THE EFFECT OF CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON THE QUALITY OF A CURED, FERMENTED PORK SAUSAGE

by

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DECLARATION

I declare that the dissertation hereby submitted by me for the degree M.Sc. Food Science in the Faculty of Natural and Agricultural Sciences at the University of the Free State is my own independent work and has not previously been submitted by me at another university or faculty. I furthermore cede copyright of this dissertation in favour of the University of the Free State.

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LIST OF ABBREVIATIONS

*a**	redness / greenness
ANOVA	Analysis of Variance
a_w	Water activity
*b**	yellowness / blueness
BC	Before Christ
BCCA	Branched-chain amino acid
BCCAs	Branched-chain amino acids
BF	Backfat
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
c	*cis*
cfu	Colony forming units
cm	Centimetre
CLA	Conjugated linoleic acid
°C	degrees Celsius
Δ	delta
DBI	Double bond index
DFD	Dark, firm and dry meat
e.g.	exempli gratia
et al.	*et alia*
F	Force
FA	Fatty acid
FA	Fatty acids
FAME	Fatty acid methyl esters

Individual FAME:

Complete Formula	*Common name*	*Systematic (IUPAC) name*
C14:0	Myristic	Tetradecanoic
C15:0	Pentadecylic	Pentadecanoic
C16:0	Palmitic	Hexadecanoic
C16:1*c*9	Palmitoleic	*cis*-9-Hexadecenoic
C17:0	Margaric	Heptadecanoic

C18:0	Stearic	Octadecanoic
C18:1*c*9	Oleic	*cis*-9-Octadecenoic
C18:1*t*9	Elaidic	*trans*-9-Octadecenoic
C18:1*c*7	Vaccenic	*cis*-7-Octadecenoic
C18:2*c*9,12(*n*-6)	Linoleic	*cis*-9,12-Octadecadienoic
C18:2*c*9,*t*11(*n*-6)	Conjugated linoleic	*cis*-9, *trans*-11Octadecadienoic
C18:2*t*10,*c*12(*n*-6)	Conjugated linoleic	*trans*-10,*cis*-12-Octadecadienoic
C18:3*c*9,12,15(*n*-3)	α-Linolenic	*cis*-9,12,15-Octadecatrienoic
C20:0	Arachidic	Eicosanoic
C20:1*c*11	Eicosenoic	*cis*-11-Eicosenoic
C20:2*c*11,14(*n*-6)	Eicosadienoic	*cis*-11,14-Eicosadienoic
C20:3*c*11,14,17(*n*-3)	Eicosatrienoic	*cis*-11,14,17-Eicosatrienoic
C20:4*c*5,8,11,14 (*n*-6)	Arachidonic	*cis*-5,8,11,14-Eicosatetraenoic
C21:0	Henicosanoic	Heneicosanoic
C22:6*c*4,7,10,13,16,19(*n*-3)	Docosahexaenoic	*cis*-4,7,10,13,16,19-Docosahexanoic

FFDM	Fat-free dry matter
g	gram
GRAS	Generally Regarded As Safe
h	hour
IMF	Intramuscular fat
IV	Iodine value
kg	kilogram
*L**	lightness
LAB	Lactic acid bacteria
M	Molar mass
µg	microgram
µl	microlitre
mEq	milliequivalents
mg	milligram
ml	millilitre

mm	millimetre
MRS	deMan, Rogosa & Sharpe
MUFA	Monounsaturated fatty acid
MUFAs	Monounsaturated fatty acids
n	sample size
n-3	Omega-3
n-6	Omega-6
NaCl	Sodium chloride
ND	Not detected
NS	Not significant
PEF	Pre-emulsified fat
pH	pH value
PI	Peroxidizability index
ppm	parts per million
PUFA	Poly-unsaturated fatty acid
PUFAs	Poly-unsaturated fatty acids
PV	Peroxide value
RBCA	Rose-Bengal Chloramphenicol Agar
RDA	Recommended Dietary Allowance
rH	relative humidity
SFA	Saturated fatty acid
SFAs	Saturated fatty acids
SFO	Sunflower oil
subsp.	Subspecies
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric acid reactive substances
t	*trans*
TFA	Trans fatty acid
TFAs	Trans fatty acids
TG	Triglyceride
TVA	Transvaccenic acid
TPA	Texture profile analysis

UFA	Unsaturated fatty acid
UFAs	Unsaturated fatty acids
UTM	Universal Testing Machine
UV	Ultraviolet
USDA	United States Department of Agriculture
VRBA + MUG	Violet Red Bile Agar + Methylumbelliferyl-β-D-glucoronide
WHC	Water holding capacity
WHO	World Health Organization
w / w	weight per weight

CHAPTER 1

INTRODUCTION

The term *sausage* can be traced back to Old Norman French *saussiche*, from the Late Latin *salsīcia* or from Latin *salsus* for “salted” (Harper, 2001-2012). Sausages were created as an innovative way to make economical use of less desirable cuts of meat and components such as blood and internal organs. The etymology suggests that sausages can be made from any salted meat. It is, however, traditionally applied to chopped pork stuffed into a casing. Sausages made from pork originated in Egypt and the Far East, as long ago as 5000 B.C., when it was discovered that salt could preserve easily perishable surplus meat. Homer’s *Odyssey* (800 B.C.), describing sausage making and consumption, is probably one of the oldest surviving documents. Consumption of sausages was even outlawed at one stage by Roman Emperor Constantine the Great and the early Christian Church due to its association with many pagan festivals. This gave rise to a black market distribution and made sausages highly sought after (Anonymous, 2007). In the Jewish and Arab cultures sausages made from pork are still ruled a taboo (Toussaint-Samat, 2009).

