \frac{\text{Nutrient intake} - \text{Faecal nutrient} \times 100}{\text{Nutrient intake}}$$

3.3. Animal production study

3.3.1. Animals

Sixty Landrace x Large White crossbred pigs, with an average weight of ± 30 kg, were randomly divided into ten groups comprising of six pigs per pen. Each pen was randomly allocated to each treatment resulting in two pens per treatment. These treatments comprised of a control diet, supplemented with 1% palm oil (BergaFat HPL-160) and four experimental diets supplemented with one of 1% soya oil, 1% linseed oil, 1% fish oil and 1% *Echium* oil. Pigs were provided ad libitum access to feed and water. The pigs' weights were recorded weekly on the same day each week and at the same time in the morning. Pigs were fed until the average live weight was ± 110 kg. The average daily gain (ADG) was expressed as live-weight gain, divided by the days in the trial. Feed conversion ratio was calculated as the total feed intake, divided by the total live-weight gain.

3.3.2. Slaughter and carcass measurements

Pigs were slaughtered when the average live weights reached ± 110 kg. Pigs were weighed and feed was removed approximately 12 hours before slaughter. Pigs were transported by trailer for approximately 500m to the abattoir where they were humanely slaughtered. All pigs were

electrically stunned (220 V @ 60Hz for 7 seconds), stuck, scalded (60 ºC) and dressed, following commercial procedures.

Ten pigs per treatment were randomly selected for meat quality and sensory analysis (n=10). The Hennessy Grading Probe was used to measure backfat thickness and the thickness of the *M. longissimus thoracis* muscle, 45 mm off the carcass midline, between the second and third last rib. The percentage lean meat content in each carcass was calculated according to the formula currently used by the South African meat industry (Bruwer, 1992): Percentage lean meat content was calculated as (LMC) = 72.5114 – 0.4618V + 0.0547S [V = fat thickness (mm) and S = muscle thickness (mm) at 45 mm from the carcass midline, between the second and third last rib]. Pigs were then classified according to lean meat content (LMC) into one of six classification groups (PORCUS), namely P = ≥ 70.1 % LMC; O = 68.1 – 70.0% LMC; R = 66.1 – 68.0% LMC; C = 64.1 – 66.0 % LMC; U = 62.1 – 64.0 % LMC; S = ≤ 62.0% LMC (Bruwer, 1992). Commercial warm carcass weights were also obtained at this time.

After hanging in a cold room at ± 1 ºC for 24 hours, commercial cold carcass weights and carcass measurements were obtained. Carcass measurements consisted of carcass length, shoulder circumference and buttock circumference. Heads were removed, carcasses split (between the second and third last rib) and the right loin portion from each carcass (last three ribs) was removed. The firmness of the subcutaneous fat was measured with a fat hardness meter MK2 (FHM) on the cross sectional surface, at the position between the second and third last rib. These values were obtained from the average of twelve readings, adjusted to 1ºC, using the equation: FHM = M – 18(1ºC - TºC), with FHM being the temperature-corrected meter reading, M the actual reading and T the actual fat temperature (Sather, Jones, Robertson, & Zawadski, 1995). Backfat and muscle (*M. longissimus thoracis*) colour (L*, a* and b* values) was determined at the same position, after a 30 min bloom time, with a Minolta CM-600d tristimulus colour analyser using illuminant A with specular component included. The colour of the belly fat was also measured at this time. Saturation index (SI), which is related to the colour intensity of the meat, was calculated according to the formula: SI = $\sqrt{a^{*2} + b^{*2}}$ (Lanari, Schaefer, & Scheller, 1995). Hue angle was calculated according to the formula $\tan^{-1}\left(\frac{b*}{a*}\right)$ (Ripoll, Joy, & Muñoz, 2011).

3.3.3. Tissue sampling

A core sample of both layers of subcutaneous fat and *M. longissimus thoracis* was taken 24 hours post mortem, from all carcasses, 45 mm from the carcass midline between the second and third last rib, on the left side of the carcass. It is known that the lipid saturation between backfat layers differ (McDonald, & Hamilton, 1976). Since both layers of backfat are used in processed meat products it was decided to use the combined fat layers. Samples for lipid extraction were stored in Nunc cryotubes (AEC-Amersham, Johannesburg, South Africa) at -20 °C pending lipid extraction. Backfat quality was determined on fifty of the carcasses obtained from the animal production study.

The right loin was removed, cut into 1.5 cm thick chops, frozen and used for stability tests of fresh and frozen pork, and sensory analysis. The left loin, including the last three ribs, was removed and deboned, while the back fat and lean muscle were separated. The lean muscle, as well as the back fat of the 10 pigs selected for meat quality work from each dietary treatment, was pooled, minced through a 13 mm OKTO mincer plate (OKTO No. 32 mincer, Crown National, Johannesburg, South Africa), vacuum sealed and stored at -18 °C, until used for the preparation of pork sausages and salami.

3.4. Intramuscular-, back- and belly fat quality

3.4.1. Lipid extraction and fractionation

Extraction of total lipids from muscle (±5 g) and the subcutaneous fat (±1g) was performed quantitatively according to Folch, Lees, & Sloane-Stanley (1957), using chloroform and methanol in a ratio of 2:1. Buthylated hydroxytoluene (BHT) was added as an antioxidant, at a concentration of 0.001%. The extracts were dried under vacuum in a rotary evaporator and further dried in a vacuum oven at 50 ºC overnight with phosphorous pentoxide as moisture adsorbent. Total extractable fat content (EFC) was determined by weighing and expressed as % fat (w/w) per 100 g tissue. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g tissue. The moisture content of the backfat was determined by subtraction (100% – % lipid – % FFDM) and expressed as % moisture (w/w) per 100 g tissue. The extracted fat samples was stored in a polytop (glass tube, with push-in top) under a blanket of nitrogen (N_2) and frozen at -20 ºC pending chemical and fatty acid analysis.

3.4.2. Fatty acid analysis

Total lipid (± 40 mg), extracted by the Folch et al. (1957) method, was converted to methyl esters by base-catalysed transesterification. Fatty acid methyl esters were quantified using a Varian 430-GC flame ionization GC, with a fused silica capillary column, (Chrompack CPSIL 88, 100 m length, 0.25 mm ID, 0.2 µm film thicknesses). Analysis was performed by using an initial isothermic period (40 °C for 2 minutes). The temperature was thereafter increased at a rate of 4 °C/minute to 230 °C. Finally an isothermic period of 230 °C for 10 minutes followed. Fatty acid methyl esters in n-hexane (1 µl) were injected into the column using a Varian CP-8400 Autosampler. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas and N_2 was employed as the makeup gas. Galaxy Chromatography Data System Software recorded the chromatograms. Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Nonadecanoic acid (C19:0) (SIGMA N553377-1G) was used as the internal standard to improve

quantitative FAME estimation. Fatty acid data was used to calculate the following ratios of fatty acids: C16:0 + C18:0; (C16:1+C18:1c9)/(C16:0 + C18:0); C16:0/C18:2; C18:0/C18:2; C18:2/C18:1 total MUFA; total SFA; total PUFA; total UFA; MUFA/SFA; PUFA/SFA; and the ratio of omega-6 to omega-3 (n-6)/(n-3) fatty acids. Double bond index (DBI) was calculated as: DBI = Σ% of UFA x number of double bonds of each UFA (Alam, & Alam, 1986).

3.5. Physical and chemical properties of *M. longissimus thoracis*

3.5.1. M. longissimus thoracis and backfat area measurements

The eye muscle area and backfat from *M. longissimus thoracis* chops were traced onto paper. The areas were measured by means of a Video Image Analyser (VIA) (analySIS Life Science system). The composition of chops was expressed as % lean and % fat.

3.5.2. Drip loss of M. longissimus thoracis

Drip loss of pork was measured in duplicate. Fifty grams of fresh meat (24 h post mortem) was sliced into cubes of 10 mm x 10 mm x 20 mm. Each cube was hung onto a pin, secured to the cap, inside a sample bottle (200 ml), ensuring that the meat did not touch the sides of the bottle. The samples were stored for 3 days at 4 °C ± 1 °C. The amount of drip loss was measured as the difference between the sample mass before and after. Drip loss was expressed as a percentage of the starting mass (Honikel, 1987).

3.5.3. Water-holding capacity of M. longissimus thoracis

A 400-600 mg meat sample was placed on a filter paper (Whatman 4, Whatman International Limited, Maidstone, England), sandwiched between two perspex plates and pressed at constant pressure for 5 min, according to the method described by Grau, & Hamm (1953). The water holding capacity (WHC) was determined by calculating the ratio of meat area to liquid area after pressing. The areas were measured by means of a VIA (analySIS Life Science system), described by Irie, Izumo, & Mohri (1996). The WHC was expressed as the area of the meat, divided by the area of the moisture (including meat area).

3.6. Sensory analysis of pork

3.6.1. Descriptive sensory analysis of the sensory properties of fresh pork

3.6.1.1. Training of the sensory panel

A trained, 10 member sensory panel was used for the sensory analysis of the pork. The purpose of the descriptive analysis was to determine how the samples from the experimental treatments differed in terms of specific sensory characteristics. The 10 panellists were selected based on their previous participation in descriptive sensory panels, taste and smell acuity, interest, ability to discriminate between the four basic tastes and being available for the entire study. Evaluations were performed in individual sensory booths. Samples were evaluated under red light conditions

to mask colour differences. The sensory analysis facilities conform to the American Society for Testing and Materials (ASTM) (1996) design guidelines for sensory facilities. The analyses were conducted over an 8 day period (including training). Samples from all treatments were randomly assigned to five sessions (10 minutes apart) per day. All samples were coded with random three digit codes. Water, at room temperature, was served as palate cleanser in between evaluation sessions.

During the training sessions, panellists were exposed to the samples to be evaluated, in order to develop relevant terminology (Table 3.5). The 10 panellists received a representative sample of pork meat and fat from the five dietary treatments and were then trained to increase sensitivity and ability to discriminate between specific samples and sensory attributes. In order to ensure that panellists were not influenced in any way, no information, with regard to the nature of the samples, was provided. A clear definition of each attribute was developed to describe the specific attribute to be evaluated. Panellists were instructed to give a detailed description of the aroma, flavour and aftertaste attributes of the pork meat and fat samples (Table 3.5). An eight-point intensity scale was used for scoring the different characteristics of the pork meat and fat from the experimental treatments (Table 3.6).

3.6.1.2. *Preparation of sensory samples*

The frozen pork loin chops (stored at -18 °C) were thawed over a period of 24 hours at 5 °C before cooking. The cuts were prepared and evaluated according to the American Meat Science Association and National Live Stock and Meat Board (Chicago, Illinois, 1995) research guidelines for the cookery and sensory evaluation measurements of fresh meat. Consumers usually roast meat that is low in connective tissue, such as pork chops. The samples were cooked according to an oven-broiling method using direct radiant heat. Four chops from a single treatment were placed on an oven pan on a rack to allow meat juices to drain during cooking. The chops were positioned 90 mm below the pre-heated element of an electric oven (Mielé, H217 ovens) at an oven temperature of 170 °C. As the heat radiates from only one direction, samples were turned during cooking. The samples were cooked to an internal temperature of 75-77 °C at the geometric centre of the chop. Three of the hot samples were prepared immediately for sensory evaluation. The fourth chop was used for shear force determination. Each panellist received standardised cubes measuring 12 mm x 12 mm x 12 mm of each cooked sample. Only the centre cubes were used and the outer sides were avoided. The meat cubes were wrapped in three-digit coded foil squares (90 mm x 90 mm) and presented at 55 °C on pre-warmed plates to the panel. For the analysis of the fat, cubes measuring 12 mm x 12 mm x 12 mm were used. The pieces of fat were each placed into pre-

Table 3.5: Descriptions and definitions of attributes used by members of the trained sensory panel to evaluate pork fat and meat samples.

DESCRIPTOR	DEFINITION
FAT AROMA:	
Fresh pork fat	Aromatic associated with fresh pork fat (cooked)
Roast pork fat (caramel)	Aromatic associated with cooked, roasted pork meat, meat extract (browned meat, caramel and sweet)
Rancid / old fat	Aromatic associated with rancid oil and fat (distinctly like linseed oil)
Fishy	Aromatic associated with some rancid fats and oils (similar to old fish)
Sour	Aromatic reminiscent of fruit
Piggy (old / musty)	Aromatic associated with a live pig or its habitat, or wet pig hair also associated with close air spaces such as attics, closets (dry) and basements and turned soil (wet)
Chemical	General term for aromatics associated with many different types of compounds, such as solvents, cleaning compounds and hydrocarbons
MEAT AROMA:	
Cooked pork meat (fresh pork)	Aroma associated with freshly cooked pork meat
Roast pork meat	Aroma associated with cooked, roasted pork meat, meat extract (browned meat, caramel and sweet)
Rancid / old	Aromatic associated with rancid oil and fat (distinctly like linseed oil)
Sour	Aroma reminiscent of fruit
Fishy	Aromatic associated with some rancid fats and oils (similar to old fish)
Piggy (old / musty)	Aroma associated with a live pig or its habitat, or wet pig hair also associated with close air spaces such as attics, closets (dry) and basements and turned soil (wet)
Livery (metallic / bloody)	An aroma associated with the inside of an empty can, tinny. Regarded as a negative attribute
JUICYNESS OF MEAT:	
Initial impression of juiciness	The amount of fluid exuded on the cut surface when pressed between thumb and forefinger
MEAT TEXTURE:	
FIRST BITE:	
Tender	The impression of tenderness of the meat when biting into the meat and evaluate whether the meat breaks easily between the teeth (tender) or has become tough / difficult to bite through
Muscle fibre & overall tenderness – Residue	Chew sample with light chewing action. The impression of tenderness that you form of the meat when chewing
Sustained impression of juiciness - meat	The impression of juiciness that is formed when chewing. It is either dry with no fluid or juicy with moisture
MEAT FLAVOUR	
Cooked pork meat	Flavour associated with freshly cooked pork meat
Roast pork meat	Flavour associated with cooked, roasted pork meat, meat extract (browned meat, caramel and sweet)
Rancid / old	Flavour associated with rancid oil and fat (distinctly like linseed oil)
Sour	Flavours reminiscent of fruit (fermented)
Fishy	Flavour associated with some rancid fats and oils (similar to old fish)
Piggy (old / musty)	Flavour associated with a live pig or its habitat, or wet pig hair also associated with close air spaces such as attics, closets (dry) and basements and turned soil (wet)
Livery (metallic / bloody)	A flavour associated with the inside of an empty can, tinny. Regarded as a negative attribute
AFTER TASTE	
Cooked pork meat	An aftertaste associated with freshly cooked pork meat
Rancid / old	An aftertaste associated with rancid oil and fat (distinctly like linseed oil)
Sour	An aftertaste reminiscent of fermented fruit
Fishy	An aftertaste associated with some rancid fats and oils (similar to old fish)
Metallic	An aftertaste associated with the inside of an empty can, tinny. Regarded as a negative attribute

Table 3.6: Sensory evaluation form for trained panel

Sensory evaluation of PORK and PORK FAT 18 Jan – 3 Feb 2016

Name:…… Date……...

Session…….. Sensory Code……

AROMA: Smell the pork fat by lifting the foil closure at least half way off the glass container and inserting your nose into the top part of the glass beaker. Smell the fat.								
AROMA-FAT	**Extr bland**	**Very bland**	**Fairly bland**	**Slight bland**	**Moderate**	**Fairly intense**	**Very intense**	**Extremely intense**
Fresh pork fat	1	2	3	4	5	6	7	8
Roast pork fat (caramel)	1	2	3	4	5	6	7	8
Rancid / old fat	1	2	3	4	5	6	7	8
Fishy	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
Piggy (old-musty-sweet)	1	2	3	4	5	6	7	8
Chemical	1	2	3	4	5	6	7	8
AROMA-MEAT	**Extr bland**	**Very bland**	**Fairly bland**	**Slight bland**	**Moderate**	**Fairly intense**	**Very intense**	**Extremely intense**
Fresh cooked pork meat	1	2	3	4	5	6	7	8
Roast pork meat	1	2	3	4	5	6	7	8
Rancid / old	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
Fishy	1	2	3	4	5	6	7	8
Piggy (Old- musty)	1	2	3	4	5	6	7	8
Livery (metallic/bloody)	1	2	3	4	5	6	7	8
FLAVOUR, TEXTURE, JUICINESS AND AFTERTASTE. Now bite through the meat, evaluate first bite the chew and swallow. Be cognisant of the mouth-feel and aftertaste while tasting. The aftertaste should be judged as the flavour in the mouth immediately after swallowing the product								
JUICINESS OF MEAT	**Extr. dry**	**Very dry**	**Fairly dry**	**Slight**	**Moderate juicy**	**Fairly juicy**	**Very juicy**	**Extremely juicy**
Initial impression of juiciness	1	2	3	4	5	6	7	8
FIRST BITE	**Extr. tough**	**Very tough**	**Fairly tough**	**Slight**	**Moderate**	**Fairly tender**	**Very tender**	**Extremely**
Tender	1	2	3	4	5	6	7	8
Muscle fibre & overall Tenderness - Residue	**Extr. tough**	**Very tough**	**Fairly tough**	**Slight**	**Moderate**	**Fairly tender**	**Very tender**	**Extremely**
	1	2	3	4	5	6	7	8
Sustained impression of juiciness – meat	**Extr. dry**	**Very dry**	**Fairly dry**	**Slight**	**Moderate juicy**	**Fairly juicy**	**Very juicy**	**Extremely juicy**
	1	2	3	4	5	6	7	8
FLAVOUR - MEAT	**Extr bland**	**Very bland**	**Fairly bland**	**Slight bland**	**Moderate**	**Fairly intense**	**Very intense**	**Extremely intense**
Cooked pork meat	1	2	3	4	5	6	7	8
Roast pork meat	1	2	3	4	5	6	7	8
Rancid / old	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
Fishy	1	2	3	4	5	6	7	8
Piggy (old- musty- sweet)	1	2	3	4	5	6	7	8
Livery (metallic/bloody)	1	2	3	4	5	6	7	8
AFTER TASTE	**Extr bland**	**Very bland**	**Fairly bland**	**Slight bland**	**Moderate**	**Fairly intense**	**Very intense**	**Extremely intense**
Cooked pork meat	1	2	3	4	5	6	7	8
Rancid / old	1	2	3	4	5	6	7	8
Sour	1	2	3	4	5	6	7	8
Fishy	1	2	3	4	5	6	7	8
Metallic	1	2	3	4	5	6	7	8

heated glass beakers (60 °C) and covered with similar pre-coded aluminium foil and placed in a re-heated sand bath at 120 °C and presented simultaneously with the meat sample to the panel.

3.6.2. Consumer analysis of sensory properties of fresh pork

3.6.2.1. Consumer sensory panel

Sensory analysis was carried out on all five treatment groups using a 100 member untrained consumer panel consisting of 72 females and 28 males, with 79% of panellist between the age of 20 and 29 years. A nine-point hedonic scale ranging from 1 for dislike extremely up to 9 for like extremely was used to score aroma, taste, aftertaste and overall acceptability as attributes (Table 3.7).

Table 3.7: Simplified example of the hedonic scale used for consumer sensory analysis

Nine-point hedonic scale for aroma, taste, texture and overall acceptability								
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

3.6.2.2. Preparation of sensory samples

Preparation of samples for untrained consumer sensory analysis was done as described for trained sensory panel in Section 3.4.1.2.

3.6.3. Physical texture analyses

Physical texture analyses (shear force measurements) were performed with the Instron Universal Testing Machine (UTM, Model 430) so as to correlate the results regarding tenderness with the findings of the trained sensory panel. After cooking the loin chops for sensory analysis, ten chops per treatment were cooled down at room temperature for at least 5 hours, before shear force measurements were made. Cylindrical samples (6 cores/sample), with a 12.7 mm core diameter, were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using a Warner Bratzler shear device mounted on the Instron Universal Testing Machine (UTM, Model 430). The shear force was determined using cross head speed = 200 mm/minute, test speed with a 1 kN load cell. The reported shear force value, in kilogram, represents the average of the peak force measurements of each sample.

3.6.4. Myofibrillar fragment lengths

Myofibrillar fragment lengths (MFL) determinations were done on days 1 and 5 post mortem. A frozen *M. longissimus lumborum* sample (3g) was placed in a 50 ml Bühler glass containing

myofibrillar fragment length extraction buffer (30 ml) [0.02 M Potassium phosphate buffer containing 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA and 1 mM NaN_3 (pH 7.0) (4 °C)]. Samples were allowed to thaw for 60 seconds and were then homogenised for exactly 30 seconds in a Bühler HO 4/A homogeniser, at 20000 rpm (blade turned around in order to fragment myofibrils rather than cut them). Samples were subsequently centrifuged at 3000 rpm (4 °C) for 15 minutes. The supernatant was discarded and the pellet was suspended in MFL extraction buffer (30 ml) (4 °C) and centrifuged at 3000 rpm (4 °C) for 15 minutes. The supernatant was discarded and the pellet suspended in MFL extraction buffer (10 ml) (4 °C). The suspension was filtered under vacuum through a 1000 µm polyethylene strainer. Additional MFL extraction buffer (5 ml) (4 °C) was used to facilitate the passing of myofibrils through the strainer. The filtrate was subsequently filtered under vacuum, through a 250 µm polyethylene strainer. This filtrate was used to measure MFL with the VIA. Myofibrils were extracted according to Culler, Parrish, Smith, & Cross (1978) with some modifications (Heinze, & Brueggermann, 1994). The myofibrillar fragments from the filtrate were examined with an Olympus BX40 system microscope at a 400X magnification. One hundred myofibril fragments of each sample were measured, using analySIS Life Science software package.

3.6.5. Muscle fibre typing of M. longissimus lumborum

Muscle samples (± 10 g) were cut from the *M. longissimus lumborum* and frozen in liquid N_2. The frozen samples were cut against the grain of the meat and mounted on a cryotome disc, with special mounting solution for frozen meat cuts. Cryotome discs with samples were placed in the cryotome chamber at -25 °C and cut to a thickness of 10-12 µm. Cuts were placed on microscope slides, stained by staining solution [0.2 N phosphate buffer (pH 7.65) (25 ml), 0.2 N succinic acid-NaOH buffer (pH 7.64) (25 ml) and 1 mg/ml nitroblue tetrasolium (NBT) (25 ml)] and incubated (37 °C). After incubation the slides were removed, washed [0.9 % NaCl, 1 % formal saline (10 min), 15% alcohol and distilled H_2O] and dried. Slides were then fixed by mounting in melted glycerine jelly, dried and cleaned with distilled H_2O (Barka, & Anderson, 1963). Muscle fibre types were examined with an Olympus BX40 system microscope at a 100X magnification. Fifty measurements per muscle fibre type were done and the amount of red, intermediate and white muscle fibres were counted in 5 microscopic fields to determine the percentage of each fibre type in the samples (analySIS Life Science).

3.7. Chemical and oxidative stability studies

3.7.1. Colour and lipid stability of fresh and frozen pork

Four loin chops from each pig, from each treatment group, were individually packed. One loin chops from each pig was packed into a polystyrene tray, containing an absorbent pad and overwrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap and stored at 4 °C for 7 days, under fluorescent light, for the fresh meat stability study. The remaining three chops

were vacuum packed. One chop was used for lipid stability tests at day 0 and the other two stored at -18 °C, in the dark, for frozen storage stability studies for 3 and 6 months. On days 0 and 7, one loin chop from each pig, stored at 4 °C, was opened. The colour (*L**, *a** and *b** values) of both muscle and fatty tissue were assessed, in duplicate, after 30 min, using a Minolta CM-600d chromometer with illuminant A and spectral component included. Chroma, which is related to the colour intensity of the meat, was calculated according to the formula: Chroma = $\sqrt{a^{*2} + b^{*2}}$ for both muscle and backfat (Lanari, Scheafer, & Scheller, 1995; Ripoll et al., 2011). According to Joo, Kauffman, Kim, & Kim (1995), chroma was better correlated with visual perception of pork colour than *a**-value. Hue angle was calculated according to the formula [tan-1(*b**/*a**)] (Ripoll et al., 2011).

To assess oxidative stability during frozen storage, lean meat and backfat were sampled on days 0 and 7, and at 3 and 6 months. Thiobarbituric acid reactive substances (TBARS) analyses, to determine lipid oxidation of lean meat, were done according to the following method. Two 5 g samples lean meat were removed from the middle of each loin chop and the aqueous acid extraction method of Raharjo, Sofos and Schmidt (1992) was used. A 10 g sample of backfat (inner + outer layer) was also removed for lipid extraction, using the Folch et al. (1957) method. To assess lipid oxidation in backfat, the peroxide value (PV) was determined on 5 g of the extracted lipid sample, using the AOAC (2005) method nr. 965.33.

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3.8. Processed meat products

3.8.1. Bacon

3.8.1.1. Manufacturing

Brine was formulated at a 20% injection level, according to the formulation, outlined in Table 3.8. The brine was formulated to deliver the following concentrations of the ingredients in the final products: salt (2%), sucrose (0.5%), sodium nitrite (160 mg/kg) and sodium-tripolyphosphate (0.5%). The brine solution was injected to the desired level. The meat was left overnight in a curing vat at 4 °C, to allow even distribution of the brine. The meat was then dried at 60-65 °C in a smoking chamber (Crown Mills), where after it was oak smoked at 65.5-68.5 °C, until golden brown (approximately 45 min). The smoked meat was frozen, tempered, cut and vacuum packaged in 200 g packages, as for retail purposes. The packaged bacon were stored at 4 °C until sampling, which took place on day 0 and weeks 3 and 6 of refrigerated storage. The weights of the pork loins after dressing and just before injection were recorded as the green weight of the loins.

Percentages of pump, shrinkage after smoking, processing yield, drip loss, cooking loss and total loss were calculated with the following formulas:

% Pump = (weight after pump – green weight) / green weight x 100

Drip loss (%) = [(packet weight – sliced bacon weight) / packet weight] x 100

Table 3.8: Bacon brine formulation for 20% pump

Ingredients	Inclusion level (%)
Salt	12.00
Sucrose	3.00
Sodium Nitrite	0.096
Sodium- Tri polyphosphate	3.00
Water	81.90
Total	**100.00**

Cooking loss (%) = [(uncooked bacon weight – cooked bacon weight) / uncooked bacon weight] x 100

Total loss (%) = [(packet weight – cooked bacon weight) / packet weight] x 100

3.8.1.2. Quality and oxidative stability

Colour measurements were performed on days 0 and weeks 3 and 6 on the uncooked, vacuum-packaged bacon. Four packets of each of the five treatments were used and measurements per package were done in sextuplicate. Colour measurements were made using a Minolta CR 400 Chroma Meter (Konica Minolta, Cape Town, South Africa) with 8 mm measuring area at a 0° viewing angle. On the chosen sampling days, two 5 g samples were taken from each pack of bacon of the five treatment groups for TBARS analysis (Raharjo et al., 1992).

3.8.1.3. Consumer sensory evaluation

Bacon from each treatment group was also stored at -18 °C, until used for sensory analysis. Sensory analysis were carried out on bacon from all five treatment groups using a 75 member untrained consumer panel. A nine-point hedonic scale ranging from 1 for dislike extremely up to 9 for like extremely was used to score aroma, taste, aftertaste and overall acceptability (Table 3.6). Packets of bacon from the five treatments were removed from the freezer and defrosted in a refrigerator at 4°C, one day before it was to be evaluated. The bacon from each dietary treatment were pan-fried separately and kept warm in stainless steel containers on hot trays. Care was taken not to cook the bacon any further or let it dry out on the hot plate during waiting periods. One rasher from each treatment measuring about 40 mm x 40 mm, was served to the panellists. Each sample was placed in a pre-heated 20 ml glass bowl, covered with a square of aluminium foil. Samples in glass bowls, ready to be served to the panel, were kept warm in a *bain marie*, filled with hot water (60 ºC). The five bacon samples were coded with randomized, three digit codes to prevent bias. The sensory evaluation was performed in individual booths of the sensory laboratory and the booths were fitted with red coloured bulbs emitting only red light to mask any

possible colour variations between different samples. Diluted apple juice, at room temperature, was served as palate cleanser to prevent fat build-up during tasting.

3.8.2. Pork sausages (Bangers)

3.8.2.1. Manufacturing

Lean pork and backfat were minced separately through a 13 mm mincing plate fitted to an OKTO mincer. The two separate batches of pork and backfat were then thoroughly mixed to obtain homogenous raw materials. For each treatment, specific amounts of pork, backfat, ice water, rusk, spices and additives were weighed out (Table 3.9). The spice and additive pack contained the following: sodium tripolyphosphate (21.58%), coriander (21.58%), MSG (14.39%), dextrose (7.19%), white pepper (7.19%), nutmeg (5.40%), sodium metabisulfite (5.04%), black pepper (5.04%), cardamom (4.46%), sodium ascorbate (3.60%), sage (2.16%), ginger (2.01%), and mace (0.36%). The ice water, rusk, spices and additives were mixed continuously for 1 min and left standing for 5 min to allow for proper hydration of the rusk and starch and for proper dissolving of the water-soluble additives. The preweighed pork and backfat were added to the mixture and the batter was mixed thoroughly before being minced through a 3 mm mincing plate. Natural hog casings with a diameter of 28-32 mm were then filled with the sausage batter using a manual sausage filler (Trespade, Crown National, Johannesburg, South Africa). This resulted in a single, continuous roll of sausage with a weight of 2.50 kg per treatment. Banger links were formed in the roll and 24 individual pieces of sausage were then cut from each roll, each weighing ± 80 g. Each individual sausage was then placed in an expanded polystyrene (EPS) tray containing an absorbent pad, over-wrapped with polyvinyl chloride (PVC) film and stored either at 4 °C under retail refrigeration-type conditions, including fluorescent lighting, for fresh product shelf-life determination (16 sausages per treatment) or at -18 °C for frozen product lipid stability determination (8 sausages per treatment). Twenty of the sausages were refrigerated under the same conditions as for the fresh product self-life determination. After the 9 days the sausages were removed from their packaging and weighed.