Sausage has a long and respected history throughout the world. The ancient Romans prepared a sausage from *mortarium* (salted pork) and myrtle berries which was the ancestor of the modern Mortadella, an Italian pistachio-flavoured sausage. In the fifth century B.C., reference is made to salami which is thought to have originated in the city of Salamis on the east coast of Cyprus (Predika, 1983). In one of the oldest recipe books, *Liber de Coquina* (Kitchen Handbook, circa 1300s), a fresh fish sausage prepared from fresh fish and herbs fried in cheesecloth is described. In Germany, each geographical location specializes in a *wurst* (sausage). Eastern Europeans make use of sausage recipes passed down across generations without any changes to the recipes. The Bedouin from North-Africa favour *mirqaz* (lamb and mutton), a popular sausage dating back to the Middle Ages (Snodgras, 2004). In South Africa *boerewors* is the main regional sausage (Hugo, Roberts & Smith, 1993). The recipe for boerewors appeared in the first Afrikaans cookbook in 1891 (Steyn, 1989).

Of major importance to the manufacturing of a large group of sausages (dry and semi-dry sausages) is the use of fermentation technology (Bacus & Brown, 1986). The term “fermented foods” can be defined as: “those foods which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food”. Food fermentations are regarded as the oldest use of biotechnology and although initially carried out unconsciously, now contribute 20-40% of our modern day food supply (Campbell-Platt, 1994).

Various spices also became known which contributed to preservation and flavour enhancement and led to the eventual creation of a category now known as dry sausages. Also known as summer sausages, dry sausages are prepared from cured meat without any heat treatment, although some form of smoking may be applied. Dry sausages are defined as “chopped or ground meat products, that as a result of bacterial action, reach a pH of 5.3 or less and are dried to remove 20-50% of the moisture” (Bacus & Brown, 1986). Characteristically, dry sausages have a tangy flavour due to fermentation and may be stored at room temperature or can be refrigerated for a longer shelf life (Anonymous, 2007). The term “curing” refers to the treatment of meat with salt and sodium nitrate and/or sodium nitrite. The curing process imparts a characteristic pink colour and flavour to the products which differ from products that do not contain nitrate and/or nitrite (Wirth, 1991b). The pink colour is due to the formation of red nitric oxide myoglobin from a reaction between nitrous acid and/or nitric oxide with metmyoglobin (Lücke, 1998). Famous examples of this type of sausage are primarily cervelat, pepperoni and salami (Predika, 1983).

As salami is a fermented food that does not undergo heat treatment and may enhance the survival of probiotic bacteria in the digestive system, it may be viewed as a probiotic food (Arihara, 2006; De Vuyst, Falony & Leroy, 2008). The probiotic status of salami coupled with numerous studies (listed by Beriain, Gómez, Petri, Insausti & Sarriés, 2011) in reformulating cured, raw sausages opened up a door towards functional meat products (Corino, Magni, Pastorelli, Rossi & Mourot, 2003). These meat-based functional foods can fulfil a number of new roles such as: improving the image of meat products; addressing modern day needs of consumers; and probably the most important, bringing meat products in line with new dietary recommendations. Consumers are known to be unwilling to change their dietary habits, thus considerable market potential exists for frequently consumed meat products with incorporated health benefits (Jiménez-Colmenero, 2007).

The most well known complaint surrounding pork is a high saturated fat content (Warnants, Van Oeckel & Boucqué, 1998). The fat content in formulating fermented, dry sausages such as salami is high and increases to 45-50% in some cases due to the drying process (Wirth, 1988). A simple reduction in fat content or use of more unsaturated fatty acids (UFAs) seems logical, but this creates other problems. Fat is vital to the rheological, structural and technological properties of meat products, thus low-fat products were found to develop technological problems (Keeton, 1994). The same problems develop at high levels of fat replacement (40-50% of the total fat) with more unsaturated oils (Del Nobile, Conte, Incoronato, Panza, Sevi & Marino, 2009). Use of leaner pigs has also been suggested. In leaner pigs a thinner and less saturated backfat (BF) layer leads to an increase in polyunsaturated fatty acids (PUFAs) with an inverse reduction in saturated fatty acids (SFAs). This strategy is, however, plagued with the deteriorating effects of PUFAs on the fat quality (Warnants *et al*., 1998). On the other hand, with increasing slaughter weight feed is converted with less efficiency and pigs produce carcasses with increased subcutaneous and

intramuscular fat (IMF) content (García-Macias, Gispert, Oliver, Diestre, Alonso, Muñoz-Luna, Siggens & Cuthbert-Heavens, 1996; Candek-Potokar, Zlender, Lefaucheur & Bonneau, 1998). Another result of increased slaughter weight is the increased degree of saturation of the BF and muscle lipids (Babatunde, Pond, Van Vleck, Kroening, Reid, Stouffer & Wellington, 1966; Allen, Bray & Cassens, 1967; Staun, 1972; Martin, Fredeen, Weiss & Carson, 1972). Increased slaughter weight improves fat quality (García-Macias *et al*., 1996) and heavier carcasses with more saturated and thus harder fat are seen as more desirable to the meat processor (Bruwer, Heinze, Zondagh & Naudé, 1991). It is clear that an inverse relationship between nutritional and technological qualities exists (Hugo & Roodt, 2007).

Another solution may be the use of conjugated linoleic acid (CLA) which is the collective name for a group of positional and geometric (*cis* and *trans*) isomers of linoleic acid (Thiel-Cooper, Parrish, Sparks, Wiegand & Ewan, 2001). The most representative isomers and also the most biologically active forms are the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. These isomers are known to be synthesized by the ruminal bacteria of herbivores and are also the main source of CLA in the human diet (Sirri, Tallarico, Meluzzi & Franchini, 2003). Various positive effects on human health such as anticarcinogenic, antioxidant and antiatherosclerotic effects as well as improvement of immune-responses have been linked to CLA (Hur, Park & Joo, 2007). With regards to animal feeding CLA is known to increase fat firmness and muscle marbling, both parameters that increase the economic value of pork. It also results in improvement of the technological and nutritional quality of lipids (Joo, Lee, Hah, Ha & Park, 2000; Corino *et al*., 2003). Very little CLA is synthesized in pigs (Chin, Storkson & Pariza, 1990) and, therefore, needs to be supplemented through the diet (Schmid, Collomb, Sieber & Bee, 2006). Conjugated linoleic acid supplementation in pig diets have already been shown to have no negative effects on the derived meat products such as fresh loin chops (Martín, Antequera, Muriel, Andrés & Ruiz, 2008a; Martín, Antequera, Muriel, Perez-Palacios & Ruiz, 2008c) or dry-cured hams (Corino *et al*., 2003). No information could be found in literature regarding the effect of dietary CLA supplementation of pork on the quality of cured fermented sausages.

The purpose and objectives of this study

The effect of CLA supplementation of pork (through dietary and direct addition) on the production of a fermented, dried sausage product (salami) will be evaluated to establish which possible changes may occur in CLA concentration in the end product. If the increase in CLA concentration is significant, this product may be regarded as a functional food due to the various therapeutic effects attributed to CLA. The possible effects of CLA on the fermentation and production of the salami as well as stability parameters of the fat content of the product will also be investigated. Furthermore, sensory evaluation and textural measurements will be carried out to determine if CLA

supplementation of pork affects the technological or organoleptic properties of a fermented dried sausage.

Objectives

I) To utilise dietary CLA supplemented pork to create a novel, cured and fermented pork sausage (salami) and to determine if quality (defined as: lipid composition; lipid oxidative stability; chemical parameters; microbial parameters; colour parameters; textural parameters and sensory attributes) is influenced by slaughter weight and dietary supplementation of CLA.

II) To utilise CLA supplemented pork (through the direct incremental addition of CLA) to create a novel, cured and fermented pork sausage (salami) and to determine if quality (defined as: lipid composition; lipid oxidative stability; chemical parameters; microbial parameters; colour parameters; textural parameters and sensory attributes) is influenced by direct addition of CLA.

CHAPTER 2

LITERATURE REVIEW

ABSTRACT

The aim of this literature survey was to provide an overview of pork fat with special emphasis on its use in the manufacturing of cured, fermented pork sausages. Various negative characteristics of pork fat such as its high saturated fatty acid content and the negative effects thereof on human health were discussed. In contrast to this, the technological importance of this type of fat was addressed together with a variety of quality criteria associated with pork fat. The discovery of conjugated linoleic acid, its numerous positive effects on human health and an overview of research carried out on this subject was discussed. This literature survey revealed that the use of CLA in improving the fatty acid composition and fatty acid content of salami has not yet been reported. There was also no information on how CLA could impart functional food characteristics on this type of product. Thereafter the literature review focused on an in depth discussion of salami and the underlying aspects of fermented sausage technology in an attempt to identify if CLA can be used to improve this premium type of meat product and what possible interactions could be expected between CLA supplementation and fermented meat technology.

Keywords: pork fat; nutrition; health; technological properties; fermented sausage; salami; functional food

2.1 Introduction

Meat and the consumption of meat products are increasingly being seen as the causes for increased risk of contracting chronic diseases such as obesity, cancer and stroke (Weiss, Gibis, Schuh & Salminen, 2010). In Europe, fresh meat consumption is already being depressed and a variety of factors are responsible. The individual consumer is consuming less meat, concerns about food safety are on the rise, and environmental concerns are becoming more important (Verbeke, Van Oeckel, Warnants, Viaene & Boucqué, 1999). Worldwide consumers increasingly demand healthier meat and meat products that have reduced levels of fat, cholesterol, sodium chloride and nitrite. They also want an improved fatty acid profile and expect the incorporation of health enhancing ingredients (Zhang, Xiao, Samaraweera, Lee & Ahn, 2010). For pork this is especially important as pork has gained a largely negative public image because of its high saturated fat content and a general misconception about the safety of pork as part of the human diet in certain populations (Aida, Che Man, Wong, Raha & Son, 2005.)

These views often neglect the importance of meat in maintaining human health and have forced not only the meat and meat product industry to react, but the food industry as a whole (Weiss *et al.,* 2010). Meat and meat products are major sources of protein, essential amino acids, lipids, vitamins, minerals and other nutrients (Biesalski, 2005). Salami type meat products are viewed as important sources of the above mentioned nutrients (Severini, De Pilli & Baiano, 2003). In the last decade there has been increased interest in the dietary supplementation of CLA in pigs due to improvements in carcass and meat quality traits and the enrichment of meat and meat products with CLA (Schmid *et al*., 2006; Martín *et al.*, 2008a; Marco, Juárez, Brunton, Wasilewski, Lynch, Moon, Troy & Mullen, 2009).

In 1979, Michael Pariza and fellow researchers found an antimutagenic substance in pan-fried hamburger. Almost a decade later they identified the substance as conjugated linoleic acid (CLA). A lot of research has been done on CLA in the first 15 years since its discovery and positive effects on cancer, cardiovascular disease, diabetes, body composition, immune system and bone health have been identified (Schmid *et al.*, 2006). Over the last decades interests in CLA have increased as a result of its potential health-related effects on humans and animal production (Khanal, Roy & Antolic; according to Zhang *et al*., 2010). It is viewed as an interesting new component in a completely new approach to reducing cholesterol uptake in mice (Weiss *et al.*, 2010). Since CLA was discovered in beef, a lot of research has been done on this animal model and it was found that the substance is actively synthesized in not only cattle but different species of ruminants (Schmid *et al.*, 2006).