Refrigeration loss was then calculated with the formula:

$$\text{Refrigeration loss (\%)} = [(\text{initial weight} - \text{weight after refrigeration}) / \text{initial weight}] \times 100$$

The other 20 sausages of each treatment group were kept frozen at −18 °C for 9 days to simulate a short-term home-freezing scenario. After 9 days the samples were kept at 4 °C for 24 h to allow for gradual thaw, after which the samples were removed from their packaging and weighed. The sausages were then dry-cooked in a convection oven pre-heated to 160 °C until an internal temperature of 72 °C was reached. During cooking the baking tray was rotated 90° every 2 min for even cooking conditions. Afterwards, the sausages were removed from the oven and air cooled to room temperature before being weighed again.

Table 3.9: Banger formulation

Ingredients	Inclusion (%)
Lean pork 90/10	54.81
Pork backfat	22.50
Ice water	15.00
Rusk	3.50
Spices and additives	1.39
Starch	0.80
NaCl	2.00
Total	**100.00**

Thaw loss was calculated with the formula

Thaw loss (%) = [(initial weight – weight after thawing) / initial weight] x 100

And cooking loss was calculated with the formula:

Cooking loss (%) = [(weight after thawing – weight after cooking) / weight after thawing] x 100

The total loss percentage was calculated as the sum of the thaw and cooking losses.

3.8.2.2. Quality and oxidative stability

Colour measurements were performed only on the refrigerated bangers on days 0, 3, 6 and 9 before the PVC film was removed and samples for chemical analysis were taken. Four sausage samples of each of the five treatment groups were used and measurements per sausage were done in sextuplicate. An individual sausage was gently pressed along the length to obtain a flatter surface before measurements were taken through the PVC film. The white calibration plate was covered in the same film, any air bubbles between the film and plate were rubbed-out and the plate was used to calibrate the colorimeter. Colour measurements were made using a Minolta CR 400 Chroma Meter (Konica Minolta, Cape Town, South Africa) with 8 mm measuring area at a 90° angle perpendicular to the sample surface. The same colour parameters were measured as for the bacon in 3.6.1.2. TBARS analysis (Raharjo et al., 1992) were done on both the fresh (days 0, 3, 6 and 9) and frozen (days 90 and 180) bangers

3.8.2.3. Consumer sensory evaluation

Samples of each treatment group were defrosted overnight at 4 °C. The sausages of different treatment groups were dry-cooked on separate baking trays in a convection oven pre-heated to 160 °C until an internal temperature of 72 °C was reached. The cooked sausages were cut into

pieces each with a length of ± 2 cm and placed individually in small glass bowls that were covered with squares of aluminium foil. The bowls were kept warm at 55 °C until just before serving. A 100 member consumer panel of staff and students from the Agriculture Building of the University of the Free State was used. All other procedures were carried out as for the sensory analysis of the bacon in 3.6.1.3.

3.8.3. Salami

3.8.3.1. Manufacturing

Minced lean meat and backfat, earmarked for the manufacturing of processed meat products, were used to manufacture 2 x 2.5 kg batches of salami from each treatment, according to the formulation outlined in Table 3.10. The lean meat and backfat were vacuum-packed and stored at - 18 °C, prior to the manufacturing of the salami. The first 40% of the frozen lean meat was chopped to a 10 mm particle size in a 20 L OKTO Bowl Cutter. The starter culture (0.03%) [Bactoferm™ T-D-66, which consisted of a mixed culture of *Staphylococcus carnosus* (good flavour development and stable red colour) and *Lactobacillus plantarum* (medium to fast initiation of acidification, which results in a medium to low pH)] from CHR HANSEN, Lake Foods, Sandton, Johannesburg, was used. The starter culture and spice mixture (2.18%) (Table 3.11) was added and the meat was chopped to a fine consistency. The remaining frozen lean pork (39.75%) was added and chopped to a particle size of 20 mm. The backfat (15%) and curing salt (3.05%) (Table 3.12) were added and the mixture was chopped at low speed. After the mixture was properly mixed, it was chopped at high speed until a particle size of 4-5 mm was reached. The meat mixture was filled into wetted Colpak Fibrous Bak 65/50 casings (Crown National, Bloemfontein) to produce 12 salamis per batch, with an average weight of ± 200 g each. The individually labelled salamis were fermented for 48 h at 22 °C with a relative humidity (rH) of 90%. After fermentation, the salamis were oak smoked in a Crown Mills smoking chamber for 10 min at 18-22 °C. After smoking, the salamis were ripened at 12 °C at a rH of 75%. The weight loss, as loss in moisture, was monitored at time 0, 12 h, 24 h, 36 h, 48 h and then every 48 h, until a final weight loss (20%) was achieved, which indicatedthe end of the ripening process. After manufacturing, 3 salamis from each batch were selected for chemical analysis. After the ripening period, the remaining salamis were vacuum-packed and stored for 1 month at 4 °C. After fermentation and drying and after one month storage, chemical analyses and physical measurements were done on salamis from the experimental treatment groups.

3.8.3.2. Quality and oxidative stability

Two 5 g samples, from each of the salamis, were taken for TBARS analysis as an indicator of oxidative stability (Raharjo et al., 1992). A 10 g sample from each salami, was taken for Folch et al. (1957) lipid extraction. Peroxide value was determined on 0.5 g extracted lipid, using the AOAC (2005) method nr. 965.33, to assess primary lipid oxidation. Free fatty acids were determined

Table 3.10: Salami formulation

Ingredients	Inclusion level (%)
Pork 90/10	40.00
Pork 90/10	39.75
Pork backfat	15.00
Curing salts	3.05
Spice mixture	2.18
Starter culture	0.03
Total	**100.00**

Table 3.11: Spice mixture used for salami manufacturing

Ingredient or spice	Inclusion level (%)
Dextrose	45.95
Sucrose	45.95
White pepper	4.59
Garlic powder	2.76
Nutmeg	0.75
Total	**100.00**

Table 3.12: Curing salt mixture used for salami manufacturing

Additive	Inclusion level (%)
Sodium chloride	99.30
Sodium nitrate (formulated to 120 ppm in end product)	0.39
Sodium nitrite (formulated to 92 ppm in end product)	0.30
Total	**100.00**

according to Pearson (1968) on 0.5 g extracted lipid. A 10 g sample, per salami, was taken to determine total acidity (TA) according to Konieko (1985). The pH of three models from each dietary treatment group was also determined at the same intervals as for the weight loss determinations. The pH measurements were performed at room temperature (25 °C), using a THERMO Orion STAR SERIES 3 (USA) digital pH meter equipped with probe (model MA 920). The probe was calibrated with pH 4.00 and 7.00 buffer solutions from Merck (uniVAR®, Wadeville, Gauteng, SA) each day before measurement. The water activity (aw) of each sample was measured after sample collection, using a Novasina Thermoconstanter aw meter (model No. TH200). Water activity measurements were carried out at room temperature (25°C) after the machine was allowed to reach equilibrium with deionised distilled water. Readings given as % rH were later converted to aw value.

3.8.3.3. Consumer sensory evaluation

Sensory analyses were carried out on all five treatments of salamis after manufacturing, using a 75 member untrained consumer panel. A nine-point hedonic scale ranging from 1 for dislike extremely up to 9 for like extremely was used to score aroma, taste, aftertaste, texture and overall acceptability as attributes (Table 3.6). The samples were encoded with a randomized three-digit code, unique to each sample, to prevent the development of bias by the consumers. Each sample consisted of a 2 mm slice of salami with a 35 mm diameter. Samples were removed from refrigerated storage, allowed to reach room temperature and then served at a room temperature of 22 °C under red lighting to mask any possible colour differences. Diluted apple juice was used between each sample as a palate cleanser.

3.9. Statistical analysis

One-way Analysis of Variance (ANOVA) was performed to determine significant differences between the digestibility of diets, growth performance and feed efficiency of animals, carcass characteristics and the chemical, physical, histological, chemical stability and sensory properties of meat and meat products from the five dietary treatments. (NCSS 11 Statistical Software 2016). The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to identify significant differences between the treatment means (NCSS 11 Statistical Software 2016). Pearson's correlation analysis was performed to determine the relationship between selected quality characteristics (NCSS 11 Statistical Software 2016). Sensory data were visualised in a 2-dimensional space by principal component analysis (PCA) (XLSTAT, 2018). In tables statistically analysed data are represented with ± standard deviations. Where data are presented as figures error bars represent standard deviation

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Feed composition and quality

The formulation and nutrient composition of the grower and finisher diets for all five experimental treatments are shown in Tables 3.1 to 3.3. The control diet contained 1% Bergafat HPL 106 (palm oil) and the experimental diets contained 1% soya oil, 1% linseed oil, 1% fish oil or 1% *Echium* oil in both the grower and finisher diet phases (Table 4.1).

The major differences in the fatty acid compositions between the five diets were: C16:0, C18:2, C18:3, C18:4 and the long chain (LC) n-3 fatty acids C20:5 (EPA), C22:5 (DPA) and C22:6 (DHA) (Table 4.1). The experimental diets were specifically chosen because of their very different fatty acid profiles as illustrated in Table 3.4. The C16:0 was the highest in the control diet for both feeding phases, due to the high levels of C16:0 in palm oil. The control diet also had the highest saturated fatty acid (SFA) content which resulted in it having the lowest PUFA:SFA and highest n-6:n-3 ratios. Soya oil is rich in C18:2 and therefore the soya oil diet had the highest levels of C18:2. It also had the highest levels of total n-6 and polyunsaturated fatty acids (PUFA) of all treatments. Adding linseed oil to the feed resulted in high levels of C18:3. The linseed diet also had the highest levels of total n-3 and therefore the lowest n-6:n-3 ratio. The longer chain n-3 fatty acids (FA), EPA, DPA and DHA, were only seen in the fish oil diet. It is evident from Table 4.1 that the *Echium* oil diet had high levels of C18:3 and the highest levels of all five treatments for C18:4.

The more double bonds present in unsaturated fatty acids, the more susceptible it is to lipid oxidation (Monahan et al., 1992). This is clearly evident in the iodine values (IV) of the different treatments with the control having the lowest IV.

Free fatty acid (FFA) measurement is the simplest method to monitor hydrolytic rancidity in lipid-containing foods and is a key feature linked with the quality and commercial value of oils and fats. In the present study the FFA content of the finisher phase from diets containing increased n-3 FA was higher than the grower phase. A possible explanation could be that the oils used in the feed oxidised during storage. The feed from the control group had much higher levels of FFA compared to the other treatments. Tropical fruits such as palm are particularly prone to high FFA levels in crude oils as the fruit mesocarp contains high lipase activity (Azeman & Yusof, 2015).

Primary oxidation products in oil, mainly hydroperoxides, are measured by the peroxide value (PV). In general, high quality oil will have a lower PV. However PV decreases as secondary oxidation products appear (Miller, 2010). The secondary stage of oxidation occurs when the hydroperoxides decompose to form carbonyls and other compounds, in particular aldehydes. These are what give the oil a rancid smell, and they are measured by the p-anisidine value (AV). A lower AV indicates better quality oil, therefore the AV test is a good way to measure secondary

Table 4.1: Fatty acid composition, fatty acid ratios and lipid quality of the five diets used in this experiment.

	Control		Soya oil		Linseed oil		Fish oil		*Echium* oil	
	Grower	**Finisher**	**Grower**	**Finisher**	**Grower**	**Finisher**	**Grower**	**Finisher**	**Grower**	**Finisher**
Fatty acid composition (%)										
C14:0	0.57 ± 0.01	0.63 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	2.42 ± 0.02	2.62 ± 0.03	0.02 ± 0.03	0.03 ± 0.01
C15:0	0.03 ± 0.01	0.04 ± 0.01	ND	ND	ND	ND	0.04 ± 0.06	0.09 ± 0.01	ND	ND
C16:0	33.27 ± 0.12	33.63 ± 0.46	12.20 ± 0.01	11.70 ± 0.04	10.82 ± 0.04	10.47 ± 0.05	12.59 ± 0.11	12.46 ± 0.08	10.60 ± 0.04	10.33 ± 0.01
C16:1c9	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	3.01 ± 0.03	3.23 ± 0.01	0.05 ± 0.07	0.08 ± .00
C17:0	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.03 ± 0.04	0.06 ± 0.01
C17:1c10	ND	0.01 ± 0.01	ND	0.01 ± 0.02	ND	0.01 ± 0.02	0.26 ± 0.01	ND	ND	ND
C18:0	1.87 ± 0.01	1.69 ± 0.02	3.17 ± 0.01	2.91 ± 0.01	2.62 ± 0.02	2.50 ± 0.02	2.36 ± 0.01	2.26 ± 0.01	2.48 ± 0.01	2.37 ± 0.01
C18:1t9	ND	ND	ND	ND	0.01 ± 0.01	ND	0.19 ± 0.01	0.20 ± 0.01	ND	ND
C18:1c9	27.12 ± 0.05	25.42 ± 0.22	27.23 ± 0.05	26.41 ± 0.04	31.80 ± 0.17	30.87 ± 0.01	27.22 ± 0.16	25.60 ± 0.05	30.12 ± 0.19	29.49 ± 0.01
C18:1c7	ND	ND	ND	ND	ND	ND	3.09 ± 0.02	3.20 ± 0.01	ND	ND
C18:2c9,12(n-6)	35.31 ± 0.08	36.50 ± 0.24	52.87 ± 0.05	54.36 ± 0.09	42.84 ± 0.21	43.63 ± 0.03	39.14 ± 0.22	39.76 ± 0.04	40.10 ± 0.21	41.18 ± 0.01
C18:3c6,9,12(n-6)	ND	ND	0.03 ± 0.05	ND	0.01 ± 0.02	ND	0.04 ± 0.01	0.04 ± 0.01	2.85 ± 0.01	2.72 ± 0.01
C18:3c9,12,15(n-3)	1.20 ± 0.01	1.37 ± 0.01	3.43 ± 0.02	3.45 ± 0.01	11.23 ± 0.05	11.47 ± 0.03	1.52 ± 0.01	1.68 ± 0.01	9.85 ± 0.01	9.52 ± 0.01
C18:4c6,9,12,15(n-3)	ND	ND	ND	ND	ND	ND	0.88 ± 0.01	0.90 ± 0.01	3.49 ± 0.02	3.29 ± 0.01
C20:0	0.27 ± 0.01	0.24 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	0.17 ± 0.23	0.31 ± 0.01	0.21 ± 0.29	0.41 ± 0.01	0.15 ± 0.21	0.29 ± 0.01
C20:1c11	ND	0.13 ± 0.01	0.08 ± 0.12	0.18 ± 0.01	0.07 ± 0.10	0.15 ± 0.01	ND	0.26 ± 0.01	ND	0.27 ± 0.01
C20:2c11,14(n-6)	0.11 ± 0.01	ND	0.24 ± 0.01	ND	ND	ND	ND	ND	ND	ND
C20:3c8,11,14(n-6)	ND	0.09 ± 0.01	ND	0.22 ± 0.01	0.09 ± 0.13	0.18 ± 0.01	0.07 ± 0.10	0.14 ± 0.01	0.06 ± 0.09	0.12 ± 0.01
C20:3c11,14,17(n-3)	ND	ND	ND	ND	ND	ND	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
C20:4c5,8,11,14(n-6)	ND	ND	ND	ND	ND	ND	0.07 ± 0.10	0.14 ± 0.01	ND	ND
C20:5c5,8,11,14,17(n-3)(EPA)	0.10 ± 0.01	ND	0.15 ± 0.01	ND	0.13 ± 0.01	ND	5.03 ± 0.02	5.07 ± 0.02	0.11 ± 0.01	ND
C22:0	ND	ND	ND	0.01 ± 0.01	ND	ND	ND	0.01 ± 0.01	ND	ND
C22:1c13	ND	ND	ND	ND	ND	ND	0.02 ± 0.01	0.02 ± 0.01	ND	ND
C22:5c7,10,13,16,19(n-3)(DPA)	ND	ND	ND	ND	0.01 ± 0.01	0.02 ± 0.01	0.46 ± 0.01	0.46 ± 0.01	ND	0.01 ± 0.01
C22:6c4,7,10,13,16,19(n-3)(DHA)	ND	ND	ND	ND	ND	ND	1.21 ± 0.02	1.22 ± 0.01	ND	ND
C23:0	ND	ND	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C24:0	ND	0.09 ±0.01	ND	0.14 ± 0.01	ND	0.13 ± 0.01	ND	ND	ND	0.11 ± 0.01
C24:1c15	ND	ND	ND	0.03 ± 0.01	0.03 ± 0.04	0.08 ± 0.01	0.03 ± 0.04	0.09 ± 0.01	0.03 ± 0.04	0.07 ± 0.01
Fatty acid ratios:										
SFA(%)	36.06 ± 0.13	36.37 ± 0.46	15.89 ± 0.01	15.26 ± 0.03	13.70 ± 0.17	13.53 ± 0.03	17.72 ± 0.20	17.97 ± 0.11	13.28 ± 0.24	13.19 ± 0.01
MUFA(%)	28.42 ± 0.05	26.90 ± 0.22	27.40 ± 0.07	26.71 ± 0.06	31.98 ± 0.02	31.19 ± 0.03	33.83 ± 0.17	32.60 ± 0.04	30.19 ± 0.09	29.91 ± 0.01
PUFA(%)	36.72 ± 0.08	37.97 ± 0.23	56.72 ± 0.08	58.03 ± 0.09	54.32 ± 0.14	55.29 ± 0.01	48.45 ± 0.02	49.44 ± 0.07	56.52 ± 0.15	56.90 ± 0.01
UFA(%)	65.14 ± 0.13	64.87 ± 0.46	84.12 ± 0.01	84.74 ± 0.03	86.30 ± 0.17	86.47 ± 0.03	82.28 ± 0.20	82.04 ± 0.11	86.71 ± 0.24	86.81 ± 0.01
C18:0/C18:2	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
DBI	102.16 ± 0.22	103.06 ± 0.69	144.74 ± 0.13	146.44 ± 0.13	152.38 ± .23	153.46 ± 0.01	155.60 ± 0.10	156.92 ± 0.29	163.37 ± 0.35	162.73 ± 0.02
PI	39.13 ± 0.10	40.10 ± 0.23	61.60 ± 0.13	62.37 ± 0.09	67.16 ± 0.05	67.80 ± 0.03	89.73 ± 0.41	91.46 ± 0.26	81.11 ± 0.13	79.97 ± 0.01
PUFA/SFA	1.02 ± 0.01	1.04 ± 0.02	3.57 ± 0.01	3.80 ± 0.01	3.96 ± 0.06	4.09 ± 0.01	2.73 ± 0.03	2.75 ± 0.02	4.26 ± 0.09	4.31 ± 0.01
n-6(%)	35.42 ± 0.08	36.60 ± 0.24	53.14 ± 0.10	54.59 ± 0.09	42.95 ± 0.10	43.81 ± 0.03	39.32 ± 0.02	40.09 ± 0.05	43.01 ± 0.14	44.02 ± 0.01
n-3(%)	1.30 ± 0.01	1.37 ± 0.01	3.58 ± 0.01	3.45 ± 0.01	11.37 ± 0.04	11.48 ± 0.03	8.24 ± 0.01	8.45 ± 0.02	10.020.01	9.59 ± 0.01
n-6/*n*-3	54.50 ± 0.05	54.66 ± 0.25	14.85 ± 0.08	15.84 ± 0.01	3.78 ± 0.01	3.81 ± 0.01	4.77 ± 0.01	4.74 ± 0.01	4.29 ± 0.01	4.59 ± 0.01`
IV	78.12 ± 0.17	78.83 ± 0.53	110.74 ± 0.11	112.06 ± 0.10	116.70 ± 0.22	117.54 ± 0.01	116.94 ± 0.01	117.88 ± 0.20	125.3 ± 10.28	124.80 ± 0.02
Lipid Stability of Feed:										
% Free Fatty acids	37.68 ± 3.18	38.99 ± 4.05	14.86 ± 1.34	9.29 ± 2.18	16.85 ± 1.54	20.85 ± 1.87	13.33 ± 1.47	17.71 ± 1.94	14.70 ± 1.65	17.57 ± 2.05
Peroxide value	3.26 ± 0.03	4.13 ± 0.54	2.76 ± 0.08	11.12 ± 1.83	22.24 ± 2.16	15.13 ± 2.14	12.58 ± 1.18	10.05 ± 1.16	18.59 ± 2.09	11.93 ± 2.03
p-Anisidine value	15.01 ± 0.02	16.15 ± 0.19	15.09 ± 0.15	8.92 ± 2.00	23.91 ± 5.28	34.32 ± 1.86	23.55 ± 0.86	58.73 ± 3.18	17.33 ± 0.61	46.91 ± 0.01

ND = Not detected

oxidation products and should be used together with the PV (Miller, 2010). It is clear from the results in Table 4.1 that secondary oxidation products have formed in the finisher feed from the linseed-, fish- and *Echium* oil treatments due to the decrease in PV between the grower and the finisher diet for these three dietary treatments. Also, the p-anisidine value from these three diets has substantially increased between the grower and the finisher diets. These three diets also had the highest IV, double bond index (DBI) and n-3 PUFA which are all known for higher susceptibility for oxidation. Even though the PV is relatively low, the secondary oxidation products are much higher and therefore the lipids will likely be rancid (Table 4.1).

4.2 Digestibility analysis of feed

The total tract digestibility of nutrients in pigs can be influenced by the characteristics of the feed (chemical composition and processing treatment) as well as by animal factors (body weight, sex, physiological stage, and genotype) (Le Goff & Noblet, 2001).

Most literature (Dierick, Vervaeke, Decuypere, & Henderickx, 1983; Stanogias & Pearce, 1985; Fernandez & Jorgensen, 1986; Chabeauti, Noblet, & Carré 1991), indicate that the presence of fibre in the diet of pigs reduces the apparent feacal digestibility of nitrogen. The analysed nutrient composition and digestibility coefficients of nutrients of this study are given in Table 4.2 and 4.3. Table 4.2 indicates that the feed from the linseed treatment contained the highest concentration of acid digestible fibre (ADF) followed by the fish oil treatment. However, it must be kept in mind that the data in Table 4.2 was not statistically analysed and observed differences must be regarded as numerical. While the five experimental diets were formulated to be *iso-nutrient* during the present study, it might happen that the quality of raw materials used during manufacturing of a specific treatment varies - thus contributing to differences observed between calculated and analysed chemical composition. The linseed and fish oil treatments also resulted in the lowest protein digestibility in the present study (Table 4.3). Noblet and Perez (1993) found a strong correlation between the ash and fibre content in pig diets and that an increase in ash content would result in a decreased protein digestibility, which was not the case during the present study. The linseed treatment had the highest ADF and the lowest ash content (Table 4.2). The linseed and fish oil treatments also had the lowest protein digestibility and lowest ash (Table 4.2).

Eusebio, Hays, Speer and McCall (1965) found that chain length, degree of saturation of fatty acids and their arrangement within the triglyceride molecule are important factors in determining the degree of fat digestibility in pigs. Experimental diets during the present study differed much in their fatty acid profiles as well as degrees of FA saturation (Table 4.1). Nonetheless the FA differences recorded no significant differences were recorded in terms of fat digestibility as seen in (Table 4.3). It does seems however that the *Echium* oil treatment resulted in a slightly higher digestibility compared to the rest of the lipid source treatments. Given the proximate analysis of fat for the the *Echuim* oil treatments as illustrtated in Table 4.2, in comparison to that of the other experimental diets, it could be suggested that the slightly higher

Table 4.2: Chemical analysed nutrient composition (%) of finisher diets on an air dry basis.

	Control	**Soya oil**	**Linseed oil**	**Fish oil**	***Echium* oil**
Proximate analysis:					
Dry matter (%)	89.28	89.68	89.57	90.00	89.53
Moisture (%)	10.72	10.32	10.43	10.00	10.47
Protein (%)	16.09	16.05	15.25	16.24	16.04
Acid detergent fibre (%)	4.86	5.01	5.43	5.19	4.97
Fat (%)	3.44	2.90	2.93	3.01	2.48
Minerals:					
Ash (%)	4.11	4.31	3.72	4.07	4.33
Calcium (%)	0.59	0.44	0.50	0.61	0.57
Phosporus (%)	0.48	0.40	0.44	0.46	0.45
Magnesium (%)	0.20	0.17	0.18	0.20	0.20
Potassium (%)	0.71	0.62	0.67	0.69	0.71
Sulphur (%)	0.18	0.16	0.17	0.18	0.18
Sodium (%)	0.29	0.20	0.25	0.31	0.29
Chloride (%)	0.40	0.44	0.36	0.43	0.41
Iron (ppm)	309.20	251.90	287.10	347.50	339.90
Manganese (ppm)	131.60	104.40	121.20	135.80	139.20
Zinc (ppm)	151.80	117.50	161.70	174.80	157.10
Copper (ppm)	174.20	120.40	153.30	173.60	188.10

Table 4.3: Digestibility coefficients of nutrients in pigs fed the five diets used in this experiment.

Nutrient	Control (n=4)	Soya oil (n=4)	Linseed oil (n=4)	Fish oil (n=4)	*Echium* oil (n=4)	Sign. level
Protein	$0.80^{ab} \pm 0.02$	$0.82^{b} \pm 0.08$	$0.76^{ab} \pm 0.03$	$0.69^{a} \pm 0.04$	$0.82^{b} \pm 0.07$	$P = 0.014$
Fat	0.57 ± 0.11	0.57 ± 0.03	0.59 ± 0.05	0.55 ± 0.06	0.72 ± 0.07	$P = 0.061$
Acid detergent fibre	$0.37^{abc} \pm 0.09$	$0.56^{c} \pm 0.04$	$0.35^{ab} \pm 0.02$	$0.24^{a} \pm 0.04$	$0.53^{bc} \pm 0.14$	$P = 0.001$
Calcium	0.71 ± 0.05	0.71 ± 0.12	0.70 ± 0.03	0.65 ± 0.06	0.62 ± 0.12	$P = 0.623$
Phosphorous	0.56 ± 0.08	0.60 ± 0.14	0.56 ± 0.04	0.51 ± 0.06	0.68 ± 0.10	$P = 0.297$

Means with different superscripts in the same row differ significantly.

digestibility coefficient (Table 4.3) recorded for the *Echium* oil treatment is related the lower total fat content of the diet, and consequently more efficient utilisation thereof. However, the relative low digestibility coefficients (Table 4.3) of the experimental diets was not anticipated and difficult to explain as Stahly (1984) found that the probable digestibility of short- or medium-chain FA is high and range between 80 to 95%. The fact that the total tract digestibility coefficients were determined during this study, and not the ileal nutrient digestibility, might indicate that the contribution of microbial fat digestion in the large intestinal tract (caecum & colon) was underestimated during the present study.

Increasing the fat content of the diet of pigs will reduce fermentation in the hindgut and will result in a lower fibre digestibility (Dégen, Halas & Babinszky, 2007). Since neither the dietary fat concentrations nor the fibre levels were in the extremities, it was expected that these two dietary characteristics would not have influenced each other in a negative manner. Despite the differences in the total ash content of the experimental diets (Table 4.2), it is clearly evident from Table 4.3 that there were no significant differences between dietary treatments for either calcium or phosphorous digestibility.

4.3 Animal production performance

4.3.1 Growth performance and feed efficiency

Feed conversion ratio (FCR), is defined as the amount of feed (kg) required to increase body weight by one kg (Losinger, 1998). It is an important determinant of profitability for pork producers (Edwards, van der Sluis & Stevemer, 1989). The cost of feed accounts for approximately two-thirds of the total cost of pork production, therefore small changes in the FCR can have a considerable impact on the profitability of an operation. In the present study there were no significant differences between dietary treatments for average daily feed intake (ADFI) and FCR (Table 4.4).

It is further evident from Table 4.5 that the total growth rate as well as average daily gain (ADG) were not influenced by dietary treatments. The only significant difference (P = 0.049) was the increase in weight over the finisher period whereby the control treatment were the highest and the linseed treatment had the lowest weight gain. However, this did not result in any differences between treatments for final slaughter weight as well as the average daily gain during the finisher phase. These findings agree with numerous studies where authors used various dietary oils such as palm, soya, linseed, olive, flaxseed, rapeseed, fish and sunflower oil and found that different diets had no effect on growth performance (Bryhni, Kjos, Ofstad & Hunt, 2002; Gläser, Wenk & Scheeder, 2002; Nüernberg et al., 2005; Teye et al., 2006a; Mitchaothai et al., 2007; Haak, De Smet, Fremaut, Van Walleghem & Reas, 2008; Valencia, O'Grady, Ansorena, Astiasarán & Kerry, 2008). According to available literature, it seems that the present is the first to investigate the effect of *Echium* oil on the growth performance of pigs. Van Oekel and Boucqué (1992) found that feeding pigs diets containing more than 5% fish oil causes depression of feed intake and growth of

Table 4.4: Effect of dietary treatment on growth performance and feed efficiency of pigs.

	Control (n = 12)	**Soya oil** (n = 12)	**Linseed oil** (n = 12)	**Fish oil** (n = 12)	***Echium* oil** (n = 12)	**Sign. level**
Average daily feed Intake over grower phase (kg)	2.27 ± 0.08	2.12 ± 0.28	2.25 ± 0.18	2.30 ± 0.03	2.23 ± 0.08	*P* = 0.804
Average daily feed Intake during finishing phase (kg)	2.85 ± 0.04	2.57 ± 0.25	2.40 ± 0.06	2.61 ± 0.03	2.62 ± 0.16	*P* = 0.149
Average daily feed Intake during total feeding period(kg)	2.70 ± 0.05	2.45 ± 0.26	2.37 ± 0.01	2.53 ± 0.01	2.50 ± 0.10	*P* = 0.249
Feed conversion ratio during grower phase (kg feed/kg gain)	2.08 ± 0.22	1.98 ± 0.11	2.01 ± 0.14	2.03 ± 0.06	2.09 ± 0.06	*P* = 0.899
Feed conversion ratio during finishing phase (kg feed/kg gain)	3.21 ± 0.01	3.06 ± 0.43	3.02 ± 0.14	3.10 ± 0.03	3.08 ± 0.01	*P* = 0.907
Feed conversion ratio during total feeding period (kg feed/kg gain)	2.86 ± 0.08	2.72 ± 0.30	2.67 ± 0.14	2.75 ± 0.01	2.77 ± 0.04	*P* = 0.801

Means with different superscripts in the same row differ significantly.

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Table 4.5: Effect of dietary treatment on growth performance of pigs.

	Control (n = 12)	**Soya oil** (n = 12)	**Linseed oil** (n = 12)	**Fish oil** (n = 12)	***Echium* oil** (n = 12)	**Sign. level**
Initial weight (kg)	32.75 ± 3.00	32.48 ± 2.67	32.57 ± 2.63	33.25 ± 3.01	33.15 ± 2.75	*P* = 0.948
Weight at end of grower phase (kg)	55.78 ± 3.97	54.95 ± 4.28	56.49 ± 3.00	57.08 ± 4.02	55.63 ± 3.42	*P* = 0.685
Slaughter weight at end of finisher phase (kg)	107.80 ± 3.96	104.22 ± 3.84	102.89 ± 5.10	106.45 ± 4.94	104.83 ± 3.42	*P* = 0.062
Weight increase during grower phase (kg)	23.03 ± 2.04	22.47 ± 2.36	23.56 ± 2.34	23.83 ± 1.90	22.48 ± 2.41	*P* = 0.450
Weight increase during finishing phase (kg)	52.02^{b} ± 5.43	49.27^{ab} ± 3.90	46.40^{a} ± 5.23	49.37^{ab} ± 3.59	49.20^{ab} ± 2.81	*P* = 0.049
Weight increase during total feeding period (kg)	75.05 ± 5.27	71.73 ± 3.91	69.96 ± 6.03	73.20 ± 4.68	71.68 ±3.69	*P* = 0.124
Average daily gain during grower phase (kg)	1.10 ± 0.10	1.07 ± 0.11	1.12 ± 0.11	1.13 ±0.09	1.07 ± 0.11	*P* = 0.450
Average daily gain during finisher phase (kg)	0.89 ± 0.09	0.84 ± 0.07	0.80 ± 0.11	0.85 ± 0.07	0.84 ±0.06	*P* = 0.140
Average daily gain during total feeding period (kg)	0.95 ± 0.07	0.90 ± 0.07	0.89 ± 0.10	0.92 ± 0.07	0.90 ± 0.07	*P* = 0.415

Means with different superscripts in the same row differ significantly

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pigs while it is clear from the present study that the 1% fish oil inclusion had no effect on growth performance or ADFI (Tables. 4.4 and 4.5).

4.3.2. Carcass characteristics

The effect of dietary treatment on carcass characteristics and Hennessey grading measurements of the pigs can be seen in Table 4.6. Diet had a significant influence on both the warm (P = 0.049), and cold (P = 0.040) carcass weights, with the control treatment producing significantly heavier carcasses than that of the linseed treatment. Although the slaughter weight results (Table 4.5) did not differ significantly (P = 0.062), it is clear that the linseed treatment had the lowest slaughter weight, while the control treatment had the highest slaughter weight. It is interesting to note that despite these significant differences recorded between the linseed and control treatments in terms of warm and cold carcass weights (Table 4.6), no significant (P= 0.402) differences were recorded for the dressing percentage. Additionally, there were no significant differences between treatments for carcass length, buttock circumference and shoulder circumference (Table 4.6). There were also no differences between treatments for the Hennessey colour score or Hennessey colour variance (Table 4.6).

Due to the production of leaner pigs the processed meat manufacturers face the problem of inconsistency of adipose tissue. Leaner pigs result in fat with higher levels of PUFA and lower concentrations of SFA (Wood et al., 1989). This is known as soft fat and is unsuitable for the use in processed meat products. Lean meat content is defined as the proportion lean meat weight to the total weight of the carcass (Commission of the European Communities (EC), 2008). It is the main classification variable used in the European Union (EU) to predict the fat quality of the pig carcass (Gangsei, Bjerke, Røe & Alvseike, 2018). In France they select adipose tissue for further processing using a method that is based on carcass lean content (< 57%) as well as backfat thickness (> 15 mm) (Davenel, Riaublanc, Marchal & Gandemer, 1999). In South Africa carcasses are also classified according to their lean meat content (LMC) and measured backfat thickness (BFT). Carcasses can be divided into six groups (PORCUS; Table 4.7) based on their calculated lean meat content and backfat thickness (SAMIC, 2015). In South Africa most pigs are classed P (LMC > 70% and BFT between 1 and 12) and O (LMC between 68 to 69 and BFT between 12 and 17) (Table 4.7; Hugo & Roodt, 2015). From Table 4.6 it is evident that the linseed treatment had the highest (P < 0.05) LMC, smallest BFT and 91.7% of the pigs in the linseed treatment ere either a P or an O classification in terms of SAMIC classification (Table 4.8). The fat from the linseed treatment would therefore not be suitable for use in processed meat products but due to the smaller BFT would meet the consumers demand for leaner meat. The control diet had the lowest LMC and highest BFT but would, from a lean meat point of view, still not be suitable for use in meat products according to the French method of selecting adipose tissue. Hugo and Roodt (2015) proposed that South African pigs with a BFT of 17.8mm and LMC of no more than 66.8% (R classification) would have a high probability of producing fat with good technological quality. Most

Table 4.6: Effect of dietary treatment on carcass characteristics and Hennessey grading measurements of pigs.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Carcass characteristics:						
Warm mass weight (kg)	$90.62^{b} \pm 3.30$	$87.59^{ab} \pm 3.57$	$86.42^{a} \pm 3.33$	$89.03^{ab} \pm 3.99$	$87.24^{ab} \pm 3.70$	$P = 0.049$
Cold carcass weight (kg)	$89.30^{b} \pm 3.45$	$86.39^{ab} \pm 3.64$	$85.00^{a} \pm 4.32$	$87.88^{ab} \pm 3.99$	$85.70^{ab} \pm 3.79$	$P = 0.040$
Dressing percentage	84.08 ± 1.74	84.05 ± 1.75	83.27 ± 1.74	84.47 ± 2.46	83.21 ± 1.63	$P = 0.402$
Carcass length (cm)	102.90 ± 2.64	100.90 ± 3.51	102.85 ± 2.87	100.90 ± 2.64	103.30 ± 2.58	$P = 0.174$
Shoulder circumference (cm)	106.55 ± 2.31	104.65 ± 2.01	103.81 ± 2.28	106.00 ± 1.81	105.15 ± 3.03	$P = 0.088$
Buttock circumference (cm)	107.70 ± 2.03	106.10 ± 2.29	106.40a ± 2.53	106.55ab ± 2.03	105.05ab ± 2.29	$P = 0.146$
Hennessey grading measurements:						
Hennessey colour score	96.00 ± 32.72	75.42 ± 19.81	80.91 ± 23.33	90.17 ± 27.52	80.09 ± 24.62	$P = 0.314$
Hennessey colour Variance	98.28 ± 61.89	72.08 ± 33.46	102.82 ± 56.50	93.50 ± 50.03	92.08 ± 50.76	$P = 0.634$
Lean meat content (%)	$66.70^{a} \pm 2.32$	$67.44^{ab} \pm 1.39$	$69.16^{b} \pm 1.46$	$67.51^{ab} \pm 2.04$	$67.11^{a} \pm 1.47$	$P = 0.016$
Backfat thickness (mm)	$18.70^{b} \pm 4.09$	$17.63^{ab} \pm 2.99$	$14.70^{a} \pm 2.19$	$17.00^{ab} \pm 3.88$	$17.71^{ab} \pm 2.72$	$P = 0.048$
Eye muscle thickness (mm)	54.08 ± 7.50	55.90 ± 8.92	58.00 ± 5.64	54.22 ± 5.84	50.73 ± 9.40	$P = 0.221$
Conformation score	3.42 ± 0.51	3.17 ± 0.39	3.18 ± 0.39	3.25 ± 0.45	3.08 ± 0.29	$P = 0.366$

Means with different superscripts in the same row differ significantly

Table 4.7: South African classes for pork carcasses. (SAMIC, 2015).

Class	Calculated percentage meat of carcass (%)	Fat thickness measures by means of instruments (mm)
P	70 and more	At least 1 but not more than 12
O	At least 68 but not more than 69	More than 12 but not more than 17
R	At least 66 but not more than 67	More than 17 but not more than 22
C	At least 64 but not more than 65	More than 22 but no more than 27
U	At least 62 but not more than 63	More than 27 but no more than 32
S	61 and less	More than 32

Table 4.8: Percentage of pig carcasses in different yield classes according to treatment group.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil
P	0.0	0.0	8.3	8.3	0.0
O	50.0	50.0	83.4	41.7	41.7
P+O	50.0	50.0	91.7	50.0	41.7
R	25.0	41.7	8.3	41.7	58.3
C	25.0	8.3	0.0	8.3	0.0

of the carcasses from the *Echium* oil treatment were R classification (58.3%), while it had the fewest number of carcasses in the P+O classification (41.7%) when compared to the other. treatments (Table 4.8). The soya and fish oil treatments had 41.7% pigs in the R classification, followed by the control with 25% and the linseed treatment had the least with 8.3% (Table 4.8)

There were no significant difference between treatments for eye muscle thickness and the conformation score. The average conformation score of the treatments was a 3, which is a medium conformation.

4.4. Backfat quality

4.4.1. Physical and chemical properties of backfat

Changing the fatty acid composition of subcutaneous adipose tissue using different dietary lipid sources also changes the lipid melting point and fat firmness. Fat firmness forms part of the definition for good quality fat (Wood, 1984) and is especially important for the use in processed meat products. There was a mild positive correlation between backfat thickness and fat hardness / firmness (r = 0.4251) as illustrated in Table 4.9. The linseed treatment recorded a lower value for BFT than the control (Table 4.6). Wood et al (1989) also found that there is a strong inverse relationship between the BFT of pork and the consentration of C18:2 in the tissue. Results of the current study also recorded an inverse relationship r = -0.4274 between the BFT and the C18:0 concentration in the fat (Table 4.9). From the present results it seem that the C18:0 concentration had the strongest correlation with fat hardness (r = 0.5083). Pork fat becomes increasingly soft when pigs are fed unsaturated fats/oils (Lee & Li, 2011) and feeds high in unsaturated fatty acids (Madden, Jakobsen, & Mortensen, 1992). Even though the differences were not statistically significant, the four experimental treatments had lower scores for fat hardness as illustrated in Figure 4.1 while there was a negative relationship (r = -0.4215) between fat hardness and total PUFA concentration (Table 4.9).

Table 4.10 indicates that there were no significant differences between treatments for the percentage extractible fat content (EFC), free fat dry matter (FFDM) or the percentage moisture in the backfat of the pork. Prabucki (1991) proposed an EFC of at least 84% for good quality fat since the EFC is of economic importance for the production of lard (Barton-Gade, 1983). Results of the

Table 4.9: Pearson correlation analysis between backfat firmness and selected fat quality parameters.

	Fat hardness	**Backfat thickness (mm)**	**Extractable fat content (%)**	**C16:0 (%)**	**C18:0 (%)**	**C18:2 (%)**	**SFA (%)**	**PUFA (%)**	**UFA (%)**	**DBI**	**Iodine value**
Fat hardness	1	0.4251**	0.3512*	0.2718NS	0.5083***	-0.3203*	0.4452**	-0.4215**	-0.4454**	-0.4917***	-0.4928***
Backfat thickness (mm)		1	0.4276**	0.4000**	0.2120NS	-0.4274**	0.3605*	-0.4562***	-0.3605*	-0.4627***	-0.4625***
Extractable fat content (%)			1	0.3104*	0.2878*	-0.3328*	0.3471*	-0.3153*	-0.3469*	-0.3770**	-0.3777**
C16:0 (%)				1	0.3411*	-0.6171***	0.8479***	-0.7216***	-0.8478***	-0.8269***	-0.8277***
C18:0 (%)					1	-0.4973***	0.7843***	-0.4493**	-0.7844***	-0.5100***	-0.5367***
C18:2 (%)						1	-0.6693***	0.8835***	0.6694***	0.7403***	0.7594***
SFA (%)							1	-0.7150***	-1.0000***	-0.8199***	-0.8359***
PUFA (%)								1	0.7151***	0.9357***	0.9421***
UFA (%)									1	0.8200***	0.8359***
DBI										1	0.9990***
Iodine value											1

NS = Not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

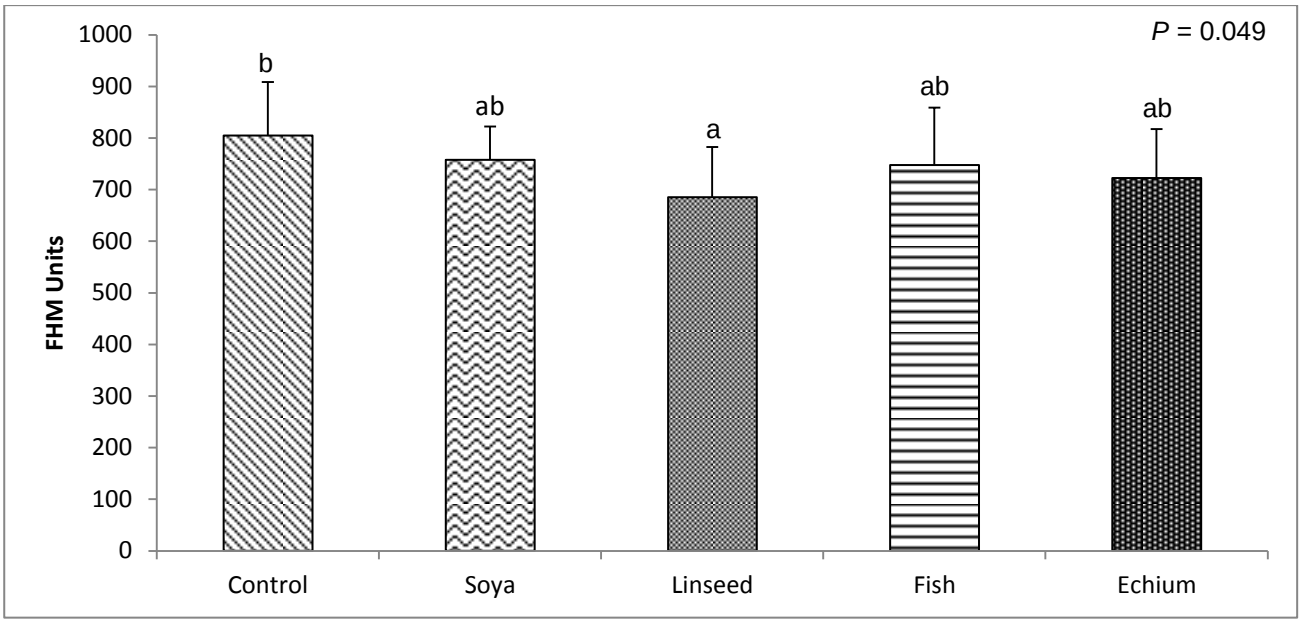

Figure 4.1: Fat hardness op pork backfat from the experimental treatments. Bars with different superscripts differ significantly.

present study indicate that none of the treatments conformed to this criteria, suggesting the processing potential of backfat was less than optimal.

Iodine value (IV) has emerged as the single most accepted method to evaluate pork fat quality. It was originally assessed by a titration method that measured the unsaturation of the fat by reacting it with iodine. Today, it is usually performed by measuring the fatty acid profile (gas chromatograph) of the fat and calculating the IV from various fatty acids (AOCS, 1998). This method has gained acceptance because it is objective, repeatable, and provides more information about the fatty acids present than the titration method. The greater the iodine value, the more unsaturated the fat is and the higher the susceptibility to oxidation. Iodine value is not only an index for rancidity but also for fat hardness and the IV should be less than 70 for good quality fat(Barton-Gade, 1983). Only the control and fish oil treatments conform to this parameter.

Consumers regard colour as a quality attribute and an indicator for eating quality, freshness and meat safety (Glitsch, 2000a, b). The absence of a dietary effect on all colour measurements (L*, a*, b*, chroma and hue) on pork backfat (Table 4.10) are in agreement with the findings of Screeder, Gläser, Eichenberger and Wenk (2000), Nüernberg et al., (2005) and Haak et al., (2008).

4.4.2 Fatty acid composition of backfat

Consumer trends indicate a shift towards wellness and therefore the meat industry is increasingly directing its efforts to meet consumer demands for healthier pork. Strategies to produce healthier meat include reducing the amount of saturated fats in meat and meat products

Table 4.10: Physical and chemical characteristics of backfat of importance in the manufacturing of processed meat products.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Physical properties:						
Colour L* - Value	71.31 ± 3.47	70.33 ± 3.77	67.89 ± 4.13	69.90 ± 2.87	69.65 ± 2.42	*P* = 0.263
Colour a* - Value	7.51 ± 0.68	8.95 ± 1.26	8.87 ± 1.55	8.90 ± 1.30	8.86 ± 1.51	*P* = 0.075
Colour b* - Value	9.73 ± 0.95	10.74 ± 1.20	11.04 ± 1.67	10.94 ± 1.19	10.56 ± 1.44	*P* = 0.196
Chroma	12.31 ± 1.02	14.00 ± 1.65	14.00 ± 2.03	13.81 ± 2.04	13.82 ± 1.85	*P* = 0.172
Hue angle	52.40 ± 2.74	50.36 ± 2.37	50.72 ± 2.97	49.65 ± 3.69	50.05 ± 3.97	*P* = 0.369
Chemical Properties						
Extractable Fat content (%)	79.51 ± 0.48	75.72 ± 3.84	74.74 ± 6.72	72.95 ± 9.89	76.34 ± 2.90	*P* = 0.168
Fat free dry matter (%)	7.22 ± 0.84	7.49 ± 2.07	8.36 ± 2.40	9.20 ± 2.74	8.08 ± 1.69	*P* = 0.237
Moisture (%)	13.27 ± 2.04	16.80 ± 2.68	16.90 ± 4.51	17.85 ± 7.42	15.58 ± 2.32	*P* = 0.169
Iodine value	64.27^{a} ± 1.84	71.22^{bc} ± 3.63	75.27^{d} ± 3.55	69.23^{b} ± 3.41	74.47^{cd} ± 2.89	*P* < 0.001

Means with different superscripts in the same row differ significantly.

and producing leaner pigs with an increased PUFA content (Wood & Enser, 1997). It is well established that the fatty acid profile of pork adipose tissue mirrors that of the dietary fatty acids (Morgan et al., 1992; Warnants et al., 1996; Sheard et al., 2000). Feeding diets high in PUFA resulted in increased levels of PUFA in the backfat of pigs. Backfat fatty acid results presented in Table 4.11 clearly indicate that the soya, linseed and *Echium* oil treatments had a significantly (P < 0.001) higher levels of PUFA than the control and fish oil treatments. Adding *Echium* oil to the diets resulted in a significant (P < 0.001) increased the levels of C18:4c6,9,12,15 (SDA). It also resulted in significantly higher levels of EPA (P < 0.001) and DPA (P < 0.001) when compared to the control, soya and linseed treatments. It was however unable to increase the EPA and DPA concentrations to the same level as the fish oil treatment (Table 4.11). *Echium* oil supplementation had no effect on the DHA levels of backfat (Table 4.11). This result agreed with Whelan (2009), who found that the only way to increase tissue levels of DHA is by dietary supplementation of preformed DHA. The fish oil treatment had the highest levels of total long chain n-3 FA followed by the *Echium* and linseed oil treatments.

Various fatty acid ratios are used to evaluate the health and nutritional properties of the lipid component of meat (Varela et al., 2004). The PUFA: SFA ratio is commonly used to calculate the risk factor of foods with regard to increase in blood cholesterol. Therefore, due to reasons of human health there is interest in trying to increase the proportion of PUFA to SFA. Ulbricht and Southgate (1991) suggested that the ratio of PUFA:SFA should be increased to above 0.4. Both the control and the fish oil treatments were unable to increase the PUFA:SFA ratio to above this value, but all other treatments had higher ratios than 0.4. Another important ratio is the n-6:n-3 ratio. Replacing SFA with UFA increased the intake of n-6 FA (Higgs & Mulvihill, 2002). This ratio is especially important in relation to the incidence of cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser, 2001). The recommended ratio is less than 4:1 (Simopoulos 1996; Scollan et al., 2001). Most Western diets can have a ratio of 10:1 or up to 20:1. From Table 4.11 it is evident that only the linseed and *Echium* oil treatments had a n-6:n-3 ratio of below 4:1.

In 2007 a UK workshop which analysed the usefulness of the n-6/n-3 ratio in understanding n-3 metabolism or addressing cardiovascular risk concluded that the focus should be on the absolute amount rather than the ratio of n-6:n-3 (Stanley, et al., 2007). Even though there is no official recommended daily allowence (RDA) for n-3 fatty acids, most organizations recommend a minimum of 250-500 mg combined EPA and DHA each day for healthy adults. Results in Table 4.12 indicate the actual n-3 fatty acid content of backfat from the five different experimental treatments expressed as mg/100g fat The *Echium* oil treatment had a significantly (P < 0.001) higher level of SDA than all other treatments. The *Echium* oil treatment also had significantly (P < 0.001) higher levels of EPA and DPA when compared to the control, soya and linseed treatments. However, these EPA and DPA concentrations of the *Echium* oil treatment is still significantly lower

Table 4.11: Fatty acid composition and fatty acid ratios of subcutaneous fat of pigs from the different experimental treatments.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Fatty acid composition (%):						
C14:0	1.25^{ab} ± 0.15	1.25^{ab} ± 0.21	1.17^{a} ± 0.13	1.37^{b} ± 0.17	1.18^{a} ± 0.07	*P* = 0.047
C16:0	25.72^{b} ± 0.90	24.26^{ab} ± 1.60	23.32^{a} ± 1.03	24.65^{ab} ± 1.37	23.98^{a} ± 0.76	*P* < 0.001
C18:0	12.90^{ab} ± 1.25	11.68^{a} ± 0.80	12.04^{ab} ± 1.17	13.34^{b} ± 1.37	12.05^{ab} ± 1.42	*P* = 0.022
∑ SFA	40.42^{c} ± 1.57	37.73^{ab} ± 1.90	37.06^{a} ± 1.78	39.84^{bc} ± 2.29	37.68^{ab} ± 2.13	*P* < 0.001
C16:1c9	2.00^{ab} ± 0.39	1.88^{ab} ± 0.36	1.67^{a} ± 0.30	2.25^{b} ± 0.38	1.77^{a} ± 0.26	*P* = 0.004
C18:1c9	38.58^{b} ± 1.29	36.78^{ab} ± 1.24	36.42^{ab} ± 1.76	36.64^{ab} ± 2.40	36.29^{a} ± 1.75	*P* = 0.033
∑ MUFA	46.09^{b} ± 1.20	43.84^{ab} ± 1.19	42.96^{a} ± 2.01	44.89^{ab} ± 2.61	43.35^{a} ± 2.07	*P* = 0.004
C18:2c9,12 (n-6)	11.70^{a} ± 0.70	16.03^{c} ± 2.11	14.71^{bc} ± 1.75	12.27^{a} ± 1.79	13.68^{ab} ± 1.09	*P* < 0.001
C18:3c9,12,15 (n-3)	1.11^{a} ± 0.07	1.52^{b} ± 0.14	4.13^{d} ± 0.52	1.05^{a} ± 0.10	3.57^{c} ± 0.27	*P* < 0.001
C18:4c6,9,12,15(n-3)	0.01^{a} ± 0.01	0.01^{a} ± 0.01^{a}	0.01^{a} ± 0.01	0.06^{b} ± 0.02	0.21^{c} ± 0.05	*P* < 0.001
C20:2c11,14 (n-6)	0.42^{ab} ± 0.03	0.55^{c}± 0.08	0.49^{bc} ± 0.07	0.36^{a} ± 0.06	0.41^{a} ± 0.04	*P* < 0.001
C20:3c11,14,17 (n-3)	0.07^{a} ± 0.01	0.11^{a} ± 0.02	0.38^{c} ± 0.08	0.06^{a} ± 0.01	0.28^{b} ± 0.03	*P* < 0.001
C20:4c5,8,11,14 (n-6)	0.14^{abc} ± 0.02	0.16^{c} ± 0.03	0.12^{ab} ± 0.02	0.11^{a} ± 0.02	0.15^{bc} ± 0.03	*P* < 0.001
C20:5c5,8,11,14,17 (n-3)(EPA)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{ab} ± 0.01	0.47^{c} ± 0.08	0.06^{b} ± 0.02	*P* < 0.001
C22:5c7,10,13,16,19 (n-3)(DPA)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.09^{a} ± 0.02	0.67^{c} ± 0.14	0.18^{b} ± 0.04	*P* < 0.001
C22:6c4,7,10,13,16,19 (n-3)(DHA)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.19^{b} ± 0.08	0.01^{a} ± 0.01	*P* < 0.001
∑ UFA	59.58^{a} ± 1.57	62.27^{bc} ± 1.90	62.94^{c} ± 1.78	60.16^{ab} ± 2.29	62.32^{bc} ± 2.13	*P* = 0.001
∑ PUFA	13.49^{a} ± 0.70	18.43^{b} ± 2.34	19.98^{b} ± 2.35	15.27^{a} ± 2.13	18.97^{b} ± 1.46	*P* < 0.001
∑ *n*-6	12.29^{a} ± 0.71	16.77^{d} ± 2.20	15.37^{cd} ± 1.81	12.77^{ab} ± 1.85	14.67^{bc} ± 1.16	*P* < 0.001
∑ *n*-3	1.20^{a} ± 0.07	1.66^{b} ± 0.16	4.61^{e} ± 0.59	2.45^{c} ± 0.33	4.09^{d} ± 0.33	*P* < 0.001
∑ long chain n-3	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{ab} ± 0.02	1.33^{c} ± 0.26	0.24^{b} ± 0.06	*P* < 0.001
Fatty acid ratios:						
C16:0+C18:0(%)	38.62^{c} ± 1.54	35.94^{ab} ± 1.74	35.36^{a} ± 1.74	37.98^{bc} ± 2.21	36.02^{ab} ± 2.10	*P* = 0.001
C16:1+C18:1/C16:0+C18:0	1.19 ± 0.08	1.22 ± 0.06	1.22 ± 0.09	1.19 ± 0.13	1.20 ± 0.11	*P* = 0.930
C18:0/C18:2	1.11^{b} ± 0.17	0.74^{a} ± 0.15	0.83^{a} ± 0.14	1.11^{b} ± 0.21	0.89^{a} ± 0.14	*P* < 0.001
C16:0/C18:2	2.20^{c} ± 0.16	1.55^{a} ± 0.33	1.60^{a} ± 0.21	2.05^{bc} ± 0.36	1.76^{ab} ± 0.17	*P* < 0.001
C18:2/C18:1	0.27^{a} ± 0.02	0.38^{c} ± 0.06	0.36^{c} ± 0.06	0.29^{ab} ± 0.05	0.33^{bc} ± 0.03	*P* < 0.001
Dienoic(%)	12.15^{a} ± 0.69	16.60^{c} ± 2.18	15.24^{bc} ± 1.80	12.66^{a} ± 1.83	14.13^{ab} ± 1.10	*P* < 0.001
Trienoic(%)	1.17^{a} ± 0.08	1.64^{b} ± 0.16	4.51^{c} ± 0.58	1.12^{a} ± 0.11	4.24^{c} ± 0.32	*P* < 0.001
Tetraenoic(%)	0.14^{a} ± 0.02	0.16^{a} ± 0.03	0.12^{a} ± 0.02	0.16^{a} ± 0.04	0.36^{b} ± 0.07	*P* < 0.001
Pentaenoic(%)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{a} ± 0.02	1.14^{c} ± 0.19	0.24^{b} ± 0.06	*P* < 0.001
Hexaenoic(%)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.19^{b} ± 0.08	0.01^{a} ± 0.01	*P* < 0.001
Penta+Hexaenoic (%)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{ab} ± 0.02	1.33^{c} ± 0.26	0.24^{b} ± 0.06	*P* < 0.001
MUFA/SFA	1.14 ± 0.07	1.16 ± 0.06	1.16 ± 0.09	1.13 ± 0.13	1.16 ± 0.11	*P* = 0.934
DBI	74.59^{a} ± 2.13	82.74^{bc} ± 4.26	87.99^{d} ± 4.19	81.06^{b} ± 4.03	86.96^{cd} ± 3.37	*P* < 0.001
PUFA/SFA	0.33^{a} ± 0.03	0.49^{b} ± 0.08	0.54^{b} ± 0.09	0.39^{a} ± 0.06	0.51^{b} ± 0.06	*P* < 0.001
n-6/*n*-3	10.31^{a} ± 0.93	10.06^{c} ± 0.59	3.34^{a} ± 0.22	5.24^{b} ± 0.53	3.59^{a} ± 0.18	*P* < 0.001

Means with different superscripts in the same row differ significantly.

Table 4.12: Actual omega-3 fatty acid content of backfat of pigs from the different experimental treatments (mg/100 g fat tissue). (n=10 per treatment)

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
C18:3c9,12,15	$861.37^{ab} \pm 93.55$	$1095.30^{b} \pm 121.71$	$2953.41^{c} \pm 422.87$	$734.88^{a} \pm 92.72$	$2732.16^{c} \pm 396.18$	$P < 0.001$
C18:4c6,9,12,15	$1.29^{a} \pm 4.09$	$0.96^{a} \pm 3.04$	$2.95^{a} \pm 4.81$	$38.67^{b} \pm 11.26$	$162.03^{c} \pm 44.52$	$P < 0.001$
C20:3c11,14,17	$50.86^{a} \pm 7.91$	$79.74^{b} \pm 11.58$	$272.47^{d} \pm 59.11$	$44.72^{a} \pm 9.17$	$211.73^{c} \pm 26.72$	$P < 0.001$
C20:5c5,8,11,14,17 (EPA)	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$10.17^{a} \pm 5.68$	$327.86^{c} \pm 58.61$	$45.13^{b} \pm 19.96$	$P < 0.001$
C22:5c7,10,13,16,19 (DPA)	$0.01^{a} \pm 0.01$	$18.16^{ab} \pm 7.99$	$65.20^{b} \pm 14.16$	$469.99^{d} \pm 117.81$	$137.99^{c} \pm 46.07$	$P < 0.001$
C22:6c4,7,10,13,16,19 (DHA)	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$145.38^{b} \pm 34.76$	$0.01^{a} \pm 0.01$	$P < 0.001$
Total n-3 fatty acids	$913.52^{a} \pm 101.29$	$1194.16^{a} \pm 134.28$	$3304.21^{c} \pm 482.27$	$1761.50^{b} \pm 289.80$	$3289.04^{c} \pm 504.58$	$P < 0.001$
EPA+DPA+DHA	$0.01^{a} \pm 0.01$	$18.16^{a} \pm 7.99$	$75.37^{a} \pm 16.18$	$943.22^{c} \pm 196.81$	$183.12^{b} \pm 64.90$	$P < 0.001$

Means with different superscripts in the same row differ significantly.