Since different health benefits has been identified and attributed to CLA (Pariza & Hargraves, 1985; Lee, Kritchevsky & Pariza, 1994; Belury, Nickel, Bird & Wu, 1996; Park, Albright, Storkson, Liu, Cook & Pariza, 1999; Miller, Stanton & Devery, 2001; Smedman & Vessby, 2001), research moved away from only identifying sources of CLA to identifying ways of increasing the levels of CLA found in animal products with the goals to change animal fat composition and increase levels of CLA in the human diet. It was found that monogastric animals such as pigs do not actively synthesize significant amounts of CLA by themselves, but that supplementation of CLA in their diets led to measurable increases in CLA content (Chin, Liu, Storkson, Ha & Pariza, 1992; Schmid *et al.,* 2006). Furthermore, it was found that CLA supplementation increases fat firmness and muscle marbling, both parameters that increase the economic value of pork. CLA supplementation in pig diets have already been shown to have no negative effects on the derived dry cured meat products such as dry-cured loin (Martín *et al.*, 2008c). Antioxidant effects have also been reported for meat products from other animal types (Ip, Chin, Scimeca & Pariza, 1991; Du, Ahn, Nam & Sell, 2001; Hur, Ye, Lee, Ha, Park & Joo, 2004). A lot of research has been done on the supplementation of CLA in pigs, to increase the level of CLA in food products from these animals (Dugan, Aalhus & Kramer, 2004).

Increased attention paid to the relationship between food and human health and wellness has led to the creation of a whole new food class, known as functional foods. These foods are purported to contain components that have beneficial physiological and or therapeutic effects or are devoid of components that may have negative health effects depending on intake levels. Increasing amounts of clinical research are demonstrating tangible health benefits from the intake of bioactive compounds as part of a daily diet (Weiss *et al.,* 2010).

Bioactive compounds are defined by The Foundation for Innovation in Medicine as "...any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease" (IFIC, according to Weiss *et al.*, 2010). Various authors cited by Weiss *et al.* (2010) have found bioactive compounds to exhibit physiological beneficial effects after ingestion. Most of these bioactive compounds occur naturally, can be extracted from plant or animal sources and may add value to the commodities from which they are extracted. Most meat products inherently contain the potent bioactive compound CLA at around 0.3-5.6 mg/g of fat (Chin *et al*., 1992) with a variety of health benefits previously mentioned.

The aim of this literature survey was to provide an overview of pork fat with special emphasis on its use in the manufacturing of cured, fermented pork sausages.

2.2 Composition of fat in meat

Dietary recommendations have focused on the consumption of less saturated fat in the human diet and this led to increased focus on meats that contain more UFAs or PUFAs. On average, pork fat is composed of 47% mono-unsaturated fatty acids (MUFAs), 43% (SFAs) and 10% (PUFAs) (Warnants, Van Oeckel & Boucqué, 1998). A greater level of unsaturated lipids is expected to have a positive impact on human health (Byers, Turner & Cross, 1993; Cardenia, Rodriguez-Estrada, Cumella, Sardi, Casa & Lercker, 2011). Dry fermented sausages are one class of meat products with a high fat content of which the fat become visible when the product is sliced (Muguerza, Fista, Ansorena, Astiasaran & Bloukas, 2002).

Saturated fatty acids are converted to their corresponding MUFAs through the activity of the Δ^9-desaturase enzyme encoded by the stearoyl coenzyme A desaturase (SCD) gene (Smith, Lunt, Chung, Choi, Tume & Zembayashi, 2006). Almost half to more than half of the fatty acids (FAs) in pork fat consist of MUFAs, which are neutral to favourable with regard to cardiovascular diseases. Saturated fatty acids are the second largest group and are generally cholesterol-raising, except for stearic acid which in contrast to other SFAs, is not hypercholesterolemic. An increase in PUFAs, with a corresponding lowering of SFAs in pork fat may better follow the dietary guidelines of 10% energy from each group of FAs in the human diet (Warnants *et al*., 1998). Fat is an important part

of the diet as it has three physiological functions: it is a source of essential FAs, it is a carrier of fat soluble vitamins and it is an energy source (Mela, according to Muguerza *et al*., 2002).

Quality of the fat tissue in pork is highly influenced by the amount of dietary FAs and the composition thereof (St. John, Young, Knabe, Thompson, Schelling, Grundy & Smith, 1987; Cardenia *et al.,* 2011). Good quality fat from pork is defined as firm and white while poor quality fat is defined as being soft, oily, wet, grey and floppy (Wood, 1984). Bryhni, Kjos, Ofstad & Hunt (2002) found that backfat from pigs fed a high PUFA diet had less SFAs such as C16:0 and C18:0 and more C18:2, C18:3 and total PUFAs than BF from pigs fed a low PUFA diet. Increased PUFAs in feed were highly correlated with PUFAs in backfat. Levels of PUFA and C18:2 were around 50% lower in the BF than the levels in the feed.

With increased slaughter weight feed is converted with less efficiency and pigs produce carcasses with increased subcutaneous and intramuscular fat (IMF) content (García-Macias *et al*., 1996; Candek-Potokar *et al*., 1998). Increased slaughter weight also result in increased degree of saturation of the BF and muscle lipids (Babatunde *et al*., 1966; Allen *et al*., 1967; Staun, 1972; Martin *et al*., 1972). Increased slaughter weight improves fat quality (Garcia-Macias *et al*., 1996) and heavier carcasses with more saturated and thus harder fat are seen as more desirable to the meat processor (Bruwer *et al.*, 1991).

The amount of PUFAs that may be present in meat is limited, as increased concentration leads to decreased lipid oxidative stability. Lipid oxidation in meat products originate in the highly unsaturated phospholipid fraction of cell membranes (Boselli, Caboni, Rodriguez-Estrada, Toschi, Daniel & Lercker, 2005). The reduction of fat in meat products or the replacement with less saturated fat might have negative effects on the technological or sensory characteristics, especially in meat products such as frankfurters, sausages, patties and liver pâtés (Jiménez-Colmenero, 2000). Microbial spoilage and lipid oxidation are the primary processes for decline in sensory quality of meat (Gray, Gomaa & Buckley, 1996). Precooked products and products made from restructured meat are susceptible to lipid oxidation (Warnants *et al.*, 1998). The fatty acid (FA) composition of fat tissue may even have an effect on the microbiological flora associated with it. Increasing the oleic acid (C18:1) content of fat in ground pork was shown to alter the type of microflora during refrigerated storage (Moran, 1996). Fat contributes to the flavour, texture, mouth feel, juiciness and sense of lubricity of meat products. Any abrupt changes or reduction in fat can affect the acceptability of the products (Huffman & Egbert according to Muguerza *et al*., 2002).

2.3 Human dietary intake of CLA

The estimated optimal intake of CLA for humans is still inconclusive due to inconsistent results

found in scientific literature. Some doses proposed vary from 0.095 g CLA/day (Enser *et al*.; Knekt, Jarvinen, Seppanen, Pukkala & Aromma; according to Martín Ruiz, Kivikari & Puolanne, 2008b) to 3.5 g CLA/day (Ha, Grimm & Pariza, 1989). Current daily consumption by the average adult in the United States is about three times less than the amount of 3.5 g CLA/day. It is, therefore necessary to increase the CLA level in foods (Ip *et al.*, according to Hur *et al*., 2007). As there are no real measures of usual or actual intake of CLA, most values are only estimates. It is reported that the average estimated intake of CLA in the United States may be as low as 0.2 g/day (Ritzenthaler, McGuire, Falen, Shultz, Dasgupta & McGuire, 2001). In other countries such as Germany where the energy consumption from ruminant fat is much higher, intake of CLA is also much higher, at about 0.4 g/day (Steinhart, Rickert & Winkler, 2003).

A study done on a small group (22 persons) of free-living Canadians monitored their intake of *cis*-9,*trans*-11-Octadecadienoic acid (CLA) by analyzing two 70 day diet records taken six months apart. It was found that average intake of the isomer was about 0.1 g/day (Ens, Ma, Cole, Field & Clandinin, 2001). It was also found that the intake of CLA in men is higher than in women; this is probably due to the fact that men are more likely to consume fat from meat and dairy products and more of it (Ritzenthaler *et al.,* 2001; Steinhart *et al.*, 2003).

Foods from ruminant animals provide by far the most CLA in the diet (Lin, Lin & Lee, 1999; Parodi, 2003; Wahle, Heys & Rotondo, 2004). By estimates it was found that in the United States beef provided 32% and dairy products provided 60% of the intake of CLA (Ritzenthaler *et al.*, 2001). Interestingly, because products from ruminants contain two times or more vaccenic acid (the most predominant *trans* MUFA in ruminant fat) than CLA and ±20% of this vaccenic acid is converted endogenously to CLA in humans (by the Δ9-desaturase enzyme in tissues), it is estimated that the effective dose of CLA is in fact higher than that ingested (Parodi, 2003).

Commercial sources of CLA, such as those found in weight loss products (mostly in the form of capsules) provide additional CLA supplementation in the diet and complement the amount contained in food. The total CLA content and the isomeric distribution of CLA in these products are two important issues surrounding commercial CLA products. Until 2003 these issues were not closely examined. A study was carried out on four commercial preparations of CLA supplements to establish total CLA content, CLA isomeric distribution, FA composition, colour and hexane solubility (Yu, Adams & Watkins, 2003). The study indicated that attributes mentioned above, may differ significantly between commercial CLA supplements. For example, the total CLA contents were 65.1–77.9 mg/100 mg total fatty acids, while the *cis*-9,*trans*-11-CLA isomer accounted for 24.3–37.7 mg/100 mg total fatty acids. Differences may be explained by the composition of the fatty acids used in the original oils, the conditions under which isomerization takes place and other ingredients used in the different formulations (Yu *et al.*, 2003).

2.4 Biosynthesis of CLA in monogastric animals

Conjugated linoleic acids is made up of a family of isomers of linoleic acid (C18:2*c*9,12) that differ positionally and geometrically and are formed by biohydrogenation and oxidation processes that occur in ruminants (Figure 2.1; Dhiman, Nam & Ure, 2005*)*.

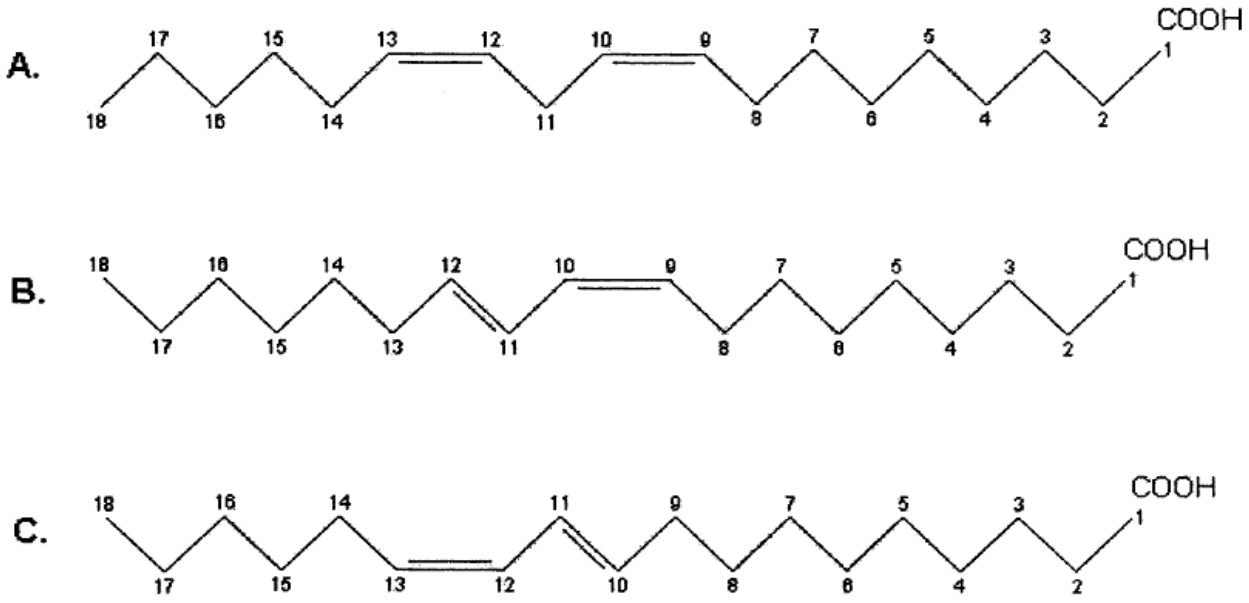


Figure 2.1: Abbreviated chemical structures of ordinary C18:2*c*9,12 (A) and the two major conjugated linoleic acids: C18:2*c*9,*t*11 (B) and C18:2*t*10,*c*12 (C) (Dhiman *et al.,* 2005).