($P < 0.001$) than that of the fish oil treatment. Only the fish oil treatment was able to increase the levels of DHA in backfat. This resulted in the fish oil treatment having the highest concentration ($P < 0.001$) of EPA+DPA+DHA. The linseed and *Echium* oil treatments had significantly ($P < 0.001$) higher levels of total n-3 fatty acids than all other treatments. This agrees with the finding of Kitessa et al. (2012) who found that *Echium* oil was not superior to linseed oil in increasing the LC n-3 PUFA. However in our study, *Echium* oil was more effective than linseed oil in increasing the sought after LC n-3 FA and the levels of EPA+DPA+DHA was significantly ($P < 0.001$) higher than that of the linseed treatment.

The healthiness of pork fat is not as important as that of meat because most consumers will remove all visible fat. However, processed meat products contain a lot of pork backfat. At least 30% of the raw material of salami is pork backfat so it is possible to calculate the percentage contribution of total LC n-3 FA of a 28g portion of salami from the different dietary treatments to the 500mg recommendation. Table 4.13 indicates that if you were to consume a portion (28g) of salami from the linseed and *Echium* treatment it will contribute 41.0 and 41.1% respectively to your 500mg recommendation for LC n-3 PUFA. On the other hand salami from the fish oil treatment will contribute the most (12.6%) to the concentration of EPA+DPA+DHA at 12.6%, followed by the *Echium* treatment (2.8%). The improvement of health characteristics of fat is usually accompanied by the deterioration in the technological quality of fat (Hugo & Roodt, 2007). Pork backfat is the main fat used in the manufacturing of processed meat products. Dry fermented sausages, like salami, have a fat content of around 32%, and after drying, the fat content increase to about 40-50% (Wirth, 1988). It is therefore clear that the quality of fat used in processed meat products is of utmost importance as it contributes to several quality attributes. Fat firmness is the most important technological property of fat (Wood, 1984) and is positively correlated with individual FA as well as FA ratios. Over the years various authors have set quality parameters that would result in good quality fat for further processing. Backfat of good technological quality should have a C18:0 content of more than 12% and C18:2 content between 12-15% (Lizardo et al., 2002). All treatments except the soya treatment were able to conform to the C18:0 thresholds. The linseed, fish and *Echium* oil treatments fell within the parameters for good quality fat with regard to C18:2. However the control was lower and the soya oil treatment higher than the set parameters. Backfat with a higher SFA content will usually be firmer and of better technological quality than backfat with a higher UFA concentration. Ultimately, for technological quality purposes the SFA content should be more than 41% (Häuser & Prabucki , 1990), a UFA content less than 59% (Prabucki, 1991), MUFA less than 57% (Lizardo et al., 2002) and PUFA less than 15% (Houben & Krol, 1983). Results (Table 4.11) from this study indicate that none of the treatments could conform to these specific SFA or UFA parameters regarding technological quality. All the treatments had a MUFA concentration of less than 57% but only the control had a PUFA content of less than 15%. According to Prabucki

Table 4.13: Percentage contribution of consumption of a 28 g portion of salami from the different treatments to the 500 mg recommendation for omega-3 fatty acids.

Treatment	Total n-3 consumed as % of 500 mg recommendation	Total EPA+DPA+DHA consumed as % of 500 mg recommendation
Control	11.6	0.1
Soya oil	15.0	0.4
Linseed oil	41.0	1.3
Fish oil	22.8	12.6
***Echium* oil**	41.1	2.8

(1991) good quality backfat should have a DBI of less than 80, again only the control was able to conform to this quality parameter.

The ratios of C16:0 + C18:0, C16:0/C18:2 and C16:1 + C18:1/C16:0 + C18:0 were all correlated with fat firmness and used as an indicator of fat firmness (Lea et al., 1970; Enser, 1984; Cameron et al., 1990). There was no significant difference (P = 0.930) between treatments for the C16:1 + C18:1/C16:0 + C18:0 ratio. The control diet had the highest ratio for both the C16:0 + C18:0 (P = 0.001) and C16:0/C18:2 (P < 0.001) ratios. For backfat of good quality, the dienoic fatty acid (PUFA with 2 double bonds) content should be less than 10%, trienoic fatty acid (PUFA with 3 double bonds) content less than 1%, tetraenoic fatty acid (PUFA with 4 double bonds) content less than 0.5% and pentanoic + hexaenoic fatty acid (PUFA with 5 and 6 double bonds) content less than 1 % (Häuser & Prabucki, 1990). From Table 4.11 it is evident that all the treatments had a tetraenoic fatty acid content of less than 0.5%. Only the fish oil treatment was unable to conform to the pentanoic + hexaenoic fatty acid parameter due to the high levels of EPA and DHA.

4.5. Belly fat quality

4.5.1. Physical and chemical properties of belly fat

Pork belly accounts for approximately 18% by weight of the entire carcass, with a very high proportion (approximately 40–50%) of fat (Pulkrábek, Pavlík, Valis, & Vítek, 2006). In a study by Lee and co-workers (2018), the fat volume of a whole pork belly was more than the meat volume. The Pork Chain Quality Audit in the USA (Cannon et al., 1996) concluded that 10% of bellies were too thin for bacon production and an additional 2% were too soft/ oily to be used in bacon manufacturing.

Even though the values were a bit lower than that of the backfat (Figure 4.1) the fat hardness of belly fat (Figure 4.2) followed a similar trend. The linseed treatment had the least firm fat and differed significantly (P = 0.024) from the control treatment which are in agreement with the results of Madden et al. (1992) who reported that higher levels of PUFA result in softer fat. As in the case of backfat (Table 4.10), all colour measurements (L*, a*, b*, chroma or hue angle), EFC, FFDM and moisture was not affected by dietary treatment (Table 4.14). The EFC of all treatments were below the recommended value of 84% (Prabucki 1991). However, the iodine value was influenced by dietary treatment (P < 0.001) with the linseed recording the highest and the control treatment the lowest IV. Barton-Gade (1983) recommended an IV of less than 70 for good fat quality, while it is clear that both the linseed and *Echium* oil treatments recorded IV of slightly higher than 70.

4.5.2. Belly fat fatty acid composition

Pork bellies are mainly used for the manufacturing of streaky bacon and since bellies contain approximately 40-50% fat, the quality of the fat is of utmost importance. It is well know that

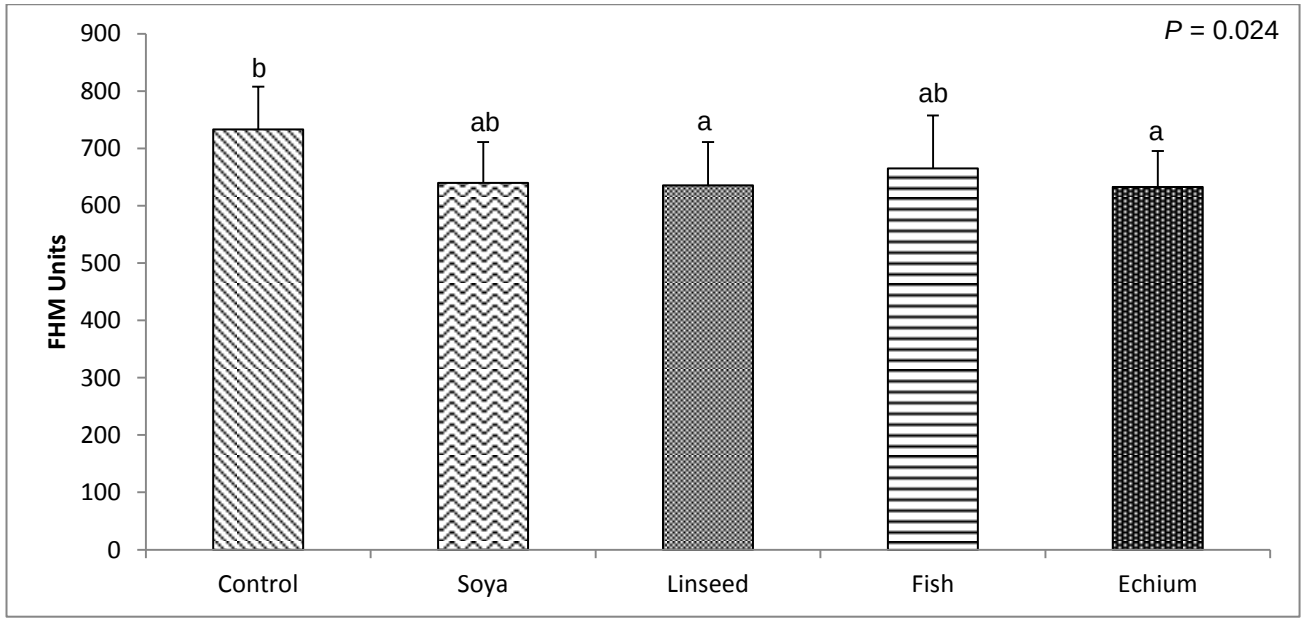

Figure 4.2: Fat hardness of belly fat from the experimental treatments. Bars with different superscripts differ significantly

Table 4.14: Physical and chemical characteristics of belly fat of importance in the manufacturing of processed meat products.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Physical properties:						
Colour L* - Value	74.56 ± 2.86	74.20 ± 3.12	71.97 ± 4.13	72.65 ± 3.87	74.31 ± 2.78	*P* = 0.343
Colour a* - Value	5.15 ± 1.13	5.22 ± 1.37	6.10 ± 1.48	6.58 ± 1.67	5.47 ± 1.03	*P* = 0.103
Colour b* - Value	7.32 ± 2.22	7.14 ± 2.59	8.10 ± 2.72	8.86 ± 2.57	7.57 ± 1.82	*P* = 0.511
Chroma	8.98 ± 2.40	9.27 ± 3.50	10.17 ± 3.03	11.06 ± 2.99	9.36 ± 2.02	*P* = 0.475
Hue angle	54.12 ± 4.47	50.86 ± 3.52	52.02 ± 4.57	53.15 ± 3.90	53.79 ± 3.60	*P* = 0.364
Chemical Properties						
Extractable Fat content (%)	71.89 ± 3.49	72.19 ± 6.36	71.69 ± 4.08	72.98 ± 4.00	72.37 ± 3.11	*P* = 0.971
Fat free dry matter (%)	9.13 ± 1.05	8.77 ± 1.12	10.29 ± 1.93	10.07 ± 2.34	9.27 ± 1.64	*P* = 0.230
Moisture (%)	18.98 ± 3.03	19.04 ± 6.04	18.03 ± 3.22	16.95 ± 3.17	18.36 ± 2.95	*P* = 0.746
Iodine value	63.88^{a} ± 2.07	68.32^{bc} ± 2.29	72.13^{c} ± 5.73	67.71^{ab} ± 2.95	70.95^{bc} ± 3.00	*P* < 0.001

Means with different superscripts in the same row differ significantly

soft pork fat leads to poorer slicing of bacon (Wood, 1984). Therefore, as in the case of backfat, the firmness of belly fat is one of the main quality parameters. A more SFA profile will have better technological properties (Gandemer 2002), however this will result in deterioration of the health properties for consumers. A SFA content of more than 41% and UFA content of less than 59% is advised for good quality belly fat (Häuser & Prabucki, 1990; Prabucki et al., 1991). It is evident from Table 4.15 that none of the dietary treatments conformed to either the SFA or UFA parameters. The double bond index (DBI) should be less than 80 for good quality fat, while it is evident (Table 4.15) that both the linseed and *Echium* oil treatments had a DBI of more than 80. For the manufacturing of good quality bacon, Enser and co-workers (1983) reported that the C18:2 content should be less than 15% and the C18:0/C18:2 ratio should be less than 1.47. All of the dietary treatments (Table 4.15) had a C18:2 content of less than 15% and a C18:0/C18:2 of less than 1.47. The C18:0 content for good quality fat should be more than 12% (Lizardo et al., 2002), with only the fish oil treatment that was able to conform to this criteria.

There were no significant differences between treatments for either the C16:0+C18:0 (P = 0.246) or C16:1+C18:1/C16:0+C18:0 (P = 0.395). As in the case of the fatty acids from the backfat, good quality belly fat should have a dienoic fatty acid content of less than 10%, trienoic fatty acid content of less than 1%, tetraenoic fatty acid content of less than 0.5% and pentanoic + hexaenoic fatty acid content of less than 1 % (Häuser & Prabucki., 1990). None of the treatments could conform to the denoic and trienoic fatty acid parameters. As in the case of backfat, all treatments had a tetraenoic fatty acid content of less than 0.5. The fish oil treatment was unable to conform to the pentanoic + hexaenoic fatty acid concentration due to the high levels of EPA and DHA. From a health point of view, the PUFA:SFA ratio should be increased to above 0.4 (Ulbricht & Southgate, 1991). Both the control and the fish oil treatments were unable to increase the PUFA:SFA ratio to above this value. Replacing SFA with UFA in the diet increases the intake of n-6 FA (Higgs & Mulvihill, 2002). Simopoulos (1996) and Scollan et al., (2001) recommended that the ratio of n-6:n-3 should be reduced to less than 4:1. Only the linseed and *Echium* oil treatments had a n-6:n-3 ratio of less than the recommended ratio of 4:1. It was recommended by Stanley and co-workers (2007) that the primary focus on the absolute amount of n-3 FA rather than the ratio of n-6:n-3. The amount of LC n-3 PUFA in belly fat is important as it contributes to the recommended amount of LC n-3 PUFA when streaky bacon is consumed. The *Echium* oil treatment had a significantly higher (P < 0.001) level of SDA in the belly fat than all other treatments (Table 4.16). It also had significantly higher (P < 0.001) levels of EPA and DPA when compared to the control, soya and linseed treatments. However, the *Echium* oil treatment again had no effect on the levels of DHA (Table 4.16). The total n-3 FA was highest in the linseed treatment followed by the *Echium* oil treatment (Table 4.17). The levels of EPA+DPA+DHA was highest in the fish oil treatment, it was significantly higher (P < 0.001) than the levels of the *Echium* oil treatment. However, the *Echium* oil treatment levels were significantly higher than the control, soya and linseed treatments (Table 4.17).

Table 4.15: Fatty acid composition and fatty acid ratios of belly fat of pigs from the different experimental treatments.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Fatty acid composition (%):						
C14:0	1.37 ± 0.14	1.34 ± 0.20	1.31 ± 0.13	1.42 ± 0.15	1.31 ± 0.10	*P* = 0.400
C16:0	26.06 ± 0.80	25.29 ± 1.28	24.71 ± 1.58	25.26 ± 1.18	25.00 ± 0.90	*P* = 0.141
C18:0	11.30 ± 0.77	11.58 ± 1.26	11.15 ± 0.96	12.76 ± 1.75	11.71 ± 1.48	*P* = 0.064
∑ SFA	39.18 ± 1.44	38.69 ± 1.99	37.62 ± 2.42	39.88 ± 2.74	38.46 ± 2.29	*P* = 0.236
C16:1c9	2.54 ± 0.25	2.21 ± 0.42	2.23 ± 0.27	2.46 ± 0.39	2.20 ± 0.28	*P* = 0.061
C18:1c9	40.09^{b} ± 1.74	37.38^{ab} ± 2.13	36.99^{a} ± 2.31	37.39^{ab} ± 2.79	37.11^{a} ± 2.11	*P* = 0.018
∑ MUFA	49.04^{b} ± 1.97	45.21^{a} ± 2.41	45.05^{a} ± 2.19	46.18^{ab} ± 3.20	45.13^{a} ± 2.91	*P* = 0.005
C18:2c9,12 (n-6)	10.12^{a} ± 1.56	13.92^{c} ± 1.42	13.05^{bc} ± 2.62	11.16^{ab} ± 1.51	12.08^{abc} ± 1.35	*P* < 0.001
C18:3c9,12,15 (n-3)	1.06^{a} ± 0.06	1.40^{a} ± 0.09	3.39^{b} ± 0.84	1.02^{a} ± 0.08	2.90^{b} ± 0.46	*P* < 0.001
C18:4c6,9,12,15(n-3)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.04^{b} ± 0.02	0.17^{c} ± 0.06	*P* < 0.001
C20:2c11,14 (n-6)	0.34^{a} ± 0.03	0.46^{b} ± 0.05	0.42^{b} ± 0.08	0.32^{a} ± 0.04	0.34^{a} ± 0.04	*P* < 0.001
C20:3c11,14,17 (n-3)	0.05^{a} ± 0.01	0.09^{a} ± 0.01	0.29^{c} ± 0.08	0.06^{a} ± 0.01	0.20^{b} ± 0.03	*P* < 0.001
C20:4c5,8,11,14 (n-6)	0.15^{ab} ± 0.02	0.17^{b} ± 0.03	0.14^{a} ± 0.02	0.12^{a} ± 0.02	0.17^{b} ± 0.02	*P* < 0.001
C20:5c5,8,11,14,17 (n-3) (EPA)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.42^{b} ± 0.10	0.05^{a} ± 0.02	*P* < 0.001
C22:5c7,10,13,16,19 (n-3) (DPA)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.09^{a} ± 0.02	0.58^{c} ± 0.11	0.17^{b} ± 0.04	*P* < 0.001
C22:6c4,7,10,13,16,19 (n-3) (DHA)	0.01a ± 0.01	0.01a ± 0.01	0.01a ± 0.01	0.18b ± 0.08	0.01a ± 0.01	*P* < 0.001
∑ UFA	60.82 ± 1.43	61.31 ± 1.98	62.46 ± 2.50	60.11 ± 2.74	61.54 ± 2.29	*P* = 0.213
∑ PUFA	11.77^{a} ± 1.63	16.09^{bc} ± 1.52	17.42^{c} ± 3.62	13.93^{ab} ± 1.77	16.40^{bc} ± 1.96	*P* < 0.001
∑ *n*-6	10.63^{a} ± 1.60	14.58^{c} ± 1.45	13.64^{bc} ± 2.71	11.62^{ab} ± 1.55	12.91^{abc} ± 1.43	*P* < 0.001
∑ *n*-3	1.14^{a} ± 0.06	1.51^{a} ± 0.10	3.78^{c} ± 0.94	2.27^{b} ± 0.27	3.33^{c} ± 0.52	*P* < 0.001
∑ long chain n-3	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{ab} ± 0.03	1.18^{c} ± 0.24	0.22^{b} ± 0.05	*P* < 0.001
Fatty acid ratios (%):						
C16:0+C18:0(%)	37.37 ± 1.38	36.87 ± 1.89	35.86 ± 2.35	38.02 ± 2.64	36.71 ± 2.18	*P* = 0.246
C16:1+C18:1/C16:0+C18:0	1.31 ± 0.09	1.23 ± 0.11	1.26 ± 0.09	1.22 ± 0.15	1.23 ± 0.13	*P* = 0.395
C18:0/C18:2	1.14^{b} ± 0.22	0.84^{a} ± 0.12	0.90^{ab} ± 0.28	1.16^{b} ± 0.22	0.98^{ab} ± 0.16	*P* = 0.002
C16:0/C18:2	2.63^{b} ± 0.43	1.83^{a} ± 0.25	1.98^{a} ± 0.53	2.30^{ab} ± 0.35	2.09^{a} ± 0.28	*P* < 0.001
C18:2/C18:1	0.22^{a} ± 0.04	0.33^{c} ± 0.05	0.31^{bc} ± 0.07	0.26^{ab} ± 0.04	0.29^{bc} ± 0.04	*P* < 0.001
Dienoic(%)	10.49^{a} ± 1.59	14.41^{c} ± 1.45	13.50^{bc} ± 2.70	11.50^{ab} ± 1.53	12.43^{abc} ± 1.36	*P* < 0.001
Trienoic(%)	1.11^{a} ± 0.06	1.49^{a} ± 0.10	3.69^{b} ± 0.91	1.09^{a} ± 0.10	3.41^{b} ± 0.55	*P* < 0.001
Tetraenoic(%)	0.15^{a} ± 0.02	0.17^{a} ± 0.03	0.14^{a} ± 0.02	0.16^{a} ± 0.03	0.34^{b} ± 0.06	*P* < 0.001
Pentaenoic(%)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{a} ± 0.03	1.00^{c} ± 0.19	0.22^{b} ± 0.05	*P* < 0.001
Hexaenoic(%)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.18^{b} ± 0.08	0.01^{a} ± 0.01	*P* < 0.001
Penta+Hexaenoic (%)	0.03^{a} ± 0.01	0.03^{a} ± 0.01	0.10^{ab} ± 0.03	1.18^{c} ± 0.24	0.22^{b} ± 0.05	*P* < 0.001
MUFA/SFA	1.25 ± 0.09	1.17 ± 0.11	1.20 ± 0.08	1.17 ± 0.15	1.18 ± 0.13	*P* = 0.425
DBI	74.08^{a} ± 2.39	79.31^{b} ± 2.68	84.14^{b} ± 6.79	79.18^{ab} ± 3.44	82.69^{b} ± 3.56	*P* < 0.001
PUFA/SFA	0.30^{a} ± 0.04	0.42^{bc} ± 0.05	0.47^{c} ± 0.12	0.35^{ab} ± 0.05	0.43^{bc} ± 0.06	*P* < 0.001
n-6/*n*-3	9.33^{c} ± 1.34	9.62^{c} ± 0.75	3.65^{a} ± 0.28	5.14^{b} ± 0.49	3.91^{a} ± 0.28	*P* < 0.001

Means with different superscripts in the same row differ significantly.

Table 4.16: Actual omega-3 fatty acid content of belly fat of pigs from the different experimental treatments. (n=10 per treatment) (mg/100 g belly fat).

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
C18:3c9,12,15	695.04^{a} ± 59.48	947.53^{a} ± 147.32	2306.39^{c} ± 689.13	691.32^{a} ± 90.87	1898.88^{b} ± 364.82	*P* < 0.001
C18:4c6,9,12,15	5.14^{a} ± 5.61	3.94^{a} ± 5.36	3.77^{a} ± 5.09	29.18^{b} ± 12.39	111.24^{c} ± 40.92	*P* < 0.001
C20:3c11,14,17	35.01^{a} ± 4.49	62.68^{b} ± 11.73	196.58^{d} ± 60.98	39.84^{ab} *± 7.90	133.06^{c} ± 24.23	*P* < 0.001
C20:5c5,8,11,14,17 (EPA)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	6.52^{ab} ± 7.14	286.00^{c} ± 72.01	35.73^{b} ± 13.37	*P* < 0.001
C22:5c7,10,13,16,19 (DPA)	3.71^{a} ± 6.14	18.30^{a} ± 2.44	60.73^{b} ± 18.17	390.75^{d} ± 85.92	109.02^{c} ± 27.83	*P* < 0.001
C22:6c4,7,10,13,16,19 (DHA)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a} ± 0.01	138.85^{b} ± 34.92	0.01^{a} ± 0.01	*P* < 0.001
Total n-3 fatty acids	738.89^{a} ± 59.20	1032.44^{a} ± 160.21	2573.99^{c} ± 765.14	1575.94^{b} ± 250.56	2287.93^{c} ± 447.22	*P* < 0.001
EPA+DPA+DHA	3.71^{a} ± 6.14	18.30^{a} ± 2.44	67.25^{a} ± 23.38	815.60^{c} ± 180.33	144.75^{b} ± 40.09	*P* < 0.001

Means with different superscripts in the same row differ significantly.

Table 4.17: Percentage contribution of consumption of a 40 g portion of two rashers belly bacon from the different treatments to the 500 mg recommendation for omega-3 fatty acids.

Treatment	Total n-3 consumed as % of 500 mg recommendation	Total EPA+DPA+DHA consumed as % of 500 mg recommendation
Control	11.14	0.20
Soya oil	15.32	0.47
Linseed oil	37.75	1.43
Fish oil	24.11	12.97
***Echium* oil**	34.08	2.75

Belly bacon form both the linseed, fish and *Echium* oil treatments can be labelled as an excellent source of total n-3 FA, as they all contribute more than 20% to the recommended daily intake as illustrated in Table 4.17 (US Food and Drug Administration Centre, 2008). It is clear from Table 4.17 that bacon from the fish oil treatment will contribute the most to the recommendation for EPA, DPA and DHA at 12.97% and can therefore be labelled as a source of these LC PUFA (US Food and Drug Administration Centre, 2008).

4.6. Quality of *M. longissimus thoracis*

4.6.1. Physical and chemical properties of *M. longissimus thoracis*

As in the case of backfat (Table 4.9) and belly fat (Table 4.14), none of the colour measurements (L*-, a*-, b*-value, chroma and hue angle) or FFDM and moisture were significantly influenced by dietary oil treatments (Table 4.18). Intramuscular fat (IMF) is important in terms of the sensory quality of pork and has been positively related to flavour, juiciness and tenderness (Fortin, Robertson & Tong, 2005; Schwab, Baas, Stalder & Nettleton, 2009). De Vol and co-workers (1988) suggested that pork should have an IMF of 2 to 3% to ensure desirable palatability. . Only the control and *Echium* oil treatments had IMF content of above 2%, however there were no significant difference for extractable fat between treatments.

One of the ways to evaluate pork is the area of the loin eye muscle (mm2) (Lee et al., 2018). There was no significant difference between dietary treatments for loin eye muscle area, fat area of the chop, meat area of the chop, fat area (%) or meat area (%) (Table 4.18).

Meat with a pH24hours of >6.0 is classified as DFD (Dark, firm and dry) (Adzitey & Nurul, 2011). Normal meat has a pH24hours in the order of 5.5. While it is evident from Table 4.18 that none of the treatment groups could be classified as DFD and there were no significant differences between treatments. When the pH of pork decreases to about 5.4 and the muscle temp45minutes is above 25°C (Cassens, 2000; Adzitey, & Nurul, 2011) and when rigor mortis onset occurs at a pH below 5.9 at a temperature above 35 °C (Cassens, 2000) the PSE (Pale, soft and exudative) syndrome occurs. Even though the $temp_{45minutes}$ was higher than 25°C, none of the pork was classified as PSE. The pH and temperature development, early post mortem, are especially critical for the water holding capacity of pork (Olsson & Pickova, 2005).

Water holding capacity (WHC) is defined as the ability of meat to retain water under external influences such as compression or centrifugation (Swatland, 2000). Therefore, higher values for WHC mean that less moisture was pressed out of the products. WHC is important to the consumer as it influence the tenderness, juiciness, firmness and appearance of meat (Offer & Knight, 1989). A higher WHC will increase the value of pork for use in highly processed pork products (Andersen, 2000). Although statistically significant, WHC did not show a large variation among treatments (Table 4.18; $P = 0.0021$). Drip loss is influenced by the WHC of pork, whereby

Table 4.18: Physical and chemical characteristics of *M. longissimus thoracis* of importance in the manufacturing of processed meat products.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Loin chop composition:						
Eye muscle area (mm²)	4158.81 ± 517.43	4031.10 ± 467.59	4235.34 ± 566.34	3917.56 ± 574.72	3997.01 ± 358.67	*P* = 0.634
Fat area of chop Fat (mm²)	5161.41 ± 924.92	5292.29 ± 1289.01	4052.01 ± 755.90	4630.42 ± 1257.85	4482.70 ± 568.15	*P* = 0.058
Meat area of chop (mm²)	9964.84 ± 1117.85	10742.60 ± 1330.78	9559.91 ± 819.60	10180.34 ± 1249.20	9457.40 ± 1288.02	*P* = 0.121
Fat area %	34.11 ± 4.44	32.69 ± 5.54	29.75 ± 4.11	31.14 ± 6.76	32.33 ± 4.76	*P* = 0.419
Meat area %	65.89 ± 4.44	67.31 ± 5.54	70.25 ± 4.11	68.86 ± 6.76	67.67 ± 4.76	*P* = 0.419
Physical properties:						
$pH_{45\ min}$	6.36^{ab} ± 0.19	6.22^{a} ± 0.28	6.41^{ab} ± 0.25	6.23^{ab} ± 0.19	6.50^{b} ± 0.16	*P* = 0.029
$Temp_{45\ min}$ ˚C	38.03^{b} ± 1.25	36.47^{ab} ± 0.96	35.39^{a} ± 1.87	36.51^{ab} ± 0.94	36.08^{a} ± 1.28	*P* <0.001
$pH_{24\ hours}$	5.41 ± 0.05	5.41 ± 0.04	5.43 ± 0.03	5.39 ± 0.05	5.43 ± 0.05	*P* = 0.081
$Temp_{24hours}$ ˚C	3.36 ± 0.56	3.01 ± 0.56	3.38 ± 0.43	3.25 ± 0.69	3.24 ± 0.49	*P* = 0.325
Drip loss (%)	10.79^{b} ± 1.95	9.23^{ab} ± 1.53	9.42^{ab} ± 2.31	10.94^{b} ± 2.43	8.18^{a} ±2.88	*P* = 0.049
Water Holding Capacity (Meat Area / Total Area)	0.32^{a} ± 0.03	0.32^{ab} ± 0.03	0.36^{b} ± 0.04	0.35^{ab} ± 0.03	0.32^{ab} ± 0.02	*P* = 0.021
Colour L* - Value	55.59 ± 3.66	53.78 ± 2.91	52.17 ± 3.65	53.32 ± 4.43	53.49 ± 3.88	*P* = 0.372
Colour a* - Value	12.24 ± 2.43	11.09 ± 1.74	9.53 ± 1.87	10.58 ± 2.62	11.20 ± 2.96	*P* = 0.159
Colour b* - Value	12.66 ± 2.71	10.95 ± 2.00	9.46 ± 2.38	10.68 ± 2.48	10.87 ± 2.99	*P* = 0.107
Chroma	17.62 ± 3.61	15.60 ± 2.60	13.46 ± 2.92	15.07 ± 3.53	15.63 ± 4.16	*P* = 0.125
Hue angle	45.82 ± 1.68	44.44 ± 2.18	44.30 ± 3.71	45.28 ± 2.69	44.00 ± 2.58	*P* = 0.522
Chemical properties:						
Extractable Fat content (%)	2.05 ± 0.40	1.90 ± 0.49	1.86 ± 0.43	1.82 ± 0.39	2.14 ± 0.34	*P* = 0.397
Fat Free Dry Matter (%)	24.14 ± 0.56	24.88 ± 1.07	24.39 ± 0.94	25.15 ± 1.62	24.36 ± 1.06	*P* = 0.246
Moisture (%)	73.81 ± 0.64	73.22 ± 1.31	73.75 ± 1.16	73.02 ± 1.82	73.50 ± 0.99	*P* = 0.579
Iodine value (Calculated)	68.71^{a} ± 4.14	73.68^{ab} ± 5.36	74.90^{ab} ± 6.92	76.06^{b} ± 5.52	71.21^{ab} ± 4.18	*P* = 0.025

Means with different superscripts in the same row differ significantly.

the *Echium* oil treatment had a significantly lower (P = 0.049) drip loss compared to the control and fish oil treatments.