Conjugated linoleic acid may also be synthesized endogenously in both ruminant and non-ruminant animals (Khanal & Dhiman, 2004). The availability of *trans*-vaccenic acid is higher in ruminants as a result of ruminal biohydrogenation which acts as substrate for CLA biosynthesis (Bessa, Santos-Silva, Ribeiro & Portugal, 2000). In contrast to ruminant animals, pigs are monogastric animals meaning that they only have one primary digestive stomach compared to ruminants such as cattle with a four part stomach. Pigs also have a much faster rate of passage of the stomach contents which also limits the potential for biohydrogenation of precursor FAs to CLA. Only a very small amount of CLA is produced via biohydrogenation and in general the total amount of CLA normally found in pork is as low as 0.1-0.2 mg/g fatty acids. As CLA is not further saturated before absorption, pork may be an ideal candidate for dietary supplementation of CLA. Conjugated linoleic acid is also deposited in tissues with relatively high efficiency. All of this indicates that pork could become a significant source of CLA in the human diet (Dugan *et al.,* 2004).

When pure transvaccenic acid (TVA) was fed to mice it was desaturated to CLA and it is presumed that the conversion occurred in the adipose tissue even though the liver is the site for fat synthesis

in non-ruminants (Santora *et al.* according to Khanal & Dhiman, 2004). A progressive increase in tissue CLA concentration was found when rats were fed increasing amounts of TVA (Banni, Angioni, Murru, Carta, Melis, Bauman, Dong & Ip, 2001). The conversion of TVA from the diet to CLA resulted in a dose-dependent increase in the accumulation of CLA in the mammary fat pads of rats. A high correlation between concentrations of CLA in the tissues and CLA in the blood, liver and mammary fat pads of rats fed varying concentrations of TVA and CLA was found. This suggests that with TVA as precursor, endogenous synthesis of CLA took place (Corl, Barbano & Ip, 2003).

It has been shown that the synthesis of CLA from TVA occur in humans and that several intestinal species of bacteria is capable of synthesizing CLA although the precise amount of CLA synthesis that occur endogenously or from intestinal bacteria has not yet been estimated for humans and non-ruminants (Adlof, Duval & Emken, 2000; Alonso, Cuesta & Gilliand, 2003; Coakley, Ross, Nordgren, Fitzgerald, Devery & Stanton, 2003). There have been no reports of CLA synthesis in monogastric herbivores. As investigators have detected very little or no CLA in chickens (Chin *et al.*, 1992; Yang, Huang, James, Lam & Chen, 2002; Raes, Huyghebert, Smet, Nollet, Arnouts & Demeyer, 2002) and pigs (Chin *et al.,* 1992) it needs to be verified where the CLA content in pigs and poultry comes from. It may come from the feeding of ruminant products such as meat, bone meal and blood meal, or there might actually be endogenous synthesis of CLA (Khanal & Dhiman, 2004).

In humans, dietary vaccenic acid can be converted endogenously to the *c*9,*t*11 isomer of CLA by Δ9-desaturase in tissues. Although it seems that the predominant source of CLA in humans comes from dietary intake of CLA (Salminen, Mutanen, Jauhiainen & Aro, 1998; Adlof *et al.*, 2000; Turpeinen, Mutanen, Aro, Salminen, Basu, Palmquist & Griinari, 2002; Kraft & Jahreis, according to Schmid *et al.,* 2006). Salminen *et al.* (1998) indicated that the serum CLA levels increased in subjects fed a diet rich in hydrogenated oil which contained vaccenic acid. In the same study, a mixture of almost equal portions of vaccenic acid and *trans*-12-18:1 was fed to subjects at three different levels. The results showed a linear increase in serum levels between vaccenic acid and CLA. On average the conversion rate of vaccenic acid to CLA was 19% (Salminen *et al.*, 1998). In a previous study by Adlof *et al.* (2000), it was reported that deuterium labelled vaccenic acid fed to a single subject showed a 32% increase in CLA in serum lipids.

2.5 CLA content of food

The concentration of CLA in foods has previously been mentioned to be the highest in foods from ruminants (beef, lamb). On the other hand, pork, seafood, most poultry products as well as vegetable oils are not notable sources of CLA (Parodi, 2003). Schmid *et al.* (2006) reported levels

of 3-8 mg and 5.4-7 mg CLA/g of fat in beef and bovine milk respectively. In Table 2.1 a summary of this evidence is shown with the total CLA content (mg/g fat) and the concentration of the important *c9*,*t*11-isomer (%) (Chin *et al.*, 1992). It was found that most research on CLA is focused on CLA in ruminants and ruminant products.

Table 2.1: Relative concentrations of CLA in uncooked meat products (Chin *et al.,* 1992).

Food	Total CLA (mg/g fat)	*c*9,*t*11-isomer (%)
Meat:		
Fresh ground beef	4.3	85
Beef round (meat cut)	2.9	79
Beef frank (beef hotdog)	3.3	83
Beef smoked sausage	3.8	84
Veal	2.7	84
Lamb	5.6	92
Pork	0.6	82
Poultry:		
Chicken	0.9	84
Fresh ground turkey	2.5	76
Seafood:		
Salmon	0.3	n.d.*
Lake trout	0.5	n.d.
Shrimp	0.6	n.d.