Iodine value differed significantly (P = 0.025) between treatments with the control treatment having an IV of less than 70, as recommended by Barton-Gade (1983).

4.6.2. Fatty acid composition of IMF from *M. longissimus thoracis*

Although adipose tissue has a much higher fat content than muscle, it seems that the fatty acid composition of the two tissues is broadly similar. However, results in Table 4.19 illustrate that the levels of EPA, DPA and DHA were much higher in the IMF (Table 4.19) than that of backfat (Table 4.11) and belly fat (Table 4.15) of the fish oil treatment. This agrees with the findings of Øverland et al., (1996). Even though statistically significant (P < 0.001), the levels of SDA of the *Echium* oil treatment was only slightly higher than the other treatments (Table 4.19). Nevertheless, the actual SDA content (Table 4.20) of the *Echium* oil treatment, was significantly higher (P < 0.001) than that of the other treatments. The quantitative FAME estimation (Table 4.19) showed no significant difference between the linseed and *Echium* oil treatments for EPA and DPA, although the actual amounts (mg/100g muscle) showed that the *Echium* oil treatment had significantly higher (P < 0.001) levels of both EPA and DPA. From Table 4.20 it is clear that the actual levels of EPA, DPA and DHA were the highest in meat from the fish oil treatment. Adding *Echium* oil to the diets of pigs caused in increase in both the EPA and DPA content of meat. While the *Echium* oil treatment had significantly higher (P < 0.001) actual levels of DHA when compared to the control and soya treatment, it did not differ significantly (P < 0.001) from the linseed treatment (Table 4.20). Unlike the back- and belly fat, where the levels of DHA were unaffected by all dietary treatments (except for the fish oil), all treatments recorded elevated DHA levels in the *M longissimus thoracis*. These levels were still very low and only differed significantly (P < 0.001) from the fish oil treatment (Table 4.19).

Even though supplementing the diets of pigs with *Echium* oil did not result in an increase in the levels of DHA in either the fat or muscle, the enrichment achieved in terms of the SDA and EPA itself may be of some health benefits to consumers. The Japanese Lipid Study (Yokoyama & Origasa, 2003) demonstrated that long-term supplementation of pure EPA, in the absence of supplemental DHA, significantly reduced major coronary events in patients with a history of cardiovascular heart diseases (CHD). In a comparison

Table 4.19: Fatty acid composition and fatty acid ratios of muscle of pigs from the different experimental treatments.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Fatty acid composition (%):						
C14:0	1.20 ± 0.20	1.16 ± 0.26	1.08 ± 0.16	1.19 ± 0.12	1.22 ± 0.11	*P* = 0.465
C16:0	25.16 ± 1.10	24.56 ± 1.31	24.17 ± 0.93	24.82 ± 1.00	25.16 ± 0.65	*P* = 0.165
C18:0	11.31 ± 0.92	11.34 ± 0.92	11.45 ± 0.73	11.74 ± 0.86	11.38 ± 1.06	*P* = 0.827
∑ SFA	38.09 ± 1.85	37.47 ± 1.40	37.08 ± 1.51	38.10 ± 1.30	38.17 ± 1.56	*P* = 0.419
C16:1c9	3.31 ± 0.37	3.04 ± 0.65	2.88 ± 0.33	2.95 ± 0.50	3.08 ± 0.27	*P* = 0.277
C18:1c9	38.06 ± 1.72	35.68 ± 2.00	36.33 ± 3.18	35.69 ± 2.50	37.23 ± 1.78	*P* = 0.108
∑ MUFA	48.85 ± 1.70	45.81 ± 2.76	46.24 ± 3.73	45.82 ± 3.02	47.74 ± 1.93	*P* = 0.059
C18:2c9,12 (n-6)	9.89^{ab} ± 1.51	12.61^{b} ± 2.64	11.71^{ab} ± 2.97	10.94^{ab} ± 2.17	9.61^{a} ± 1.50	*P* = 0.023
C18:3c9,12,15 (n-3)	0.75^{ab} ± 0.09	0.85^{b} ± 0.08	1.57^{d} ± 0.15	0.70^{a} ± 0.07	1.35^{c} ± 0.12	*P* < 0.001
C18:4c6,9,12,15(n-3)	0.01^{a} ± 0.01	0.01^{a} ± 0.01	0.01^{a}± 0.01	0.01^{a} ± 0.01	0.03^{b} ± 0.02	*P* < 0.001
C20:2c11,14 (n-6)	0.22 ± 0.03	0.25 ± 0.06	0.24 ± 0.04	0.21 ± 0.03	0.22 ± 0.02	*P* = 0.138
C20:3c11,14,17 (n-3)	0.03^{a} ± 0.01	0.05^{a} ± 0.03	0.16^{b} ± 0.04	0.03^{a} ± 0.02	0.14^{b} ± 0.02	*P* < 0.001
C20:4c5,8,11,14 (n-6)	1.82 ± 0.61	2.45 ± 0.83	2.04 ± 0.97	1.64 ± 0.54	1.61 ± 0.46	*P* = 0.061
C20:5c5,8,11,14,17 (n-3) (EPA)	0.04^{a} ± 0.02	0.08^{ab} ± 0.03	0.29^{bc} ± 0.15	1.24^{d} ± 0.38	0.33^{c} ± 0.12	*P* < 0.001
C22:5c7,10,13,16,19 (n-3) (DPA)	0.13^{a} ± 0.05	0.22^{a} ± 0.07	0.44^{b} ± 0.21	0.84^{c} ± 0.19	0.47^{b} ± 0.14	*P* < 0.001
C22:6c4,7,10,13,16,19 (n-3) (DHA)	0.03^{a} ± 0.03	0.04^{a} ± 0.03	0.07^{a} ± 0.05	0.39^{b} ± 0.11	0.07^{a} ± 0.04	*P* < 0.001
∑ PUFA	13.06^{a} ± 2.17	16.72^{b} ± 3.54	16.68^{b} ± 4.39	16.08^{ab} ± 3.27	14.09^{a} ± 2.29	*P* = 0.047
∑ *n*-6	12.08^{ab} ± 2.13	15.48^{b} ± 3.49	14.16^{ab} ± 3.96	12.89^{ab} ± 2.70	11.69^{a} ± 1.98	*P* = 0.038
∑ *n*-3	0.98^{a} ± 0.10	1.24^{a} ± 0.08	2.52^{b} ± 0.46	3.19^{c} ± 0.63	2.37^{b} ± 0.32	*P* < 0.001
∑ long chain n-3	0.20^{a} ± 0.10	0.33^{ab} ± 0.12	0.80^{bc} ± 0.38	2.46^{d} ± 0.66	0.87^{c} ± 0.29	*P* < 0.001
Fatty acid ratios (%):						
C16:0+C18:0(%)	36.47 ± 1.69	35.89 ± 1.17	35.62 ± 1.36	36.56 ± 1.18	36.54 ± 1.56	*P* = 0.452
C16:1+C18:1/C16:0+C18:0	1.34 ± 0.09	1.27 ± 0.08	1.29 ± 0.11	1.25 ± 0.10	1.30 ± 0.08	*P* = 0.264
C18:0/C18:2	1.17 ± 0.25	0.93 ± 0.21	1.03 ± 0.25	1.11 ± 0.21	1.22 ± 0.25	*P* = 0.069
C16:0/C18:2	2.61^{b} ± 0.51	2.05^{a} ± 0.53	2.18^{a} ± 0.53	2.36^{ab} ± 0.51	2.68^{b} ± 0.45	*P* = 0.034
C18:2/C18:1	0.22^{a} ± 0.04	0.30^{b} ± 0.08	$0.28b^{b}$ ± 0.10	0.26^{ab} ± 0.07	0.22^{a} ± 0.04	*P* = 0.033
MUFA/SFA	1.29 ± 0.09	1.22 ± 0.07	1.25 ± 0.10	1.20 ± 0.09	1.25 ± 0.08	*P* = 0.266
DBI	80.17^{a} ± 5.01	86.27^{ab} ± 6.53	88.03^{ab} ± 8.51	89.86^{b} ± 6.86	83.63^{ab} ± 5.12	*P* = 0.017
PUFA/SFA	0.35 ± 0.07	0.45 ± 0.11	0.45 ± 0.13	0.42 ± 0.09	0.37 ± 0.07	*P* = 0.063
n-6/*n*-3	12.37^{b} ± 2.11	12.43^{b} ± 2.51	5.55^{a} ± 0.70	4.05^{a} ± 0.41	4.93^{a} ± 0.30	*P* < 0.001

Means with different superscripts in the same row differ significantly.

experiment Harris (2008) evaluated the impact of supplemental ALA, SDA, and EPA on changes to the omega-3 index. The omega-3 index is a clinical biomarker for cardiovascular disease and correlates well with the risk for various cardiovascular disease endpoints. It is calculated as the sum of EPA and DHA in erythrocyte membranes expressed as a percentage of total erythrocyte fatty acids (Harris & Von Schacky, 2004). Harris (2008) found that SDA and EPA were more effective than ALA (wich had no effect) in improving the omega-3 index and that SDA was 17% as effective as EPA.

The label claim categories for 'source' and 'good source' reflect the degree to which a meal contributes to the consumer achieving daily values for a nutrient. Food Standards Australia and New Zealand's cut off points for 'source' and 'good source' for n-3 LC-PUFA are 30 and 60 mg/ serve, respectively (Food Standards Australia New Zealand, 2003). By considering the criteria of the Food Standards Australia and New Zealand (2003) it is possible to categorise the meat from the *Echium*, fish and linseed oil treatments as a source of total n-3 FA (Table 4.20), while the pork meat from the fish oil treatment could additionally be labelled as a source of EPA+DPA+DHA (Table 4.20). Under the US Food and Drug Administration guidelines meals which provide 10 to 19% of the recommended daily intake can be called a 'good source', while those which provide 20% or more can be called 'high', 'rich in' or 'excellent source' (US Food and Drug Administration, 2008).

The typical South African pork chop weighs approximately 180g and is composed of 58% meat, 22% fat, 5% skin and 15% bone. Results of the present study were used to calculate the contribution of a 180g pork chop in terms of the daily 500mg recommendation for LC n-3 PUFA (Table 4.21). It is clear from Table 4.21 that pork chops from the *Echium* and linseed oil treatments will contribute the most to the 500mg recommendation for total n-3 FA of 269.1 mg/180g and 268.8 mg/180g respectively and could therefore be labelled an excellent source of LC n-3 PUFA if enriched according to the current protocol. For the longer chain EPA+DPA+DHA fatty acids, pork chops from the fish oil treatment can be labelled an excellent source as it contributes 81.2% to the 500mg daily LC n-3 PUFA recommendation. Pork chops from the *Echium* oil treatment will contribute 17.5% and can therefore be called a source of the LC n-3 PUFA EPA+DPA+DHA. This level of 17.5% of EPA+DPA+DHA achieved by using *Echium* oil will be a nutritionally significant improvement as part of a normal diet compared to the linseed treatment at 8.0%.

If the meat and fat from this experiment were used to manufacture pork bangers containing 54.81% lean pork and 22.5% backfat the bangers from the linseed and *Echium* oil treatments would contribute 91.47% and 91.50% respectively to the 500mg recommendation (Table 4.22). Pork bangers from all the experimental treatments (soya, linseed, fish and *Echium*) can be labelled as a source of total n-3 PUFA under US Food and Drug Administration guidelines and the linseed, fish and *Echium* treatments can be labelled as an excillent source. Thus, only pork bangers from.

Table 4.20: Actual omega-3 fatty acid content of *M. longissimus thoracis* of pigs from the different experimental treatments. (n=10 per treatment) (mg/100 g muscle).

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
C18:3c9,12,15	$12.46^{a} \pm 4.66$	$12.24^{a} \pm 5.03$	$22.15^{b} \pm 8.00$	$9.53^{a} \pm 3.39$	$23.62^{b} \pm 5.65$	$P < 0.001$
C18:4c6,9,12,15	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$0.59^{b} \pm 0.44$	$P < 0.001$
C20:3c11,14,17	$0.50^{a} \pm 0.33$	$0.81^{a} \pm 0.46$	$2.33^{b} \pm 1.04$	$0.41^{d} \pm 0.29$	$2.44^{c} \pm 0.55$	$P < 0.001$
C20:5c5,8,11,14,17 (EPA)	$0.64^{a} \pm 0.25$	$0.96^{a} \pm 0.19$	$3.50^{b} \pm 0.61$	$15.47^{d} \pm 2.21$	$5.47^{c} \pm 1.50$	$P < 0.001$
C22:5c7,10,13,16,19 (DPA)	$1.94^{a} \pm 0.43$	$2.74^{a} \pm 0.34$	$5.34^{b} \pm 0.80$	$10.67^{d} \pm 1.58$	$7.87^{c} \pm 1.37$	$P < 0.001$
C22:6c4,7,10,13,16,19 (DHA)	$0.33^{a} \pm 0.39$	$0.50^{ab} \pm 0.33$	$0.91^{bc} \pm 0.45$	$4.86^{d} \pm 0.85$	$1.06^{c} \pm 0.56$	$P < 0.001$
Total n-3 fatty acids	$15.87^{a} \pm 4.43$	$17.25^{a} \pm 5.17$	$34.23^{b} \pm 8.84$	$40.94^{c} \pm 6.39$	$41.05^{c} \pm 7.14$	$P < 0.001$
EPA+DPA+DHA	$2.91^{a} \pm 0.98$	$4.20^{a} \pm 0.71$	$9.75^{b} \pm 1.59$	$31.00^{d} \pm 3.97$	$14.40^{c} \pm 3.25$	$P < 0.001$

Means with different superscripts in the same row differ significantly.

Table 4.21: Percentage contribution of consumption of a 180 g pork rib chop from the different treatments to the 500 mg recommendation for omega-3 fatty acids.

Treatment	Total n-3 consumed as % of 500 mg recommendation	Total EPA+DPA+DHA consumed as % of 500 mg recommendation
Control	75.7	0.6
Soya oil	98.2	2.3
Linseed oil	268.8	8.0
Fish oil	148.1	81.2
***Echium* oil**	269.1	17.5

Table 4.22: Percentage contribution of consumption of a 60 g portion of Pork Bangers from the different treatments to the 500 mg recommendation for omega-3 fatty acids.

Treatment	Total n-3 consumed as % of 500 mg recommendation	Total EPA+DPA+DHA consumed as % of 500 mg recommendation
Control	25.71	0.19
Soya oil	33.38	0.77
Linseed oil	91.47	2.68
Fish oil	50.25	27.51
***Echium* oil**	91.50	5.89

the fish oil treatment could be labelled as a source of the LC PUFA, EPA, DPA and DHA as illustrated in Table 4.22

4.7 Sensory and physical properties of *M. longissimus lumborum*

4.7.1 Physical characteristics of *M. longissimus lumborum*

Cooking loss is relevant to the consumer as it determines the final yield of the cooked product and affects the juiciness and tenderness perceptions (Aaslyng, Bejerholm, Ertbjerg, Bertram & Anderson, 2003). According to various consumer studies, drip is one of the most relevant intrinsic cues for consumers to determine pork quality (Steenkamp & van Trijp, 1996; Bredahl & Poulsen, 2002). However, Verbeke and co workers (2005) found that drip loss is not perceived as a major hurdle for the acceptance of pork chops as it is overlooked due to the presence of other more desirable/undesirable attributes. Results in Table 4.23 indicate that there were no significant differences between dietary treatments in terms of cooking, drip, evaporation or thawing loss (Table 4.23). These findings are in agreement with the results of Miller, Shackelford, Hayden, and Reagan (1990) as well as that of Scheeder, Gläser, Eichenberger, and Wenk (2000).

It further seems that dietary oil supplementation did not affect the shear force measurements as illustrated in Figure 4.3. The lack of differences in terms of the extractable fat in muscle between treatments as seen in Table 4.18, may partly explains the similar shear force obtained for these treatments. Current results are in agreement with that of Miller and co-workers (1990) as well as Scheeder and co-workers (2000) who also did not find any effect on shear force when pigs were fed different dietary oil sources.

4.7.1.1 Descriptive sensory analysis of pork *M. longissimus lumborum* and backfat samples

The results for the descriptive sensory analysis on meat from the experimental treatments are shown in Table 4.24. Results indicate that the aroma of the fat and meat was significantly influenced by dietary treatment. The fish oil treatment had the highest scores for fishy ($P < 0.001$) aroma of fat and the lowest scores for roast pork fat ($P < 0.001$). It also had significantly higher ($P = 0.001$) scores for rancid aroma of fat when compared to the control, soya and linseed treatments. The aroma of the meat from the fish oil treatment also had a fishy aroma ($P < 0.001$) and less of a fresh cooked pork meat aroma ($P = 0.011$). The fish oil treatment had significantly higher ($P = 0.049$) scores for rancid/ old when compared to the control.

The trained panellist could not detect a significant difference between dietary oil treatments for the initial impression of juiciness or sustained impression of juiciness. This could be due to the fact that there were also no differences between treatments for cooking-, drip-, evaporation- or thawing losses as recorded previously in Table 4.23. Although the trained sensory panellist found that the meat of the *Echium* oil treatment significantly tougher ($P = 0.005$; $P = 0.009$) when compared to the control diet the results were not supported by the shear force results as illustrated in Figure 4.3.

Table 4.23: Physical characteristics of *M longissimus lumborum* from the experimental treatment groups (n = 10 per treatment)

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Total cooking loss (%)	24.11 ± 3.27	21.47 ± 2.85	21.79 ± 2.64	22.17 ± 0.95	20.62 ± 2.90	*P* = 0.065
Drip loss (%)	8.40 ± 1.29	7.59 ± 1.93	6.53 ± 1.47	7.05 ± 1.57	7.01 ± 1.72	*P* = 0.117
Evaporation loss (%)	15.71 ± 2.80	13.88 ± 2.41	15.26 ± 2.04	16.25 ± 2.08	13.61 ± 2.07	*P* = 0.055
Thawing loss (%)	1.78 ± 1.02	2.09 ± 0.80	2.04 ± 0.83	2.23 ± 0.72	2.37 ± 0.88	*P* = 0.617

Means with different superscripts in the same row differ significantly.

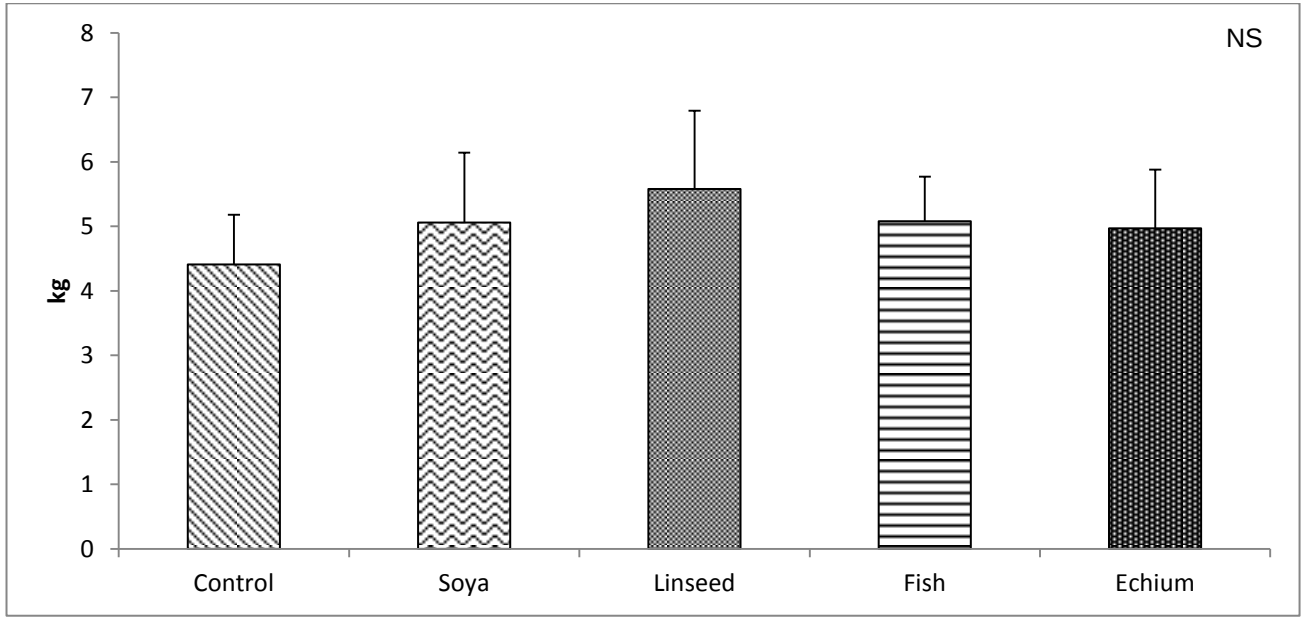

Figure 4.3: Shear force values of *M longissimus lumborum* of the experimental treatments. Bars with different superscripts differ significantly

Table 4.24: Descriptive sensory analysis of pork *M. longissimus lumborum* (pork loin) and fat samples of gilts from the experimental groups (n = 10 per treatment)

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Fat aroma:						
Fresh pork fat	4.98^{b} ± 0.84	5.00^{b} ± 1.06	4.79^{b} ± 0.91	4.30^{a} ± 1.10	4.97^{b} ± 0.87	*P* < 0.001
Roast pork fat (caramel)	5.50^{b} ± 1.35	5.21^{b} ± 1.44	5.07^{b} ± 1.30	4.48^{a} ± 1.48	5.20^{b} ± 1.26	*P* < 0.001
Rancid	1.74^{a} ± 0.71	1.70^{a} ± 0.76	1.77^{a} ± 0.68	2.08^{b} ± 0.72	1.91^{ab} ± 0.75	*P* = 0.001
Fishy	1.12^{a} ± 0.33	1.21^{a} ± 0.50	1.19^{a} ± 0.51	1.99^{b} ± 1.09	1.12^{a} ± 0.41	*P* < 0.001
Sour	1.19 ± 0.46	1.10 ± 0.30	1.17 ± 0.38	1.21 ± 0.46	1.14 ± 0.35	*P* = 0.310
Piggy (old musty)	1.53 ± 0.66	1.61 ± 0.74	1.55 ± 0.61	1.68 ± 0.75	1.65 ± 0.67	*P* = 0.486
Chemical	1.40 ± 0.62	1.35 ± 0.58	1.32 ± 0.55	1.44 ± 0.61	1.30 ± 0.50	*P* = 0.400
Meat Aroma:						
Roast pork meat	3.33 ± 1.03	3.30 ± 0.96	3.45 ± 0.99	3.27 ± 0.93	3.26 ± 0.85	*P* =0.629
Fresh cooked pork meat	5.22^{b} ± 0.91	5.23^{b} ± 0.85	5.17^{ab} ± 0.83	4.87^{a} ± 0.85	5.25^{b} ± 0.93	*P* = 0.011
Rancid / old	1.24^{a} ± 0.51	1.26^{ab} ± 0.58	134^{ab} ± 0.64	1.46^{b} ± 0.69	1.27^{ab} ± 0.45	*P* = 0.049
Sour	1.75 ± 0.64	1.77 ± 0.55	1.72 ± 0.62	1.81 ± 0.56	1.74 ± 0.60	*P* = 0.857
Fishy	1.02^{a} ± 0.14	1.05^{a} ± 0.22	1.07^{a} ± 0.26	1.29^{b} ± 0.54	1.07^{a} ± 0.29	*P* < 0.001
Piggy (old-musty)	1.37 ± 0.61	1.31 ± 0.53	1.34 ± 0.55	1.43 ± 0.67	1.33 ± 0.55	*P* = 0.637
Livery (metallic/bloody)	1.51 ± 0.58	1.49 ± 0.66	1.60 ± 0.60	1.63 ± 0.60	1.54 ± 0.59	*P* = 0.411
Juiciness of meat:						
Initial impression of juiciness	4.05 ± 1.20	4.12 ± 1.28	4.30 ± 1.08	4.00 ± 1.17	4.08 ± 1.25	*P* = 0.453
Meat Texture:						
Tenderness – First bite	4.92^{b} ± 1.12	4.60^{ab} ± 1.19	4.56^{ab} ± 1.31	4.63^{ab} ± 1.27	4.24^{a} ± 1.34	*P* = 0.005
Muscle fibre & overall tenderness – Residue	4.69^{b} ± 1.13	4.47^{ab} ± 1.22	4.26^{ab} ± 1.24	4.48^{ab} ± 1.13	4.11^{a} ± 1.32	*P* =0.009
Sustained impression of juiciness – meat	3.99 ± 1.07	4.01 ± 1.09	4.12 ± 1.10	3.85 ± 0.96	4.03 ± 1.01	*P* = 0.483
Meat Flavour:						
Roast pork meat	3.42 ± 1.02	3.33 ± 0.99	3.42 ± 1.11	3.16 ± 0.93	3.38 ± 1.00	*P* = 0.334
Cooked pork meat	5.36 ± 0.81	5.44 ± 0.87	5.26 ± 0.87	5.12 ± 0.88	5.31 ± 0.84	*P* = 0.099
Rancid / old	1.23^{a} ± 0.42	1.27^{a} ± 0.57	1.35^{ab} ± 0.70	1.52^{b} ± 0.72	1.35^{ab} ± 0.59	*P* = 0.010
Livery (Metallic / bloody)	1.87 ± 0.65	1.85 ± 0.69	1.91 ± 0.70	1.97 ± 0.64	1.99 ± 0.76	*P* = 0.534
Sour	2.43 ± 0.62	2.25 ± 0.72	2.37 ± 0.75	2.44 ± 0.72	2.28 ± 0.71	*P* = 0.201
Fishy	1.04^{a} ± 0.20	1.07^{a} ± 0.29	1.08^{a} ± 0.27	1.57^{b} ± 0.71	1.04^{a} ± 0.20	*P* <0.0001
Piggy (old – musty)	1.34 ± 0.54	1.36 ± 0.63	1.43 ± 0.59	1.52 ± 0.67	1.41 ± 0.59	*P* = 0.247
After Taste:						

Cooked pork meat	$5.04^{b} \pm 0.89$	$4.99^{b} \pm 0.87$	$4.82^{ab} \pm 0.95$	$4.58^{a} \pm 0.85$	$4.92^{ab} \pm 0.90$	$P = 0.003$
Rancid / old	1.23 ± 0.49	1.29 ± 0.66	1.32 ± 0.63	1.39 ± 0.65	1.26 ± 0.50	$P = 0.367$
Sour	2.11 ± 0.67	1.99 ± 0.61	2.01 ± 0.69	2.16 ± 0.66	1.91 ± 0.67	$P = 0.061$
Fishy	$1.04^{a} \pm 0.20$	$1.03^{a} \pm 0.17$	$1.05^{a} \pm 0.22$	$1.45^{b} \pm 0.64$	$1.05^{a} \pm 0.26$	$P < 0.001$
Metallic	1.84 ± 0.58	1.83 ± 0.60	1.86 ± 0.65	1.87 ± 0.61	1.92 ± 0.56	$P = 0.853$

Means with different superscripts in the same row differ significantly.

The fish oil treatment recorded the highest fishy taste ($P < 0.001$) and aftertaste ($P < 0.001$), wich agrees with the findings of Wood and co-workers (1999). Although the *Echium* and linseed oil treatment had higher scores for rancid/ old than the control and soya oil treatments, it did not differ significantly. On the other hand, the fish oil treatment hand had significantly higher ($P = 0.010$) scores for rancid/ old when compared to the control and soya treatments. This is due to the formation of oxidation products in these treatments with higher levels of LC PUFA (Bryhni et al., 2002). The fish oil treatment recorded significantly lower ($P = 0.023$) scores for a cooked pork meat aftertaste when compared to the control and soya treatments

Physical and descriptive sensory data were visualised in a 2-dimensional space by Principle Component Analysis (PCA) (Figure 4.4). The first dimension explained 62.61% and the second dimension 19.47% of the variance in the data. The two dimensions accounted for 82.08% of the total variation in the data. The first dimension divided the five treatments into two groups, with the control, soya, linseed and *Echium* oil treatments on the left side and the fish oil treatment on the right. This dimension indicates that the fish oil treatment can be associated with most of the negative sensory attributes such as fishy and rancid aroma and taste. Whereas the other treatments can be associated with the positive sensory attributes such as fresh cooked pork aroma and roast pork meat taste. The second dimension indicates the difference in tenderness of the meat. It is interesting to note that the fish oil treatment had the most tender meat for first bite as well as overall tenderness despite its negative sensory attributes such as fishy and rancid aroma as well as taste. The linseed and *Echium* oil treatments had the juiciest meat for initial impression as well as sustained impression of juiciness.

4.7.1.2 Consumer sensory analysis of pork *M. longissimus lumborum*

The consumer sensory analysis was carried out by a 100 member, untrained panel. The panel consisted of 72 females and 28 males. A total of 79% of the panellists were in the 20-29 year age group, 11% were under 20 years of age and 15% were between the ages of 30-59 (Table 4.25). Consumers scored the cooked pork on aroma, taste, aftertaste and overall acceptability as attributes (Figure 4.5). Consumers were unable to detect a difference in aroma between the five treatments (Figure 4.5). The consumer panel preferred the taste of the control treatment and scored the fish oil treatment the lowest for taste. The same trend for taste was seen for aftertaste with the control scoring the highest and fish oil treatment the lowest. The consumer panels' scores

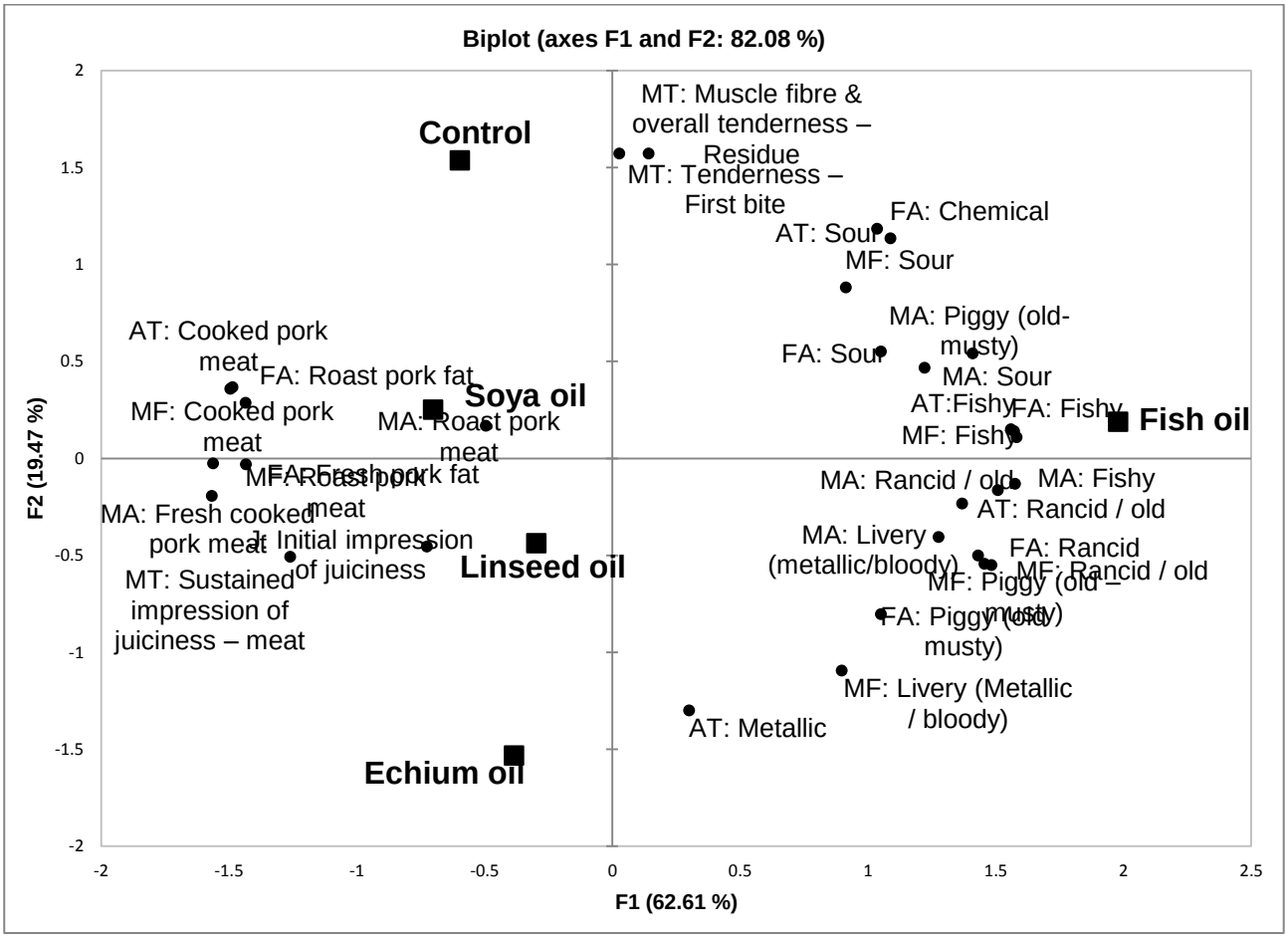

Figure 4.4: Principle Component Analysis of sensory properties of pork affected by different dietary treatments (FA = fat aroma; MA = Meat aroma; J = Juiciness; MT = Meat Texture; MF = Meat flavour; AT = aftertaste)

Table 4.25: Demographic profile of 100 member consumer sensory panel

Gender:	% of Total	Age:	% of Total
Female	72	< 20	11
Male	28	20-29	79
		30-39	3
		40-49	2
		50-59	5

agree with that of the trained panel (Table 4.24). The fishy taste and aftertaste of the meat from the fish oil treatment resulted in the consumers giving the fish oil treatment lower scores (Figure 4.5). For overall acceptability the control had significantly higher (P = 0.022) scores than the fish oil treatments (Figure 4.5).

4.7.2 Histological studies of *M. longissimus lumborum*

During the aging of meat the myofibrillar structure is broken down by calpain (a proteolytic enzyme), therefore, shorter myofibrillar fragment lengths (MFL) are normally associated with a

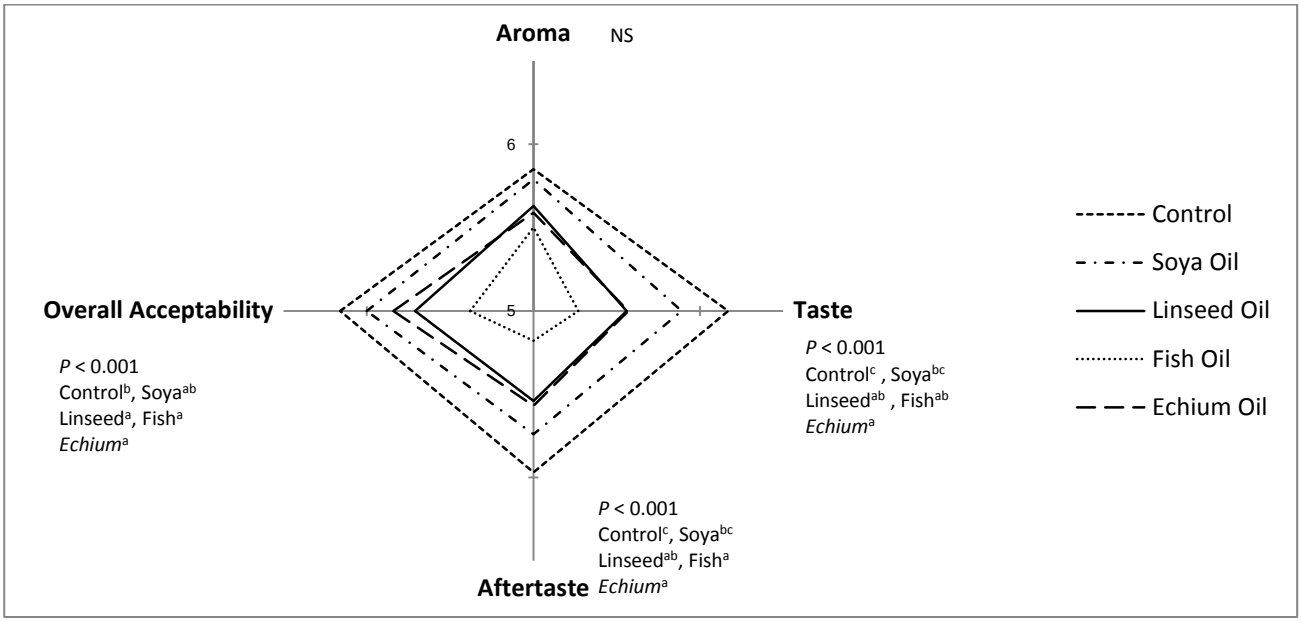

Figure 4.5: Consumer sensory analysis of *M. longissimus lumborum* from the experimental treatment groups. Different superscripts for the same attribute indicate significant differences.

higher degree of proteolysis and supposedly a larger degree of ageing and therefore tenderisation (Olson, Parish, & Stromer, 1976; Geesink et al., 2000; Geesink et al., 2006). In the present study it is evident that there were no significant differences between treatments for MFL on either day 1 or 5 post mortem (Figure 4.6). There was however a weak but significant (P < 0.01) correlation between MFL and shear force at day 1 (r = 0.3739) and no correlation at day 5 (Table 4.9).

Fibre typing is a useful tool in meat science since the classification of fibre types help to define functional and metabolic properties of muscle tissue. Table 4.25 indicates that here were no significant differences between treatments for cross sectional area of type I, type IIA or type IIB muscle fibre types. Taylor and co workers (2002) concluded that a small fibre size of muscle corresponds with toughness. The control treatment had the smallest typeI, IIA and IIB fibre size. The lack of a significant difference between treatments for shear force (Figure 4.3) is a reflection of the uniform growth and development pattern, wich indicates that muscle fibres were of similar size (Teye et al., 2006a). There was also no correlations between shear force and type I, IIA or IIB muscle fibre types as seen in Table 4.27. There were no significant differences between treatments for the amount of red fibres. Higher proportions of red fibres will increase the redness of meat due to the higher levels of Mb (Kim et al., 2010). In the present study there was a significant (P < 0.05) correlation (r = 0.3042) between the % red muscle fibres and the redness of meat as indicated by the colour a* value in Table 4.28. Furthermore there was a significant difference (P = 0.023) between treatments for the concentration of intermediate fibres. The linseed oil treatment had the highest and the *Echium* oil treatment the lowest percentage of intermediate fibres (Table 4.25). The concentration of intermediate fibres did not correlate to any of the colour

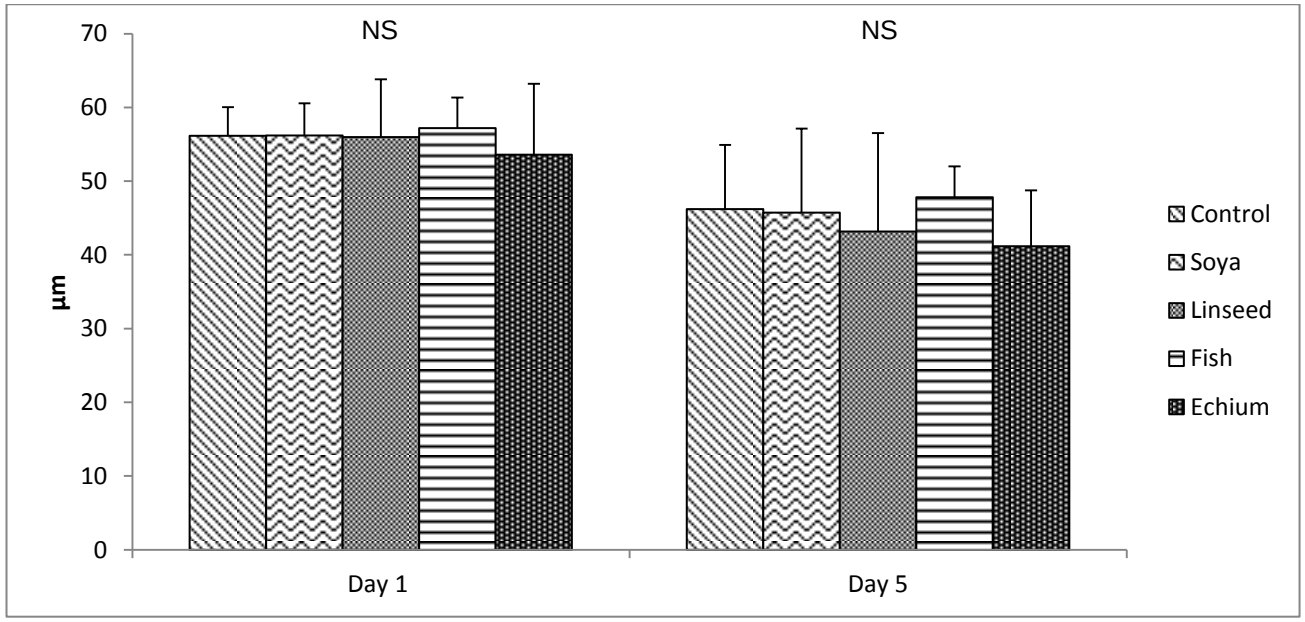

Figure 4.6: Effect of dietary treatment and aging on myofibrillar fragment lengths of *M. longissimus lumborum* of pigs on day 1 and day 5 post mortem.

Table 4.26: Effect of dietary treatment on fibre typing of *M. longissimus lumborum* of pigs.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Red fibre (Type I) area (µm²)	3952.50 ± 763.27	4435.07 ± 1029.20	4204.78 ± 1202.91	4477.97 ± 1037.66	4239.87 ± 1171.95	*P* = 0.809
Intermediate fibre (Type IIA) area (µm²)	5921.66 ± 1820.95	6617.55 ± 1708.05	6524.97 ± 2105.50	6824.72 ± 1599.64	6302.32 ± 1863.68	*P* = 0.839
White fibre (Type IIB) area (µm²)	8992.23 ± 2524.00	10334.78 ± 2788.15	9499.37 ± 2570.77	11092.35 ± 3003.04	9793.67 ± 3342.53	*P* = 0.536
% Red fibre	22.20 ± 3.38	24.17 ± 3.40	22.83 ± 2.95	22.12 ± 4.61	24.39 ± 3.17	*P* = 0.459
%Intermediate fibre	27.87^{ab} ± 9.65	26.87^{ab} ± 6.55	34.75^{b} ± 6.47	28.94^{ab} ± 5.59	24.33^{a} ± 5.24	*P* = 0.023
%White fibre	50.38^{ab} ± 6.32	48.97^{ab} ± 6.66	43.37^{a} ± 6.39	48.97^{ab} ± 4.39	51.28^{b} ± 4.99	*P* = 0.037

Means with different superscripts in the same row differ significantly.

Table 4.27: Pearson correlation analysis between selected meat quality parameters.

	Shear force measurement (kg)	Total Fat (mg/100 g muscle)	Total n-3 fatty acids (mg/100 g muscle)	EPA+DPA+DHA (mg/100 g muscle)	Water Holding Capacity (Meat Area / Total Area)	Red fibre (Type1) area (µm²)	Intermediate fibre (Type11A) area (µm²)	White fiber (Type 11B) Area (µm²)	Miofibrillar length AVG Day 1 post mortem (µm)	Miofibrillar length AVG Day 5 post mortem (µm)
Shear force measurement (kg)	1	-0.3017*	0.0403^{NS}	0.0868^{NS}	0.0313^{NS}	0.1337^{NS}	0.0938^{NS}	0.1927^{NS}	0.3739**	0.0482^{NS}
Total Fat (mg/100 g muscle)		1	0.3698**	-0.1110^{NS}	-0.1985^{NS}	-0.0393^{NS}	0.0606^{NS}	0.0556^{NS}	-0.0361^{NS}	-0.0384^{NS}
Total n-3 fatty acids (mg/100 g muscle)			1	0.7198***	0.1379^{NS}	0.0509^{NS}	0.1187^{NS}	0.1471^{NS}	-0.0097^{NS}	-0.0389^{NS}
EPA+DPA+DHA (mg/100 g muscle)				1	0.2179^{NS}	0.0750^{NS}	0.0845^{NS}	0.1723^{NS}	0.0750^{NS}	0.1110^{NS}
Water Holding Capacity (Meat Area / Total Area)					1	0.2215^{NS}	0.1494^{NS}	0.1680^{NS}	-0.0475^{NS}	-0.3792**
Red fibre (Type1) area (µm²)						1	0.7641***	0.7608***	0.2827*	-0.0465^{NS}
Intermediate fibre (Type11A) area (µm²)							1	0.8336***	0.2490^{NS}	0.0107^{NS}
White fiber (Type 11B) area (µm²)								1	0.3147*	0.0138^{NS}
Miofibrillar length AVG Day 1 post mortem (µm)									1	0.4302**
Miofibrillar length AVG Day 5 post mortem (µm)										1

NS = Not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 4.28: Pearson correlation analysis between the ratio of muscle fibre types and colour quality parameters.

	% Red Fibres	% Intermediate fibres	% White fibres	Colour L* - value	Colour a* - value	Colour b* - value	Chroma	Hue angle
% Red Fibres	1	-0.2950*	-0.1123^{NS}	0.3281*	0.3042*	0.3746**	0.3448*	0.2282^{NS}
% Intermediate fibres		1	-0.7641***	-0.1455^{NS}	-0.1583^{NS}	-0.1663^{NS}	-0.1633^{NS}	-0.1344^{NS}
% White fibres			1	0.0120^{NS}	-0.0226^{NS}	0.0069^{NS}	-0.0080^{NS}	0.1594^{NS}
Colour L* - value				1	0.7239***	0.8601***	0.8064***	0.5391***
Colour a* - value					1	0.9364***	0.9832***	0.0722^{NS}
Colour b* - value						1	0.9847***	0.4050**
Chroma							1	0.2455^{NS}
Hue angle								1

NS = Not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

4.8 Chemical and oxidative stability studies

4.8.2 Lipid and colour stability of fresh and frozen pork

The results of a chemical shelf life study, performed on pork chops for 7 days at 4 °C and -18 °C for 3 and 6 months, are shown in Figures 4.7 – 4.10 and Tables 4.28 and 4.29. It is well known that higher levels of PUFA result in accelerated oxidation (Wood et al., 1999). On both day 0 and after 7 days of refrigerated storage, the control samples had significant ($P < 0.001$) lower peroxide values (PV) than all the other treatments (Figure 4.7). A possible explanation for the lower PV recorded by the control treatments would be that the control sample contained lower levels of UFA. After 3 months of frozen storage the control treatment still had the lowest PV however, it did not differ significantly ($P < 0.001$) from the soya treatment (Figure 4.8). At 6 months of frozen storage there were no significant differences between treatments (Figure 4.8). As the secondary oxidation products form, the primary oxidation products decrease resulting in lower PV. This could possibly explain why at there were no significant differences between treatments after 6 months storage. Additionally, the increase in variation as recorded by the standard deviation (Figure 4.8) further illustrated that the PV at 6 months of storage is not a good indication for oxidation and it is therefore important to also test for the secondary oxidation products (TBARS).

TBARS values presented in Figures 4.9 and 4.10 were used to test for secondary oxidation products (malonaldehyde). From figure 4.9 it is clear that there were no significant differences between treatments for TBARS values at day 0. After 7 days of refrigerated storage, there were small, but significant differences ($P = 0.010$) between the control and the linseed treatment. The control still had the lowest TBARS values after 3 months of frozen storage as illustrated in Figure 4.10. This agrees with the findings of Beyhni and co-workers (2002) who found that after 1 month frozen storage, meat from pigs on higher PUFA diets had higher TBARS values. After another 3 months of frozen storage the TBARS values of the control treatment remained unchanged, and all other treatments increased slightly (Figure 4.10). The linseed and *Echium* oil treatments had the highest values followed by the fish- and soya oil treatments.

Meat colour is regarded as a visual measure of freshness and quality that influences the choice of consumers at the point of sale (Faustman & Cassens, 1990). Instrumental meat and fat colour measured as reflection or lightness (*L), redness (*a), yellowness (*b). The a* and *b values are used to calculate Chroma and Hue angle. Chroma is used to indicate the saturation of colour, sometimes termed vividness (Tapp, Yancey & Apple, 2011). Hue angle is used to show the development of colour from red to yellow. A larger Hue angle indicates a less red product (Tapp et al., 2011). Although these objective/instrumental measurements of colour does not by itself reflect consumer acceptance, studies have shown that thresholds for instrumental colour parameters can be used to estimate consumer acceptance (Hopkins, 1996). However, with regard to pork, Ngapo and co-workers (2007) found that even though colour is the consistently chosen characteristic for parameters (Table 4.28). There were also significant differences between treatments for the amount of white fibres. White fibres (type IIB) have been related to higher lightness (Kim et al.,

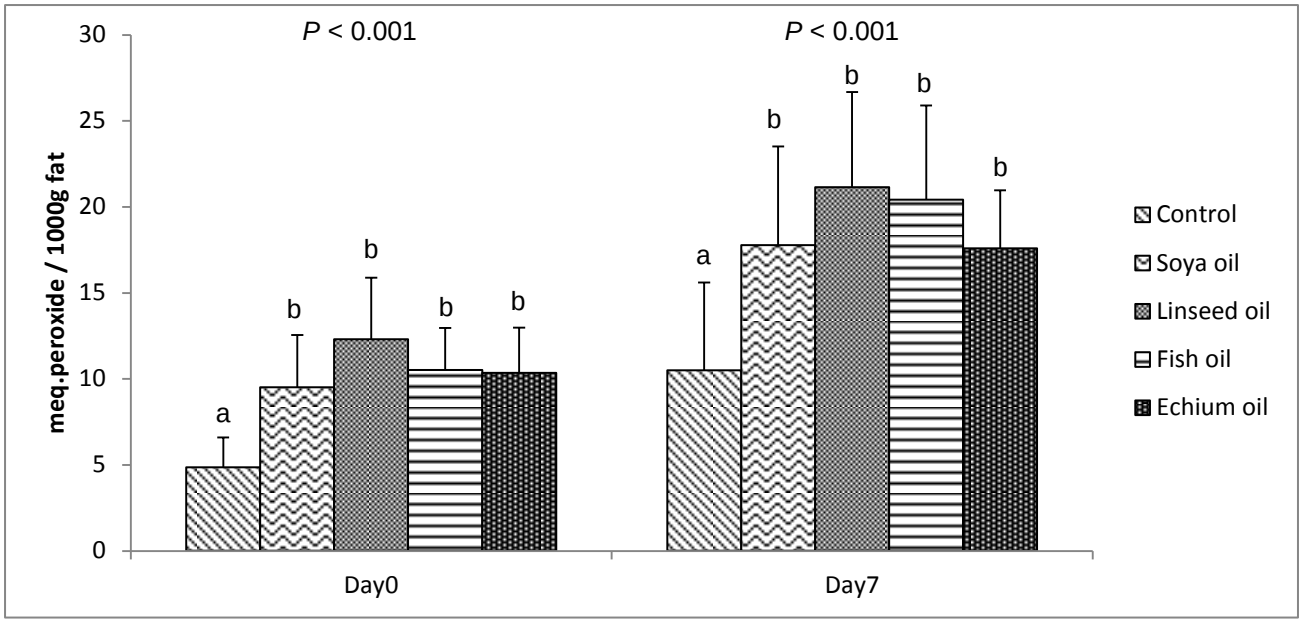

Figure 4.7: Lipid stability (PV) of muscle from fresh pork chops from the experimental treatment groups stored at 4 °C for 7 days. Bars with different superscripts for each day differ significantly

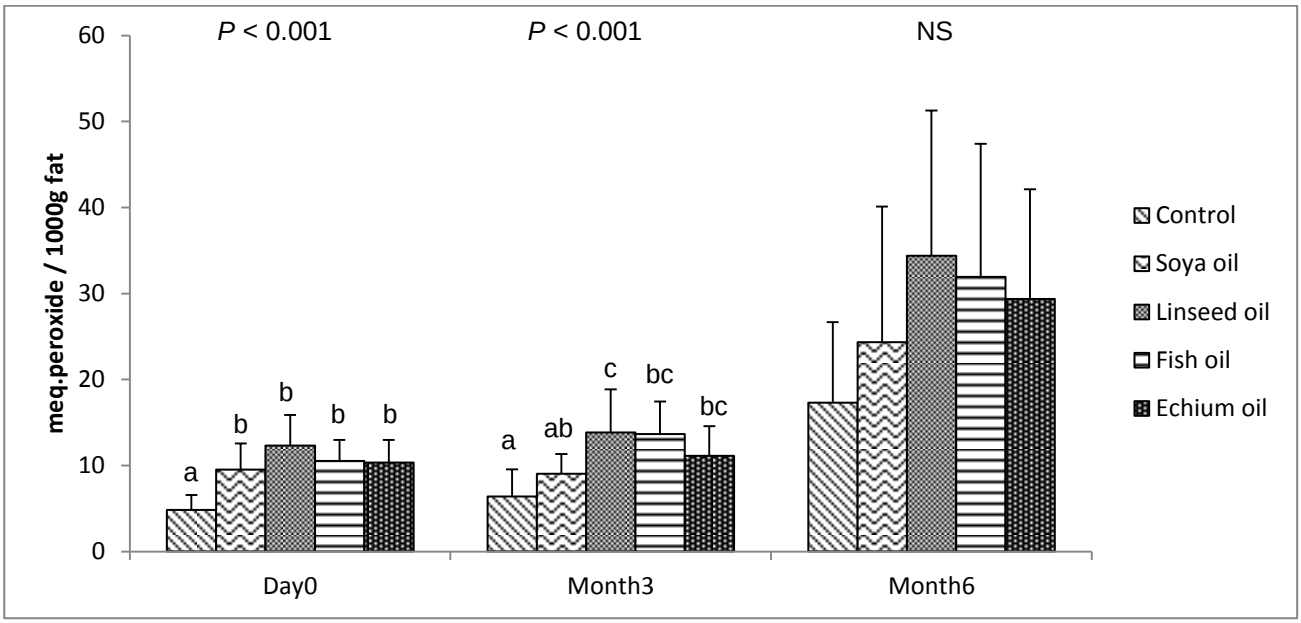

Figure 4.8: Lipid stability (PV) of muscle from fresh pork chops from the experimental treatment groups stored at -18 °C for 3 and 6 months. Bars with different superscripts for each month differ significantly

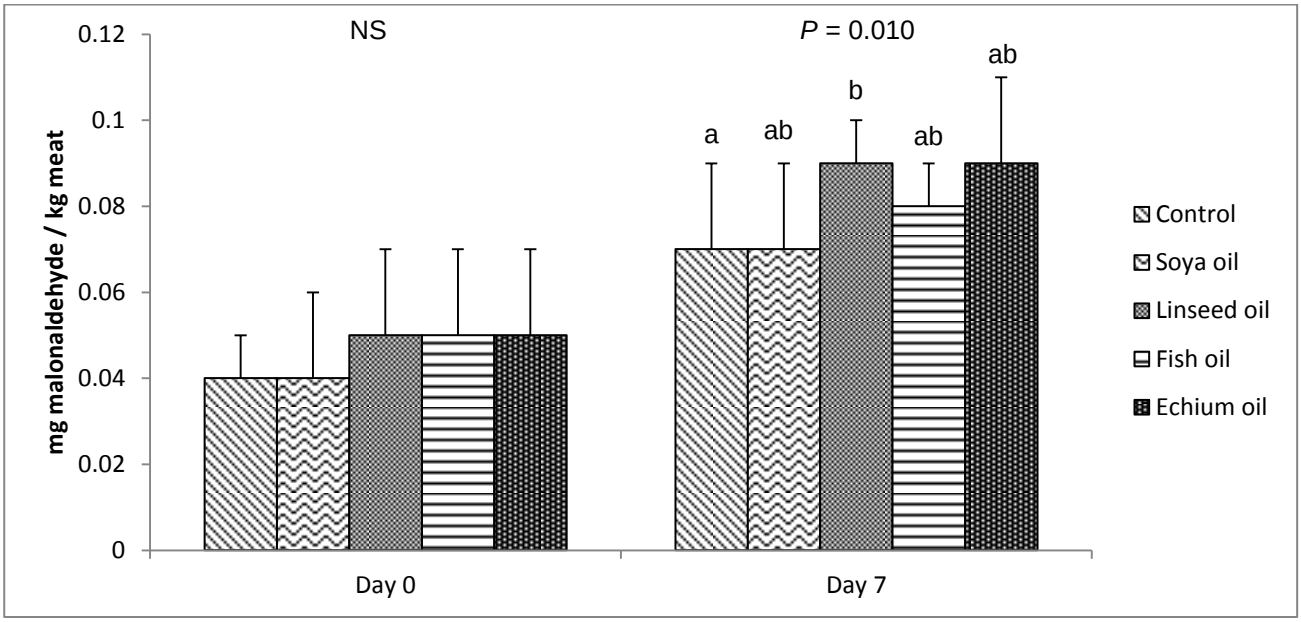

Figure 4.9: TBARS values of muscle from fresh pork chops from the experimental treatment groups stored at 4 °C for 7 days. Bars with different superscripts for each day differ significantly

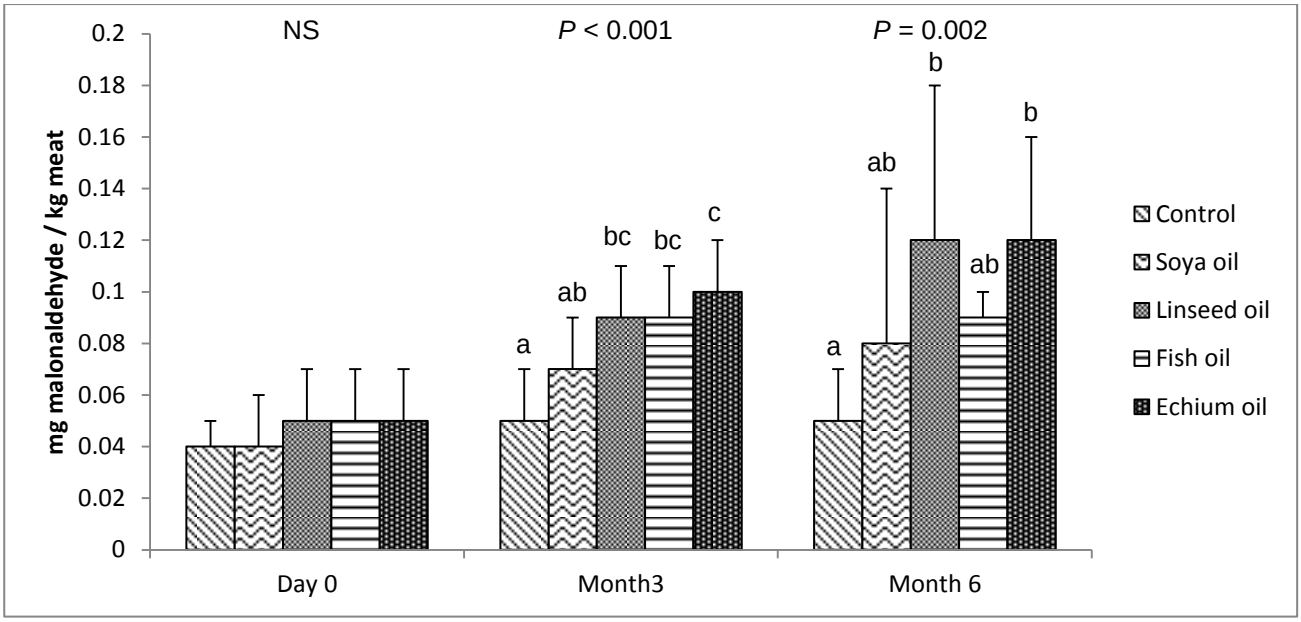

Figure 4.10: TBARS values of muscle from fresh pork chops from the experimental treatment groups stored at -18 °C for 3 and 6 months. Bars with different superscripts differ significantly

2013a), more tender meat (Taylor et al., 2002) and a lower WHC (Kim et al., 2013b). This was however not the case in the present study, whereby no correlations between type IIB fibres and lightness (L*; Table 4.28), shear force (Table 4.27), or WHC (Table 4.27) was observed

consumer choice, the consistency of choice for colour showed the largest variation among countries. Various external factors such as packaging, aging duration, display duration, slaughter

and packaging environment could have an influence on colour parameters (Mancini & Hunt, 2005). The colour of the backfat and meat of chops was rather stable after 7 day of refrigeration storage (Table 4.29 & 4.30). At day 0 there were no significant differences between treatments for any of the colour measurements in the backfat (Table 4.29). The *Echium* oil treatment had significantly (*P* = 0.027) lighter backfat (colour L*) when compared to the control at day 1. On day 2 the fish oil treatment had the lightest colour and it was significantly (*P* = 0.003) lighter than the control and the soya treatments. From days 3 to 7 there were no significant differences between treatments for lightness (Table 4.29), while the only significant difference for the redness (colour a*) of backfat was on days 3 and 5. At day 3 the fish oil treatment had highest (*P* = 0.045) scores for redness compared to the control. And on day 5 the control had the lowest scores (*P* = 0.011) compared to the soya and fish oil treatments (Table 4.29). On all 7 days there were no significant differences between the backfat of treatments for colour b* values (yellowness) or the Hue angle. On day 5 there was a significant (*P* = 0.034) difference between treatments for chroma with the control having the lowest and the soya the highest scores (Table 4.29). Even though there were some significant differences in backfat colour between treatments these differences were rather small. Over the 7 day period the lightness increased slightly in all the treatments and the redness decreased. The yellowness in all treatments, except for the linseed treatment, increased (Table 4.29).