* not detected

2.5.1 In meat

The mean CLA content in various raw meats is given in Table 2.2. The highest levels of CLA can be found in meat from ruminants, with lower levels of CLA found in non-ruminant meat. Lamb contains the highest concentration of CLA (4.3-19.0 mg/g fat) followed by lower concentrations in beef (1.2-10.0 mg/g fat). The CLA concentration then drops to about less than 2.5 mg/g fat in pork, chicken, turkey and meat from horse. It was found that the CLA concentration in turkey meat is relatively high (2-2.5 mg/g fat), although there is still no clear indication of why this is so (Chin *et al.,* 1992; Fritsche & Steinhardt, according to Schmid *et al.,* 2006).

Table 2.2: Mean CLA content of various raw meats (Schmid *et al.,* 2006).

Lamb	Beef	Veal	Pork	Chicken	Turkey	Horse
			In mg/g fat:			
5.6	2.9-4.3	2.7	0.6	0.9	2.5	
	5.8-6.8					
11.0	3.6-6.2		0.7			0.6
	1.2-3.0					
	4.0-10.0					
4.32						
			In mg/g FAME (fatty acid methyl esters):			
12.0	6.5		1.2-1.5	1.5	2.0	
	2.7-5.6			0.7		
8.8-10.8						
19.0						

Some data was gathered on meat from animals not usually found in the human diet that included elk, bison, water buffalo and zebu-type cattle (a type of domestic cattle from South Asia) that varied in CLA concentration from 1.3-2.1 mg/g CLA per FAME in elk to 2.9-4.8 mg/g FAME in bison (Rule, Broughton, Shellito & Maiorano, 2002; Giuffrida de Mendoza, Arenas de Moreno, Huerta-Leidenz, Uzcatequi-Bracho, Beriain & Smith, 2005). The highest concentration of CLA in any meat source (38 mg/g fatty acids) was found in the adipose tissues of kangaroo (Engelke, Siebert, Gregg, Wright & Vercoe, according to Schmid *et al.,* 2006).

Variation in CLA concentration was not only found between different species of animals, but also between the same muscles of different animals from the same species. One group reported a low level of CLA in beef in their study (1.2-3.0 mg/g fat) and attributed the variation to factors such as seasonal variation, animal genetics and production practices (Ma, Wierzbicki, Field & Clandinin, 1999). In a study done by Dufey (according to Schmid *et al.*, 2006) on the variation in CLA concentration of beef from different countries, it was found that the variation was as large as 70% (3.6-6.2 mg/g fat). Beef from Argentina and Brazil showed the highest levels and beef from the United States showed the lowest levels. The findings of the study were ascribed to the differences in feeding between different countries. Due to the large variations between animals it was not possible to find any significant differences in the CLA content between breeds or beef muscles (Shantha, Crum & Decker, 1994; Raes, Balcaen, Dirinck, De Winne, Claeys, Demeyer & De Smet, 2003; Schmid *et al.,* 2006).

In Table 2.3 an increase in total SFAs can be seen with increasing concentration of CLA while at the same time a decrease in total UFAs can be seen. These findings are supported when looking at the examples of individual SFAs (palmitic acid and stearic acid) and PUFAs (linoleic acid and arachidonic acid). This data supports previously cited literature stating the same results in CLA supplemented pork.

2.5.2 In meat products

According to Schmid *et al.* (2006) there is little data available on the specific CLA content of meat products. The data that is currently available is given in Table 2.4. The CLA content per gram fat of the meat product is basically the same as that of the meat used in the product and it seems that it is not influenced by the method of processing (Chin *et al.,* 1992*;* Fritsche & Steinhardt, 1998).

During the production of fermented dry sausages for long-term storage, it is preferred that the fat has a high melting point and thus, a low level of UFAs. Firm BF from pork is mostly used to produce high quality dry sausages as sausages that contain beef tallow or fatty tissues from other ruminants are organoleptically less acceptable (Lücke, 1998). It is, therefore, possible that if some

variation in CLA concentration between the meat and final meat product is found, it may be explained by the practice of using fat from another animal source.

Table 2.3: Effects of dietary CLA supplementation on intramuscular fatty acid composition of pork loin as a % of total fatty acids (adapted from Joo, Lee, Ha & Park, 2002).

	Level of CLA (% of total fatty acids)			
Fatty acid composition	**Control**	**1% CLA**	**2.5% CLA**	**5% CLA**
Myristic acid	1.29	1.29	1.31	1.26
Palmitic acid	25.60	25.93	26.15	27.06
Stearic acid	15.08	15.68	15.84	16.19
Oleic acid	40.75^{a}	39.62^{ab}	39.03^{ab}	38.13^{b}
Linoleic acid	8.73^{a}	8.26^{b}	8.00^{bc}	7.64^{c}
CLA	0.01^{a}	0.37^{b}	1.01^{c}	1.16^{c}
Linolenic acid	4.23	4.56	4.74	4.95
Arachidonic acid	1.62	1.56	1.46	1.34
Total SFA	41.47^{a}	42.53^{b}	42.97^{bc}	44.06^{c}
Total UFA	57.58^{a}	56.49^{b}	56.08^{bc}	55.13^{c}

^{a,b,c} Means with different superscripts within the same row different significantly ($p < 0.05$) (n=5)

Table 2.4: Average CLA content of different meat products (Chin *et al.,* 1992; Fritsche & Steinhardt, 1998; Zhang *et al.,* 2010).

Meat product	**N**	**CLA content (mg/g FAME)**
Salami	2	4.2
Knackwurst	2	3.7
Black pudding	2	3.0
Mortadella	2	2.9
Wiener	4	1.5/3.6
Liver sausage	2	3.3
Cooked ham	2	2.7
Beef frank	2	3.3
Turkey frank	2	1.6
Beef smoked sausage	2	3.8
Smoked bacon	7	0.8-2.6
Smoked bratwurst	3	2.4
Smoked German sausage for spreading	2	4.4
Smoked ham	2	2.9
Smoked turkey	2	2.4
Minced meat	2	3.5
Corned beef	2	6.6
Potted meat	2	3.0

2.6. Factors influencing CLA content in meat and meat products

A variety of factors such as seasonal variations, animal genetics and production practices are responsible, however the single most important factor that influences CLA concentration is diet. This is explained by the fact that the components of the diet provide the various building blocks needed for the synthesis of CLA.