The only significant difference for meat colour was on day 0 (Table 4.30), whereby the control treatment had significantly higher scores for yellowness (b*) (*P* = 0.026) and chroma (*P* = 0.033) when compared to the linseed oil treatment (Table 4.30). Over the 7 days, the lightness increased and the redness decreased, which could be mainly ascribed to the drip loss in the meat. All treatments also had an increase in yellowness (Table 4.30).

4.8.3 Quality, oxidative stability and consumer preference of pork bangers

Pork bangers as classified as a comminuted, uncured, no or partial heat treated product (SANS, 2011). In South Africa pork bangers are sold frozen (retail stores) and fresh (butcheries) and are mainly consumed as a breakfast sausage. Pork bangers were manufactured according to the formulation in Table 3.9.

According to Teye and co-workers (2006b) the fatty acid composition of UK-style sausages can affect the cooking loss whith the hardest fat loosing the most moisture and the softest the lowest (Evans & Ranken, 1975), which was not recorded during the present study (Table 4.31). Figure 4.1 indicates that in our study, the control treatment had significantly (*P* = 0.049) harder backfat than the linseed oil treatment. The only significant difference in cooking loss was between the fish oil and *Echium* oil treatments (Table 4.31). Fat hardness between the fish and *Echium* oil treatments did not differ significantly (Fig 4.2). There was a significant difference in drip loss with the *Echium* oil treatment having the least drip and the fish oil treatment the most (Table 4.18). There were also no significant differences between treatments for thawing loss. The most variation

Table 4.29: Backfat colour stability of fresh pork chops from the experimental treatment groups stored at 4 °C for 7 days. (n = 10 per treatment)

Day	Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
0	**Colour L***	71.31 ± 3.47	70.33 ± 3.77	69.80 ± 2.02	69.90 ± 2.87	69.65 ± 2.42	0.731
	Colour a*	7.51 ± 0.68	8.95 ± 1.26	8.87 ± 1.55	8.90 ± 1.30	8.86 ± 1.51	0.075
	Colour b*	9.73 ± 0.95	10.74 ± 1.20	11.04 ± 1.67	10.52 ± 1.78	10.56 ± 1.44	0.349
	Chroma	12.31 ± 1.02	14.00 ± 1.65	14.00 ± 2.03	13.81 ± 2.04	13.82 ± 1.85	0.172
	Hue angle	52.40 ± 2.74	50.36 ± 2.37	49.84 ± 4.09	49.65 ± 3.69	50.05 ± 3.97	0.392
1	**Colour L***	72.69^{b} ± 2.06	69.88^{ab} ± 3.03	69.34^{ab} ± 1.92	69.98^{ab} ± 3.18	68.90^{a} ± 3.01	0.027
	Colour a*	7.78^{a} ± 1.16	8.70^{ab} ± 0.83	8.89^{ab} ± 1.15	9.65^{b} ± 1.51	8.85^{ab} ± 1.61	0.042
	Colour b*	9.90 ± 1.16	10.60 ± 1.21	10.57 ± 1.03	11.12 ± 1.18	10.35 ± 1.51	0.284
	Chroma	12.60 ± 1.54	13.96 ± 1.66	13.83 ± 1.46	14.75 ± 1.75	13.63 ± 2.11	0.109
	Hue angle	51.98 ± 2.67	49.61 ± 3.00	50.03 ± 2.20	49.28 ± 3.10	49.68 ± 2.80	0.216
2	**Colour L***	73.49^{b} ± 2.44	73.89^{b} ± 1.41	71.50^{ab} ± 3.38	69.40^{a} ± 2.62	72.09^{ab} ± 2.71	0.003
	Colour a*	7.71 ± 1.31	8.67 ± 1.04	8.43 ± 1.15	9.14 ± 1.67	8.30 ± 1.01	0.164
	Colour b*	9.31 ± 1.58	10.55 ± 0.96	9.95 ± 1.24	10.39 ± 1.57	10.21 ± 2.06	0.412
	Chroma	12.10 ± 2.03	13.96 ± 1.86	13.31 ± 1.91	14.15 ± 2.55	12.83 ± 1.68	0.165
	Hue angle	50.39 ± 1.42	49.44 ± 2.77	48.68 ± 3.27	47.65 ± 3.04	49.67 ± 2.68	0.224
3	**Colour L***	73.95 ± 2.12	72.79 ± 2.22	70.81 ± 3.67	71.69 ± 2.88	70.47 ± 2.88	0.051
	Colour a*	7.28^{a} ± 0.63	8.50^{ab} ± 1.19	8.25^{ab} ± 1.01	8.76^{b} ± 1.30	8.35^{ab} ± 1.20	0.045
	Colour b*	9.18 ± 1.20	10.59 ± 1.57	10.03 ± 0.96	10.34 ± 1.74	10.64 ± 2.17	0.248
	Chroma	11.73 ± 1.28	13.60 ± 1.86	13.25 ± 1.63	13.56 ± 2.09	13.19 ± 2.00	0.140
	Hue angle	51.47 ± 2.24	51.12 ± 2.79	49.60 ± 3.44	49.57 ± 2.53	50.50 ± 2.29	0.403
4	**Colour L***	73.01 ± 1.89	72.69 ± 2.99	70.49 ± 4.17	73.51 ± 2.43	70.73 ± 2.80	0.085
	Colour a*	7.39 ± 0.78	8.43 ± 1.39	8.38 ± 1.48	8.66 ± 1.24	7.81 ± 1.00	0.133
	Colour b*	9.01 ± 0.76	10.40 ± 1.55	10.39 ± 1.19	9.98 ± 1.02	9.79 ± 1.29	0.075
	Chroma	11.67 ± 0.95	13.40 ± 1.99	13.37 ± 1.71	13.03 ± 1.23	12.54 ± 1.62	0.085
	Hue angle	50.63 ± 2.63	50.97 ± 2.54	51.29 ± 3.44	50.40 ± 2.12	50.93 ± 1.41	0.948
5	**Colour L***	72.34 ± 3.95	71.23 ± 3.38	69.87 ± 3.24	71.25 ± 3.03	70.77 ± 2.93	0.579
	Colour a*	6.74^{a} ± 0.87	8.30^{b} ± 0.94	7.93^{ab} ± 1.38	8.09^{b} ± 0.63	7.59^{ab} ± 1.04	0.011
	Colour b*	9.21 ± 1.69	10.62 ± 0.98	10.40 ± 1.93	10.52 ± 1.25	9.77 ± 1.81	0.236
	Chroma	11.46^{a} ± 1.64	13.50^{b} ± 1.12	13.17^{ab} ± 1.85	13.30^{ab} ± 1.14	12.41^{ab} ± 1.91	0.034
	Hue angle	53.46 ± 4.58	51.90 ± 3.18	50.84 ± 3.87	52.23 ± 3.39	51.78 ± 3.70	0.644
6	**Colour L***	73.02 ± 3.08	70.73 ± 3.33	71.30 ± 2.82	70.95 ± 3.54	71.44 ± 2.75	0.507
	Colour a*	7.05 ± 0.72	8.04 ± 1.06	7.97 ± 1.22	7.69 ± 1.12	7.37 ± 1.21	0.225
	Colour b*	10.38 ± 1.71	10.94 ± 1.74	11.26 ± 2.19	10.98 ± 1.98	9.88 ± 2.12	0.532
	Chroma	12.59 ± 1.57	13.61 ± 1.79	14.17 ± 2.23	13.11 ± 1.82	12.80 ± 2.66	0.435
	Hue angle	55.32 ± 4.38	53.35 ± 4.04	51.52 ± 3.16	54.12 ± 5.81	52.83 ± 3.21	0.356
7	**Colour L***	73.19 ± 2.63	71.69 ± 2.57	71.19 ± 2.61	70.30 ± 2.76	70.93 ± 3.43	0.219
	Colour a*	6.47 ± 0.69	7.53 ± 0.47	7.94 ± 1.58	7.75 ± 1.43	6.95 ± 1.35	0.052
	Colour b*	9.97 ± 1.83	11.29 ± 1.69	10.86 ± 1.34	11.52 ± 2.13	10.64 ± 2.27	0.401
	Chroma	12.40 ± 2.27	13.95 ± 1.88	14.20 ± 2.21	13.46 ± 1.72	13.22 ± 2.66	0.397
	Hue angle	54.80 ± 2.97	55.93 ± 3.73	51.71 ± 3.93	55.14 ± 4.27	54.40 ± 2.87	0.113

Means with different superscripts in the same row differ significantly.

in colour, between treatments, were seen after 6 and 9 days of storage. At both days 6 and 9 the bangers from the linseed and *Echium* oil treatments were significantly lighter (L*; $P < 0.001$) when compared to the control treatment (Table 4.32). Redness (a*) was the highest in the linseed oil treatment and lowest in the soya oil treatment on days 6 and 9. On day 6 the bangers from the *Echium* oil treatment had significantly lower ($P = 0.001$) yellowness compared to the control and the fish oil treatment. On day 9 the soya oil treatment had a significantly lower ($P < 0.001$) yellowness compared to all the other treatments. Various authors have found that diets high in PUFA result in greater TBARS values and therefore products

Table 4.30: Colour stability of muscle from fresh pork chops from the experimental treatment groups stored at 4 °C for 7 days. (n = 10 per treatment)

Day	Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
0	**Colour L***	55.59 ± 3.66	53.78 ± 2.91	52.17 ± 3.65	53.32 ± 4.43	53.49 ± 3.88	0.372
	Colour a*	12.24 ± 2.43	11.09 ± 1.74	9.53 ± 1.87	10.58 ± 2.62	10.77 ± 2.63	0.142
	Colour b*	13.28^{b} ± 1.87	10.95^{ab} ± 2.00	9.98^{a} ± 1.73	10.68^{ab} ± 2.48	10.87^{ab} ± 2.99	0.026
	Chroma	18.48^{b} ± 2.37	15.60^{ab} ± 2.60	14.04^{a} ± 2.26	15.07^{ab} ± 3.53	15.63^{ab} ± 4.16	0.033
	Hue angle	45.82 ± 1.68	45.00 ± 1.27	45.21 ± 2.35	45.92 ± 1.80	44.57 ± 1.85	0.435
1	**Colour L***	56.42 ± 4.54	54.35 ± 3.53	52.25 ± 4.59	53.11 ± 4.94	53.40 ± 4.32	0.284
	Colour a*	12.83 ± 1.42	11.90 ± 1.64	11.50 ± 1.51	11.40 ± 1.95	12.44 ± 1.67	0.251
	Colour b*	14.12 ± 2.23	13.17 ± 2.69	12.00 ± 2.28	12.21 ± 2.73	12.91 ± 2.29	0.332
	Chroma	19.10 ± 2.58	17.47 ± 2.80	16.64 ± 2.63	16.73 ± 3.26	17.94 ± 2.78	0.295
	Hue angle	47.54 ± 1.95	46.69 ± 2.43	45.96 ± 2.62	46.64 ± 2.79	45.84 ± 1.82	0.507
2	**Colour L***	55.94 ± 4.68	54.88 ± 3.26	53.77 ± 4.57	55.50 ± 5.39	54.51 ± 4.68	0.848
	Colour a*	11.73 ± 1.79	11.47 ± 1.42	11.29 ± 1.83	11.37 ± 1.55	11.67 ± 1.09	0.963
	Colour b*	12.97 ± 2.55	12.71 ± 2.30	12.52 ± 1.49	12.49 ± 2.20	12.74 ± 2.07	0.988
	Chroma	17.50 ± 3.07	17.38 ± 2.87	16.47 ± 2.85	16.91 ± 2.56	17.61 ± 2.66	0.886
	Hue angle	47.64 ± 1.87	46.87 ± 1.95	46.46 ± 2.61	47.51 ± 2.70	46.39 ± 2.21	0.637
3	**Colour L***	57.71 ± 4.66	55.02 ± 4.89	53.43 ± 5.03	54.29 ± 4.94	54.98 ± 4.30	0.355
	Colour a*	11.47 ± 1.14	10.97 ± 1.58	10.84 ± 1.22	10.84 ± 1.41	11.18 ± 1.26	0.803
	Colour b*	13.83 ± 1.86	12.58 ± 1.86	11.87 ± 1.70	12.35 ± 2.09	12.72 ± 2.28	0.265
	Chroma	17.99 ± 2.07	16.70 ± 2.39	16.09 ± 2.00	16.45 ± 2.45	16.96 ± 2.48	0.426
	Hue angle	50.19 ± 2.26	48.80 ± 1.72	47.49 ± 2.16	48.54 ± 2.15	48.38 ± 2.73	0.123
4	**Colour L***	58.59 ± 4.64	56.15 ± 3.75	54.20 ± 4.59	55.28 ± 5.25	55.36 ± 4.42	0.282
	Colour a*	10.97 ± 0.97	10.67 ± 1.27	10.46 ± 1.23	10.23 ± 1.31	10.66 ± 1.07	0.703
	Colour b*	13.28 ± 1.64	12.52 ± 2.04	11.57 ± 1.45	12.17 ± 2.06	12.10 ± 1.89	0.332
	Chroma	17.24 ± 1.79	16.47 ± 2.33	15.62 ± 1.83	15.92 ± 2.31	16.15 ± 2.05	0.475
	Hue angle	50.33 ± 2.21	49.38 ± 2.19	47.85 ± 1.97	49.68 ± 2.77	48.40 ± 2.61	0.153
5	**Colour L***	58.67 ± 4.24	56.63 ± 4.21	54.41 ± 4.75	55.46 ± 5.55	55.80 ± 4.56	0.342
	Colour a*	10.32 ± 0.96	10.23 ± 1.06	10.18 ± 1.21	9.70 ± 1.63	10.57 ± 0.88	0.577
	Colour b*	12.89 ± 2.01	12.49 ± 1.88	11.53 ± 1.57	11.87 ± 2.18	12.41 ± 1.82	0.530
	Chroma	16.54 ± 2.04	16.17 ± 2.01	15.41 ± 1.84	15.36 ± 2.60	16.33 ± 1.85	0.609
	Hue angle	51.08 ± 3.14	50.48 ± 2.79	48.53 ± 2.77	50.65 ± 2.86	49.39 ± 3.04	0.294
6	**Colour L***	58.91 ± 5.46	56.79 ± 4.52	55.02 ± 5.20	55.53 ± 5.27	55.71 ± 5.13	0.464
	Colour a*	9.99 ± 0.90	10.09 ± 1.24	9.68 ± 1.43	9.56 ± 1.30	10.25 ± 1.30	0.712
	Colour b*	13.27 ± 1.82	12.47 ± 1.60	11.82 ± 1.58	11.98 ± 1.90	12.50 ± 1.93	0.409
	Chroma	16.64 ± 1.78	16.05 ± 1.90	15.30 ± 1.98	15.35 ± 2.15	16.21 ± 2.09	0.506
	Hue angle	52.79 ± 3.43	50.97 ± 2.52	50.74 ± 2.96	51.30 ± 3.03	50.51 ± 3.63	0.515
7	**Colour L***	59.59 ± 4.65	56.56 ± 4.31	55.52 ± 4.94	56.04 ± 5.02	56.01 ± 4.82	0.333
	Colour a*	8.88 ± 0.99	9.44 ± 1.77	9.29 ± 1.32	9.41 ± 1.42	9.52 ± 1.91	0.894
	Colour b*	13.07 ± 2.10	11.95 ± 1.80	11.54 ± 1.97	12.18 ± 2.26	12.10 ± 2.44	0.602
	Chroma	15.85 ± 2.04	15.11 ± 2.40	15.19 ± 1.69	15.43 ± 2.55	15.47 ± 2.80	0.960
	Hue angle	53.54 ± 2.52	51.50 ± 2.27	51.83 ± 3.30	52.19 ± 2.87	50.74 ± 3.77	0.328

Means with different superscripts in the same row differ significantly.

with undesirable sensory traits (Hertzman, Göransson, & Ruderùs, 1988; Øverland, Taugbøl, Haug, & Sundstøl, 1996; Arnkværn & Bronken Lien, 1997; Bryhni et al., 2002). Results in Table 4.33 indicates that the linseed, fish and *Echium* oil treatments had significantly higher ($P < 0.001$) TBARS than the control on days 0, 3, 6 and 9. On day 9 the soya treatment also had significantly higher ($P < 0.001$) TBARS values than the control and the *Echium* oil treatments. Huang & Greene (1978) found that rancid, oxidized odours can be detected at TBARS values of above 1. Even at day 0 the pork bangers from all treatments had very high TBARS values, but from day 6 all treatments had values of above one. The frozen pork bangers (Table 4.34) followed the same trend for TBARS as the fresh pork bangers (Table 4.33). At 3 months the TBARS values from the

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Table 4.31: Thawing loss and cooking loss of pork bangers from different treatments.

	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Thawing loss (%)	3.51 ± 0.53	3.52 ± 0.57	3.68 ± 0.38	3.79 ± 0.44	3.35 ± 0.74	$P = 0.348$
Cooking loss (%)	9.62^{ab} ± 2.45	8.92^{a} ± 2.48	11.57^{ab} ± 3.52	12.57^{b} ± 2.07	9.17^{a} ± 2.16	$P = 0.003$

Means with different superscripts in the same row differ significantly.

Table 4.32: Colour measurements of pork bangers for different treatments stored at 4°C for 9 days.

Day	Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
	L*	73.73 ± 1.69	73.66 ± 1.25	73.37 ± 1.72	73.50 ± 1.47	73.82 ± 1.53	$P = 0.860$
Day 0	**a***	7.16 ± 0.80	7.72 ± 0.84	7.79 ± 0.69	7.40 ± 0.83	7.57 ± 0.80	$P = 0.052$
	b*	11.95^{b} ± 0.99	11.40^{ab} ± 0.62	11.15^{a} ± 1.22	11.94^{b} ± 0.88	11.81^{ab} ± 0.74	$P = 0.007$
	L*	73.56 ± 1.49	71.62 ± 9.22	72.18 ± 1.39	72.56 ± 1.98	72.65 ± 2.32	$P = 0.653$
Day 3	**a***	6.21 ± 0.67	6.33 ± 0.99	6.71 ± 0.65	6.26 ± 0.75	6.38 ± 0.83	$P = 0.213$
	b*	11.48^{b} ± 0.86	10.50^{a} ± 1.45	11.07^{ab} ± 0.99	11.76^{b} ± 1.02	11.27^{ab} ± 0.81	$P = 0.001$
	L*	73.42^{c} ± 2.24	73.19^{bc} ± 1.93	69.71^{a} ± 1.61	71.73^{b} ± 1.68	69.87^{a} ± 2.27	$P < 0.001$
Day 6	**a***	6.15^{ab} ± 0.87	5.74^{a} ± 0.65	6.75^{c} ± 0.63	5.75^{a} ± 0.54	6.52^{bc} ± 0.93	$P < 0.001$
	b*	11.68^{b}± 0.73	11.09^{ab} ± 0.87	11.26^{ab} ± 0.78	11.59^{b} ± 0.86	10.69^{a} ± 1.09	$P = 0.001$
	L*	73.93^{c} ± 1.65	73.50^{bc} ± 1.63	70.49^{a} ± 1.88	72.00^{ab}± 1.71	71.83^{a} ± 2.44	$P < 0.001$
Day 9	**a***	6.01^{bc} ± 0.75	5.19^{a} ± 0.62	6.57^{c} ± 0.83	5.61^{ab} ± 0.72	5.65^{ab} ± 0.86	$P < 0.001$
	b*	11.44^{b} ± 0.87	9.86^{a} ± 0.68	10.95^{b} ± 0.99	10.91^{b} ± 0.98	10.73^{b} ± 0.99	$P < 0.001$

Means with different superscripts in the same row differ significantly.

Table 4.33: Thiobarbituric acid reactive substances (TBARS) (mg malonaldehyde/kg meat) of pork bangers from different treatments during refrigerated storage at 4°C for 9 days.

Day	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Day0	$0.51^{a} \pm 0.04$	$0.58^{a} \pm 0.06$	$1.07^{b} \pm 0.06$	$1.10^{b} \pm 0.22$	$1.23^{b} \pm 0.32$	$P < 0.001$
Day3	$0.54^{a} \pm 0.12$	$1.00^{ab} \pm 0.23$	$1.77^{bc} \pm 0.34$	$1.93^{c} \pm 0.58$	$2.17^{c} \pm 0.38$	$P < 0.001$
Day6	$1.24^{a} \pm 0.13$	$2.01^{a} \pm 0.29$	$3.19^{b} \pm 0.43$	$3.05^{b} \pm 0.21$	$3.37^{b} \pm 0.71$	$P < 0.001$
Day9	$1.57^{a} \pm 0.23$	$2.87^{b} \pm 0.34$	$4.69^{c} \pm 0.62$	$4.93^{c} \pm 0.30$	$6.07^{d} \pm 0.41$	$P < 0.001$

Means with different superscripts in the same row differ significantly.

Table 4.34: Thiobarbituric acid reactive substances (TBARS) (mg malonaldehyde/kg meat) of pork bangers from different treatments during frozen storage at -18^{o}C for 6 months.

	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
3 Months	$0.57^{a} \pm 0.04$	$0.82^{b} \pm 0.08$	$1.85^{d} \pm 0.06$	$1.25^{c} \pm 0.15$	$2.91^{e} \pm 0.18$	$P < 0.001$
6 Months	$0.74^{a} \pm 0.07$	$1.21^{a} \pm 0.77$	$3.23^{b} \pm 0.74$	$3.52^{b} \pm 1.07$	$4.06^{b} \pm 0.47$	$P < 0.001$

Means with different superscripts in the same row differ significantly.

Echium oil treatment was significantly higher ($P < 0.001$) than all other treatments, followed by the linseed and then the fish oil. The control treatment had significantly lower ($P < 0.001$) values at 3 months than the soya oil treatment. After 6 months of frozen storage the *Echium*, fish and linseed oil treatments had higher ($P < 0.001$) TBARS values than the control and the soya oil treatments. These findings agree with Arnkværn & Bronken Lien (1997), who found that pork fed diets high in UFA develop more rancid odours during freezing.

A 100 member consumer panel was used to evaluate the sensory properties of the pork bangers from the different dietary treatments. The panel consisted of 81% females and 19% males and 68% of the panellist were between the ages of 20-29 (Table 4.35). The sensory properties of the pork bangers from the different dietary treatments are shown in Table 4.36. Consumers could not detect a difference in aroma between the treatments ($P = 0.190$). However, for taste and aftertaste there was a significant difference ($P < 0.001$) between the control and the linseed, fish and *Echium* oil treatments. This is due to the higher levels of PUFA in the meat and fat from these treatments use to manufacture the bangers. This agrees with the findings of Hertzman et al., (1988), Øverland et al., (1996), Arnkværn & Bronken Lien, (1997) and Bryhni et al., (2002). The lower scores for taste and aftertaste also resulted in the linseed, fish and *Echium* treatment bangers scoring lower for overall acceptability

Table 4.35: Demographic profile of consumer panel for pork bangers.

Gender:	**% of Total**	**Age:**	**% of Total**
Female	81	< 20	7
Male	19	20-29	68
		30-39	8
		40-49	5
		50-59	9
		>60	3

4.8.4 Quality, oxidative stability and consumer preference of bacon

Bacon is a high quality cured whole muscle meat product. The consumption of bacon increased during the 1990s which resulted in an increased demand of good quality pork. For good bacon quality, the pork fat should be firm and white (Wood, 1984).

It is well know that soft pork fat leads to poorer slicing qualities of bacon (Wood, 1984) and if pork fat is too soft/ oily it cannot be used in bacon manufacturing (Cannon et al., 1996). In the present study there was a significant difference ($P = 0.049$) for fat hardness between the linseed and control treatments (Figure 4.2). South African pigs with a BFT of 17.8mm and LMC of less than 66.8% (R classification) should be suitable for the use in processed meat products (Hugo & Roodt, 2015). Only the control had a BFT of more than 17.8mm. Only the linseed treatment had significantly thinner ($P = 0.048$) backfat when compared to the control treatment

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Table 4.36: Sensory properties of bangers from different treatments.

Treatment	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Aroma	6.79 ± 1.54	6.55 ± 1.51	6.49 ± 1.41	6.31 ± 1.64	6.21 ± 1.68	$P = 0.190$
Taste	7.09^{c} ± 1.30	6.97^{bc} ± 1.40	6.35^{ab} ± 1.49	6.40^{ab} ± 1.69	6.28^{a} ± 1.65	$P < 0.001$
Aftertaste	7.08^{c} ± 1.18	6.93^{bc} ± 1.27	6.37^{ab} ± 1.52	6.13^{a} ± 1.77	6.03^{a} ± 1.64	$P < 0.001$
Overall Acceptability	7.23^{b} ± 1.24	7.00^{ab} ± 1.21	6.55^{a} ± 1.27	6.40^{a} ± 1.74	6.39^{a} ± 1.55	$P < 0.001$

Means with different superscripts in the same row differ significantly

Bacons were injected to a 20% yield. After 24 hours as well as after smoking there were no significant differences between treatments for the yield (Table 4.37). There were also no significant differences between treatments for any of the preparation losses (drip-, cooking- or total loss). The colour measurements of back bacon for different treatments are shown in Table 4.38. There were no significant differences between treatments for lightness at day 0. From Table 4.38 it is evident that the control treatment had bacon with a redder ($P = 0.017$) and yellower ($P = 0.037$) colour compared to the fish oil treatment at day 0. After 3 weeks of refrigerated storage the control had significantly ($P < 0.001$) lighter bacon when compared to the soya, linseed and fish oil treatments (Table 4.38). There was no significant difference for redness between treatments after 3 weeks of refrigerated storage. The *Echium* oil treatment had the highest scores for yellowness at week 3, which was significantly higher ($P < 0.001$) than the soya, linseed and fish oil treatments. After a total of 6 weeks of refrigerated storage, the control had the lightest bacon ($P < 0.001$) compared to all the other treatments. The control also had significantly ($P < 0.001$) redder bacon compared to the fish and *Echium* oil treatments. The fish oil treatment had the lowest scores ($P < 0.001$) for yellowness compared to the control and *Echium* oil treatments.

Fat rancidity is usually not a big problem in cured meat products, due to the anti-oxidative action of nitrite (Weiss, Gibis, Schuh, & Salminen, 2010). Smoking also has a persevering effect and protects products from oxidation (Pearson & Gillett, 1996). There were no significant differences between treatments for TBARS at day 0, weeks 3 and 6. Threshold values for TBARS, where off flavours were noted by taste panels, are rare and inconclusive but Tarladgis, Watts & Younathan, (1960) reported that off flavours were first detected between TBARS values of 0.5 and 1.0. The values of the current study were much lower and values ranged from 0.07 (day 0) to 0.10 after 6 weeks of storage.

Sensory properties of bacon were evaluated by a 100 member consumer panel consisting of 75% females and 25% males (Table 4.40). The highest proportion of panel members was in the age bracket of 20-29 years (59%). From Table 4.41 it is clear that the consumers did not find any differences between the dietary supplemental oil treatments in terms of aroma, taste, aftertaste or overall acceptability. The lack of any significant differences in terms of sensory traits as illustrated in Table 4.41 is probably due to the fact that there were very little oxidation products present in the bacon as reported in Table 4.39.

4.8.5 Quality, oxidative stability and consumer preference of salami

Salami is a high value fermented meat product that must be stable at room temperature with a long shelf life. Fat-rich fermented meat products are more susceptible to oxidation, because it is more exposed to oxygen than raw meat. It is therefore recommended that the levels of PUFA in backfat used for salami manufacturing should not exceed 23% (Warnants, VanOeckel, & Boucque, 1998). In this study the linseed treatment had the highest levels of PUFA in the backfat

Table 4.37: Processing yields and preparation losses of back bacon from different treatments.

	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Processing Yield:						
Injection Yield (%)	20	20	20	20	20	
24HYield (%)	16.31 ± 1.66	16.99 ± 0.74	16.58 ± 1.72	16.79 ± 1.35	15.91 ± 1.60	*P* = 0.507
Yield after smoking (%)	13.40 ± 2.17	14.11 ± 1.00	14.24 ± 1.57	13.57 ± 1.90	13.48 ± 1.85	*P* = 0.740
Preparation losses:						
Drip loss (%)	2.82 ± 0.11	2.71 ± 0.20	2.62 ± 0.44	2.49 ± 1.10	2.62 ± 0.63	*P* = 0.794
Cooking loss (%)	26.60 ± 5.13	24.32 ± 3.81	25.84 ± 3.00	25.39 ± 4.83	23.54 ± 3.34	*P* = 0.484
Total loss (%)	29.46 ± 5.06	27.18 ± 3.71	28.63 ± 2.95	28.09 ± 5.18	26.41 ± 3.39	*P* = 0.513

Means with different superscripts in the same row differ significantly

Table 4.38: Colour measurements of back bacon for different treatments stored at 4°C for 6 weeks.

		Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Day 0	L*	54.01 ± 3.80	53.71 ± 3.25	53.17 ± 3.94	53.83 ± 2.83	53.13 ± 2.74	*P* = 0.491
	a*	6.24^{b} ± 2.64	5.81^{ab} ± 1.76	5.42^{ab} ± 1.88	5.18^{a} ± 1.71	6.06^{ab} ± 1.53	*P* = 0.017
	b*	3.86^{b} ± 2.21	3.31^{ab} ± 1.88	3.23^{ab} ± 2.09	2.74^{a} ± 1.96	3.55^{ab} ± 1.84	*P* = 0.037
Week 3	L*	54.80^{c} ± 3.15	52.74^{ab} ± 2.90	52.26^{a} ± 2.46	52.45^{a} ± 3.13	54.31^{bc} ± 4.07	*P* < 0.001
	a*	4.83 ± 1.74	5.17 ± 1.96	4.50 ± 1.59	5.12 ± 1.84	5.33 ± 1.67	*P* = 0.085
	b*	4.65^{ab} ± 1.96	3.80^{a} ± 2.49	3.66^{a} ± 1.84	3.87^{a} ± 1.94	5.40^{b} ± 2.78	*P* < 0.001
Week 6	L*	56.30^{c} ± 3.84	52.69^{a} ± 2.89	54.09^{ab} ± 4.02	54.48^{b} ± 2.97	54.38^{b} ± 2.97	*P* < 0.001
	a*	4.52^{c} ± 1.39	3.88^{bc} ± 2.48	3.65^{bc} ± 1.62	2.70^{a} ± 1.40	3.61^{b} ± 1.87	*P* < 0.001
	b*	6.75^{c} ± 2.03	5.47^{ab} ± 2.06	5.28^{ab} ± 2.29	4.59^{a} ± 2.35	5.85^{bc} ± 2.15	*P* < 0.001

Means with different superscripts in the same row differ significantly

Table 4.39: Thiobarbituric acid reactive substances (TBARS) (mg malonaldehyde/kg meat) of bacon from different treatments during refrigerated storage at 4°C for 6 weeks.

	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Day0	0.08 ± 0.02	0.07 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	*P* = 0.403
Week3	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	*P* = 0.280
Week6	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	*P* = 0.496

Means with different superscripts in the same row differ significantly

Table 4.40: Demographic profile of consumer panel for bacon.

Gender:	% of Total	Age:	% of Total
Female	75	< 20	7
Male	25	20-29	57
		30-39	8
		40-49	8
		50-59	14
		>60	6

Table 4.41: Sensory properties of bacon from different treatments.

Sample	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Aroma	6.76 ± 1.59	6.65 ± 1.77	6.55 ± 1.83	6.80 ± 1.71	6.16 ± 1.95	*P* = 0.193
Taste	6.55 ± 2.04	6.71 ± 1.58	6.45 ± 1.87	6.82 ± 1.68	6.55 ± 1.62	*P* = 0.708
Texture	6.65 ± 1.75	6.73 ± 1.57	6.43 ± 1.70	6.66 ± 1.70	6.73 ± 1.58	*P* = 0.812
Overall Acceptability	6.64 ± 1.90	6.82 ± 1.54	6.53 ± 1.65	6.76 ± 1.66	6.60 ± 1.58	*P* = 0.824

Means with different superscripts in the same row differ significantly

at 19.98% (Table 4.11). Thus, given the criteria of Warnants et al. (1998) it is expected that the backfat from all the treatments would be suitable for salami manufacturing.

The effect of the experimental treatments on various quality parameters of salami are shown in Tables 4.42 and 4.43. Results in Table 4.42 indicates that the days to 30% moisture loss was significantly (P = 0.007) influenced by dietary treatment. Salami manufactures from the fish oil treatment needed a significantly shorter time to reach a 30% moisture loss compared to the other treatments. This is due to the higher pH after drying and the higher WHC from the meat from the fish oil treatment (Table 4.18; FSRE, 2005). Results in Table 4.43 indicates that, directly after manufacturing,

pH, total acidity (TA), water activity (Aw) and FFA were all significantly influenced by dietary treatment. The pH increased after fermentation, but decreased after drying. This is an unusual occurrence and the pH after fermentation should be in the range of 5.1 (Acton & Keller 1974). Marianski & Marianski (2009) suggested that the growth of yeasts and molds can cause an increase in pH. The yeast and molds utilises the lactic acid that causes an increase in pH and also results in salami with a milder taste. Experienced salami makers follow this step to produce salami with a milder flavour. They also state that the pH should be used as the factor to determine when fermentation is complete, rather than fermentation time itself. With every stage of production the total acidity increased due to the formation of lactic acid bacteria (LAB). There were small but significant differences (P < 0.001) between treatments for Aw and at every stage of production the Aw decreased. There was a significant (P < 0.001) increase in FFA after drying. There were small but significant differences between treatments for lipid stability after one month of refrigerated storage. According to Table 4.44 treatments with higher LC PUFA (linseed, fish oil and *Echium* oil) had significantly higher (P < 0.001) TBARS values. As the secondary oxidation products (TBARS) form the primary oxidation products decrease. This is why the PV from fish and *Echium* oil salamis were so low (Table 4.44). The anisidine value also measures the secondary oxidation products and the lower the AV the better the quality of the fat. The AV followed the same trend as the PV (Table 4.44).

The demographic profile of the 100 member consumer panel is shown in Table 4.45, which consisted of 64 females and 36 males. Consumers were unable to detect a difference between treatments for aroma. As in the case of pork bangers, the treatments with higher levels of PUFA scored lower for taste and aftertaste (Table 4.46). However the only significant difference was between the salami from the control and linseed oil salami in terms of taste (P = 0.041) and between the control, linseed and *Echium* oil salami for aftertaste (P = 0.015). The dietary treatment also had no effect on the texture of the salami. Although the treatments with higher PUFA scored lower for overall acceptability, the only significant difference for overall acceptability of salami was between the control and linseed treatment (P = 0.043).

Table 4.42: The number of days needed for each salami treatment group to reach a 30 % loss in moisture.

	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Number of days to reach 30 % moisture loss	$38.00^{b} \pm 1.55$	$38.50^{b} \pm 0.55$	$37.00^{ab} \pm 1.67$	$36.00^{a} \pm 1.10$	$38.00^{b} \pm 0.50$	$P = 0.007$

Means with different superscripts in the same row differ significantly.

Table 4.43: Changes in pH, total acidity, water activity and free fatty acids of the different treatment groups during processing.

Treatment	Stage of production	pH	Total acidity	Aw	Free fatty acids
Control		$5.51^{bc} \pm 0.05$	$0.58^{a} \pm 0.03$	$0.93^{de} \pm 0.01$	$1.38^{a} \pm 0.01$
Soya oil		$5.51^{bc} \pm 0.05$	$0.58^{a} \pm 0.01$	$0.93^{cde} \pm 0.01$	$1.28^{a} \pm 0.04$
Linseed oil	**After manufacturing**	$5.46^{b} \pm 0.02$	$0.57^{a} \pm 0.01$	$0.91^{abcd} \pm 0.00$	$1.36^{a} \pm 0.04$
Fish oil		$5.47^{b} \pm 0.03$	$0.61^{a} \pm 0.01$	$0.95^{e} \pm 0.01$	$1.41^{a} \pm 0.04$
***Echium* oil**		$5.51^{bcd} \pm 0.01$	$0.57^{a} \pm 0.01$	$0.90^{abc} \pm 0.00$	$1.22^{a} \pm 0.05$
Control		$5.60^{cde} \pm 0.04$	$0.73^{b} \pm 0.01$	$0.90^{abcd} \pm 0.01$	$1.63^{a} \pm 0.02$
Soya oil		$5.63^{de} \pm 0.01$	$0.74^{b} \pm 0.01$	$0.89^{ab} \pm 0.00$	$1.73^{a} \pm 0.02$
Linseed oil	**After fermentation**	$5.59^{cde} \pm 0.04$	$0.78^{b} \pm 0.00$	$0.91^{abcd} \pm 0.01$	$1.92^{a} \pm 0.09$
Fish oil		$5.60^{cde} \pm 0.03$	$0.78^{b} \pm 0.01$	$0.92^{cde} \pm 0.00$	$1.63^{a} \pm 0.07$
***Echium* oil**		$5.64^{e} \pm 0.03$	$0.78^{b} \pm 0.03$	$0.91^{abcd} \pm 0.01$	$1.45^{a} \pm 0.04$
Control		$5.05^{a} \pm 0.07$	$1.43^{c} \pm 0.01$	$0.89^{ab} \pm 0.01$	$5.31^{b} \pm 0.64$
Soya oil		$4.99^{a} \pm 0.05$	$1.52^{d} \pm 0.06$	$0.89^{ab} \pm 0.01$	$5.43^{b} \pm 1.01$
Linseed oil	**After drying**	$4.97^{a} \pm 0.07$	$1.65^{e} \pm 0.00$	$0.92^{bcde} \pm 0.03$	$4.65^{b} \pm 0.11$
Fish oil		$5.07^{a} \pm 0.04$	$1.64^{e} \pm 0.01$	$0.89^{ab} \pm 0.01$	$4.67^{b} \pm 0.22$
***Echium* oil**		$5.01^{a} \pm 0.04$	$1.60^{e} \pm 0.00$	$0.88^{a} \pm 0.01$	$4.50^{b} \pm 0.09$
Significance level		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Means with different superscripts in the same column differ significantly

Table 4.44: Lipid stability of salami after one month of refrigerated storage as measured by peroxide value (PV), thiobarbituric acid reactive substances (TBARS) value and anisidine value (AV).

Treatment	TBARS value	Peroxide value (PV)	Anisidine value (AV)
Control	$0.23^{a} \pm 0.01$	$8.00^{b} \pm 1.18$	$3.64^{ab} \pm 0.91$
Soya oil	$0.27^{a} \pm 0.02$	$10.67^{c} \pm 0.87$	$4.13^{b} \pm 1.23$
Linseed oil	$0.49^{c} \pm 0.05$	$9.70^{c} \pm 0.67$	$3.85^{b} \pm 1.16$
Fish oil	$0.35^{b} \pm 0.03$	$6.57^{a} \pm 0.51$	$2.23^{a} \pm 0.45$
***Echium* oil**	$0.36^{b} \pm 0.03$	$5.23^{a} \pm 0.74$	$2.78^{ab} \pm 0.76$
Significance level	$P < 0.001$	$P < 0.001$	$P = 0.009$

Means with different superscripts in the same column differ significantly

Table 4.45: Demographic profile of consumer panel.

Gender:	% of Total	Age:	% of Total
Female	64	20-29	52
Male	36	30-39	19
		40-49	12
		50-59	12
		>60	5

Table 4.46: Sensory properties of salami from different treatments.

Sample	Control	Soya oil	Linseed oil	Fish oil	*Echium* oil	Sign. level
Aroma	6.99± 1.38	6.85± 1.32	6.71± 1.39	7.00± 1.30	6.48± 1.55	$P = 0.114$
Taste	6.99^{b}± 1.38	6.89^{ab}± 1.19	6.35^{a}± 1.76	6.60^{ab}± 1.59	6.43^{ab}± 1.69	$P = 0.041$
Aftertaste	6.73^{b}± 1.49	6.61^{ab}± 1.38	6.05^{a}± 1.57	6.51^{ab}± 1.46	6.09^{a}± 1.64	$P = 0.015$
Texture	6.92± 1.50	6.53± 1.34	6.36± 1.55	6.41± 1.73	6.47± 1.61	$P = 0.189$
Overall Acceptability	7.04^{b}± 1.27	6.89^{ab}± 1.19	6.43^{a}± 1.51	6.75^{ab}± 1.40	6.52^{ab}± 1.58	$P = 0.043$

Means with different superscripts in the same row differ significantly

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Since Sinclair (1953) described that Greenland Eskimos have lower death rates from CVD due to their high dietary intake of fish oil, the role of omega-3 fatty acids in reducing CVD have been widely studied (Elagizi, et al., 2018). A wealth of evidence from epidemiologic studies and randomized controlled trials have demonstrated that the consumption of the very long chain n-3 PUFA, EPA and DPA can lower the incedances of CVD morbidity and mortality (Calder, 2004; Marik & Varon, 2009). It may also have the potencial to improve inflammatory conditions such as arthritis (Calder, 2008) and aid in eye and brain development (San Giovanni, Parra-Cabrera, Colditz, Berkey, & Dwyer, 2000). Fish and seafood are the richest sources of the long chain n-3 PUFA, EPA and DHA (Calder and Yaqoob, 2009; Harris et al., 2009).

The American Heart Association (AHA) recommends an average intake of about 500 mg EPA and DHA/day for CVD risk reduction (Siscovick et al., 2017). To meet dietary EPA and DHA recommendations, the AHA recommends the consumption of two servings of fish (particularly oily fish) per week (De Caterina 2011). This recommendation, however, is problematic as some people have fish protein allergies, or simply just don't eat fish and this recommendation is unlikely to alter this habit. Hence, there is a need to find alternative sources of LC n–3 PUFA for human nutrition

Meat, both muscle and adipose tissue is a major source of fat in the human diet and there is a lot of interest in modifying the composition of meat by dietary means, to improve its nutritional value. In non-ruminants the dietary fatty acids are absorbed unchanged from the intestine and incorporated directly in die adipose tissue (Wood, Enser, Fisher, Nute, Richardson & Sheard, 1999). Pork is the most commonly consumed meat worldwide (OECD, 2017) and therefore it would make sense to increase the LC n-3 PUFA content of pork to minimise the incidences of chronic diseases (Kitessa & Young, 2009).

As early as 1932 Banks and Hilditch (1932) found that feeding pigs diets containing marine oils increase the content of EPA and DPA. Unfortunately the use of seafood products and by-products is not a sustainable strategy due to overexploitation. In light of this uncertainty in the supply of LC n-3 PUFA some authors have recommended the production of LC n-3 PUFA in land plants through plant biotechnology.

Some vegetables oil contain n-3 FA as most of them are rich in α-linolenic acid, however the conversion of ALA to EPA and DPA is rather low (5-10% for EPA and < 1% for DHA) (Botelho, da Rocha Mariano, Rogero & Castro, 2013). It has been suggested that the low conversion of ALA to EPA and DPA is due to the rate limiting initial enzyme, Δ 6 desaturase (James et al., 2003). Hence, there seems to be very limited opportunity for using ALA containing oils to influence EPA and DHA levels in animal tissue. A plant with the potential as a n-3 source is *Echium plantagineum*. The seed oil from *Echium* contains stearidonic acid (SDA). Stearidonic acid is a

precursor of EPA and DHA, but unlike ALA, does not need Δ6-desaturase activity during the conversion (Botelho, et al., 2013). Therefore it seems possible that SDA will be more effective than ALA to increase the sought after LC n-3 PUFA, EPA, DPA and DHA.

Various studies have been conducted to determine the effect of SDA on the levels of LC n-3 PUFA. In humans, SDA supplementation caused a doubling in EPA, without any effect on DPA or DHA (James et al., 2003). In chickens, Rymer and Givens (2005) and Kitessa and Young (2009) found that SDA increased the levels of EPA and DPA, but had no effect on DHA. Bernal-Santos et al. (2010) and Kitessa and Young (2011) reported greater enrichment of milk fat with EPA, and a lesser extent DPA but no effect on DHA when cows were fed SDA. A study conducted on pigs by Miller and co-workers (2007) found that the EPA and DPA concentrations increased in the sow plasma, colostrum, and piglet plasma and that the DHA concentrations increased in the plasma of the new born and weaned piglets, however there was no increase in the plasma and colostrum of sows. To our knowledge no studies have been done on pigs to determine the effect of SDA on the meat quality, technological quality, fatty acid profile of meat and fat or sensory quality.

Therefore the purpose of this study was to determine whether *Echium* oil can be used as a surrogate for fish oil to enrich tissues in EPA, DPA and DHA without any adverse effects on animal production, meat quality, sensory quality or oxidative stability. In this study I focused on the influence of different n-3 sources, with specific reference to *Echium* oil on the production and meat quality parameters of pigs under commercial production conditions as well as on the chemical and sensory stability of processed meat products manufactured from such animals. Firstly a production study was performed with a control and 4 experimental diets to determine the effect of the inclusion of different n-3 sources on production efficiency and carcass quality.

There were small but significant differences between treatments for protein, neutral detergent fibre and acid detergent fibre. Diet did not have an effect on the average daily gain, average daily feed intake of feed conversion ratio. The FCR is especially important to the pork producer as the cost of feed accounts for almost two-thirds of the total cost of pork production. Therefore small changes in the FCR can have a considerable impact on the profitability of an operation.

Carcass quality was significantly influenced by diet. The linseed treatment had the highest lean meat content and the thinnest backfat. This resulted in the linseed treatment having the softest backfat. South African pork with an R classification should be suitable for the use in processed meat products (Hugo & Roodt, 2015). Only 8.3 % of the pigs from the linseed treatment had an R classification. Higher levels of PUFA are also associated with softer fat. Although the *Echium* treatment had high levels of PUFA in the backfat, it had the most pigs with an R classification at 58.3%. The different diets had no effect on the loin chop composition. It also had no effect on any of the colour parameter of the backfat, belly fat or muscle. Diet did have a significant influence on the pH_{45min} and the $temperature_{45min.}$ However none of the pork was

classified as PSE. The *Echium* treatment had the lowest drip loss, and there were very small but significant differences between treatments for WHC with the linseed treatment having the highest WHC. The hypothesis that feeding different omega-3 containing oils will have no effect on animal performance, meat quality or eating quality was therefore accepted.

In the quantitative fatty acid composition of the backfat, belly fat and muscle the *Echium* treatment had the highest levels of SDA. The *Echium* treatment also had higher levels of EPA and DPA in both the backfat and belly fat compared to the linseed treatment, however in the muscle this was not significant. *Echium* oil had no benefits over linseed oil in increasing the levels of DHA. In the fat tissue both *Echium* oil and linseed oil had no effect on the levels of DHA, but the muscle showed a small increase in DHA levels. The same trend was seen in the actual n-3 content of backfat, belly fat and intramuscular fat. The *Echium* treatment had significantly higher levels of total n-3 FA when compared to the linseed treatment. The meat from the linseed, fish and *Echium* treatments can be labelled as a source of n-3 FA as they all contain more than 30mg of n-3 FA per 100g muscle. Also a 180g pork chop from the linseed and *Echium* treatment will contribute 268.8 and 269.1% respectively to the 500mg recommendation for n-3 PUFA. Meat from the fish oil treatment will contribute 81.2% to the 500mg recommendation for the longer chain PUFA, EPA, DPA and DHA, followed by the *Echium* treatment at 17.5% and then the linseed treatment at 8.0%. Producing processed meat products enriched with n-3 PUFA would have great health benefits for consumers. If salami was produced from the meat from this experiment, a portion of salami (28g) from the *Echium* treatment would contribute 41.1% to the 500mg recommendation for total n-3 PUFA and 2.8% to the EPA, DPA and DHA. The 2.8% is still much lower than that of fish oil (12.6%) but higher than linseed oil (1.3%). Although *Echium* oil was unable to increase the levels of DHA in fat and muscle tissue, the significant increase in SDA itself can be of some health benefits to the consumer. In a human study, Surette, Edens, Chilton and Tramposh (2004) found that *Echium* oil supplementation to hypertriacylglycerolaemic subjects significantly decreased serum triacylglycerol by about 30% from the baseline. The hypotheses that all experimental diets will increase the n-3 content of pork tissue and that feeding *Echium* oil to pigs will increase the levels of n-3 PUFA that it can be labelled as a source of n-3 PUFA was therefore accepted.

To meet the demand of consumers for healthier pork, pork producers have started to manipulate the fatty acid composition of muscle and fat tissue (Wood et al., 2003). Pork naturally has a high C18:2 content, this results in a high PUFA:SFA ratio, but an unfavourable n-6:n-3 ratio. A PUFA:SFA ratio of more than 0.4 and a n-6:n-3 ratio of below 4:1 is recommended to improve human health (Ulbricht & Southgate, 1991; Simopoulos 1996; Scollan et al., 2001). In our study the linseed and *Echium* treatments conformed to these requirements in both the backfat and belly fat. However in the muscle, only the soya, linseed and fish oil treatments had a PUFA:SFA of more than 0.4 and none of the treatments had a n-6:n-3 of below 4.

Altering the fatty acid composition of pork can have an influence on the shelf life and eating quality of the pork (Isabel et al., 2003; Wood et al., 2003). Incresing the levels of PUFA decreases

the stability of lipid oxidation which is the primary process by which sensory quality declines (Gray, Gomaa, & Buckley, 1996). This was true for the present study as the meat from the linseed, fish and *Echium* treatments had significantly higher peroxide and TBARS values when compared to the control. This also resulted in the trained panel giving higher scores for a rancid old meat flavour due to the higher oxidation products in the meat from the linseed, fish and *Echium* treatments. The trained consumer panel also gave lower scores to the treatments with higher PUFA for taste, aftertaste and overall acceptability. The dietary treatments had little effect on the colour stability during refrigerated storage. At day 7 there were no significant differences between treatments for any of the colour parameters for either muscle or fat. The hypothesis that increasing the omega-3 content of pork will result in poor technological properties or chemical stability was accepted.

The physical characteristics of *M longissimus lumborum* was also unaffected by dietary treatment. There were no significant differences for drip loss, evaporation loss, thawing loss or total cooking loss. There were also no significant differences between treatments for the shear force measurements which are an indication of tenderness. Other tenderness measurements such as MFL and muscle fibre area also showed no difference between treatments.

One of the aims of this study was to compare the quality of processed meat products manufactured from meat obtained from animals fed *Echium* oil with control animals. It has now been well established that increasing the PUFA content of pork can have unacceptable effects on the meat and sensory quality of the animal tissue. Processed meat products are even more susceptible to lipid oxidation (Bryhni et al., 2002b). This was the case for the pork bangers in the present study. All treatments had high TBARS vlaues and from day 0 the bangers from the linseed, fish and *Echium* oil treatments had the highest TBARS values. After 9 days of refrigirated storage the bangers from the *Echium* treatment had a TABRS value of 6.07. Rancid, oxidized odours kan be detected at TBARS values of above 1 (Huang & Greene 1978). These high susceptibility to lipid oxidation had an effect on the consumer sensory analysis. The bangers from the linseed, fish and *Echium* treatments had the lowest scores for taste, aftertaste and overall acceptability with the *Echium* treatment getting the lowest scores. Bacon, on the other hand, had very low TBARS values and there were no significant difference between treatments. This is probably due to the anti-oxidative action of nitrite (Weiss, Gibis, Schuh, & Salminen, 2010) and smoking also has a preservative effect and protects products from oxidation (Pearson & Gillett, 1996). Therefore consumers also could not detect differences in bacon between treatments for taste, aftertaste or overall acceptability. Salami from the linseed, fish and *Echium* oil treatments had high TBARS values with the linseed treatment having the highest values. This high susceptibility for lipid oxidation was also reflected in the consumer analysis of the sensory properties. The linseed treatment had the lowest scores for taste, aftertaste and overall acceptability even though the TBARS value was below the value of 1 were Huang and Greene (1978) already detected rancid odours. There were no differences between treatments for the texture of the salami. The null hypothesis that processed meat products manufactured from meat

obtained from animals fed *Echium* oil and other omega-3 rich oils will oxidise faster than products from the control animals was accepted.

Dietary oil type had major effects on the fatty acid composition both of muscle and fat tissue and, and therefore it affected the nutritional quality and the suitability for further processing. Although *Echium* oil was unable to increase the EPA, DPA and DHA levels of pork tissue to the same levels as fish oil it did increase the LC n-3 PUFA to levels that will benefit the consumer. The increase in SDA itself will have benefits to consumers as a source of SDA, rather than a surrogate for EPA. A valuable contribution to the needs of the human diet could thus be made through the consumption of such meat. *Echium* oil was more effective than linseed oil in increasing the EPA, DPA and DHA, however this was not the case for total n-3 PUFA.

The SDA content of *Echium* oil is less than the ALA in linseed oil, therefore feeding pigs diets with a richer source of SDA will be an interesting topic for further research. Genetically modified (GM) soyabeans engineered to produce SDA (SDASOY) contains approximately 24% SDA (Elkin, Ying, Fan, & Harvatine, 2016) which is more than double the amount in *Echium* oil. Another topic for future research is the production of transgenic pigs. Lai and co-workers (2006) was successful in transferring the *fat-1* gene into pigs, thereby dramatically reducing the n-6:n-3 ratio. Saeki and co-workers (2004) transferred the fatty acid desaturation II gene for a Δ^{12} desaturase from spinach to pigs resulting in the production of ALA. Transgenic pigs with this gene results in increased n-3 PUFA and therefore a lower n-6:n-3 ratio. This technology has the opportunity to produce meat products that are rich in n-3 fatty acids and to meet the demand for healthier pork. However, it is still very controversial and it is unsure whether consumers will accept products from transgenic pigs.

CHAPTER 6

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CHAPTER 7

SUMMARY

The objective of this study was to determine the effect of *Echium* seed oil, together with other n-3 containing oils, on the levels of long chain n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA), eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) deposited in the animal tissue. Further, to determine the effect of these experimental diets on animal production and meat quality under commercial production conditions. The study also included the manufacturing of processed meat products from experimental animals and determining the chemical and sensory stability of such products.

Sixty Landrace x Large White crossbred pigs, with an average weight of ± 30 kg, were randomly divided into five treatment groups. These treatments comprised of a control diet, supplemented with 1% palm oil (BergaFat HPL-160) and four experimental diets supplemented with one of 1% soya oil, 1% linseed oil, 1% fish oil and 1% *Echium* oil. Pigs were fed until the average live weight was ± 110 kg.

Growth performance (weight increase, ADG and FCR) showed no significant differences between treatments. The only differences in carcass characteristics were the linseed treatment which demonstrated a smaller shoulder circumference and thinner backfat thickness compared to the other treatments.

Backfat from the control had the best technological properties as it had lower levels of PUFA and a lower double bond index (DBI) compared to the other treatments. The *Echium* treatment had the highest levels of stearidonic acid (SDA) (mg/100g tissue) in the back-, belly fat and muscle. In all three these tissues the *Echium* treatment also had higher levels of EPA and DPA, however it was still significantly lower than the levels in the tissue of the fish oil treatment. In both the back- and belly fat, the *Echium* had no effect on the levels of DHA, however in the muscle it did increase the levels of DHA. The levels of EPA, DPA and DHA was the highest in the fish oil treatment, however the *Echium* treatment had significantly higher levels than the other treatments.

There were no problems with lipid stability in either the fresh or frozen pork and values were far below the threshold value. A trained panel was unable to detect any sensory differences between the *Echium* treatment and the control. Processed meat products are more susceptible to lipid oxidation and increasing the levels of PUFA normally causes accelerated rancidity problems. In general pork bangers had very high TBARS and the *Echium* treatment had the highest values. The high TBARS values had a clear influence on the sensory properties as the bangers from the *Echium* treatment scored the lowest for taste, aftertaste and overall acceptability. Bacon did not have any problems with either the lipid stability or sensory properties, due to the anti-oxidative action of nitrite and the preservative effect of smoking. TBARS values for all treatments from the salami were all below the threshold value were rancidity could be detected.

Under the US Food and Drug Administration guidelines the meat from *Echium* oil treatment in this study can be labelled as a source of EPA, DPA and DHA as it contributed more than 10% to the daily recommendation. Pork products (bangers, bacon and salami) manufactured from meat of the *Echium* treatment can be labelled an excellent source of total n-3 PUFA as they contribute 91.5, 34.08 and 41.1% respectively. Therefore, pork and pork products from animals fed *Echium* oil can be considered functional foods as it will have a positive effect on human health. South African pig producers may therefore consider using *Echium* oil to improve the healthiness of their pork and marketing it as a health food.

Keywords: Stearidonic acid, eicosapentaenoic acid, eicosapentanoic, docosahexaenoic acid, *Echium* oil, fish oil, pig production, processed meat products, sensory, stability