2.6.1 Diet of monogastric animals

In direct contrast to the sources of CLA in ruminants, the dietary fats in monogastric animals are unmodified after digestion and absorption. It then becomes necessary that the diet fed to these animals contain the desired level of CLA as it is, or that sufficient substrates for the endogenous synthesis of CLA in the animal tissues be available from the diet. The studies reported below make use of mixtures of a limited number of isomers of CLA, namely the *c9*,*t*11 and *t*10,*c*12-18:1 isomers (Schmid *et al.*, 2006).

Two studies were done on the effect of CLA supplementation on pigs by Gläser, Wenk & Scheeder (according to Schmid *et al.*, 2006). In the first study Large White pigs were fed a barley, wheat and soybean meal-based diet from 30 kg to 103 kg live weight. The diet contained either 6% high-oleic sunflower oil or various amounts of either 1.85% or 3.70% or 5.55% of a partially hydrogenated rapeseed oil which is high in transfatty acids (TFA). With increasing amounts of partially hydrogenated rapeseed oil in the diet, increasing amounts of CLA were reported, namely 3.8, 6.4 and 8.5 mg CLA/g FAME and only 0.9 mg CLA/g FAME in the sunflower oil control group. The phospholipid fractions were studied and the same type of, but more limited, effects were observed with CLA concentrations of 1.3, 3.2, 5.8, and 0 mg CLA/g FAME. In another study by the same researchers an increase in CLA concentration in the adipose tissue of Swiss Large White and Swiss Landrace pigs were found when the pigs were fed a control diet consisting of barley, wheat and soybean meal that included 5% partially hydrogenated fat (Schmid *et al.,* 2006).

The supplementation of CLA in the diet of pigs not only led to an increase of CLA concentration in adipose and muscle tissues, but also to changes in the composition of tissue FAs. There are reports that CLA supplementation increases the amount of SFAs (C14:0, C16:0 and C18:0) which leads to firmer bellies and loins and fewer problems during sausage production but SFAs is less desirable in healthy foods. Conjugated linoleic acid supplementation also leads to a decrease in the MUFA fraction (mostly C18:1) via down-regulation of the Δ9-desaturase enzyme (O'Quinn, Andrews, Goodband, Unruh, Nelssen, Woodworth, Tokach & Owen, 2000; Eggert, Belury, Kampa-Steczko, Mills & Schinckel, 2001; Ramsay, Evock-Clover, Steele & Azain, 2001; Smith, Hively, Cortese, Han, Chung, Casteñada, Gilbert, Adams & Mersman, 2002; Wiegand, Sparks, Parrish & Zimmerman, 2002; Lauridsen, Mu & Henckel, 2005; Joo *et al.,* 2002). These changes in FA composition were also seen in broilers fed a CLA supplemented diet (Du & Ahn, 2002; Aletor, Eder, Becker, Paulicks, Roth & Roth-Maier, 2003*;* Sirri *et al*., 2003).

Zhang *et al.* (2010) also came to the conclusion that using synthesized CLA in dietary supplementation can increase the content of CLA and change the FA profile of fat and muscle in non-ruminants. They concluded that dietary supplementation of CLA is a reasonable way of developing a value added meat product. Another option for increasing the level of CLA in meat products might be the direct addition of CLA as an ingredient during the manufacturing process. At

this stage there is only one study by Hah, Yang, Hur, Moon, Ha, Park & Joo (2006) where direct addition of CLA isomers to meat products was investigated.

2.6.2 Lactic acid cultures

In previous studies, it was found that some fermented dairy products contained higher levels of CLA than the non-fermented milk (Shantha, Ram, O'Leary, Hicks & Decker, 1995). An increase in CLA concentration was found from 4.4 mg CLA/g fat in the unfermented milk to 5.3 mg CLA/g in the final yogurt product, both with a fat content of 0.05%. A higher level of 8.81 mg CLA/g fat was found in Cheese Whiz than in unprocessed milk with 0.83 mg CLA/g fat (Ha *et al.,* 1989). An evaluation of cheese before and after 4 to 8 weeks of ripening found an increase in the CLA concentration due to the formation of CLA from linoleic acid in the cheese (Colbert & Decker, 1991). Jiang, Bjröck & Fondén (1998) also reported an increase in CLA in certain fermented dairy products. Werner, Luedecke & Shultz (1992) reported that neither different starter cultures nor aging caused any increase in the CLA concentration in Cheddar-type cheeses. There were also no changes observed in the CLA concentration of fermented dairy products such as low fat and regular yogurts or cheeses.

Six most commonly used lactic acid cultures (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *lactis, Lactococcus lactis* subsp. *cremoris*, *Lactobacillus lactis* subsp. *lactis* and *Streptococcus salivarius* subsp. *thermophilus)* were examined together with the addition of linoleic acid and different incubation times to determine the effects on CLA production in sterilized skim milk *in vitro.* The yield of CLA after fermentation was primarily dependant on the addition of linoleic acid as well as the strain of starter culture used. The added linoleic acid was effectively converted to CLA during fermentation. An incubation time of 24h and a concentration of 1000 µg/ml culture medium were most effective. Neither an increase in incubation time, nor an increase in the linoleic acid concentration in the medium showed meaningful enhancements (Lin *et al.,* 1999). Another possible effect of LAB on CLA is the possible oxidation of the free PUFAs. In a recent study it was reported that lactic acid oxidizes CLA isomers to oxylipins in the absence of sufficient antioxidants (Toomik, Lepp, Lepasalu & Püssa, 2012). Some of these oxylipins such as leukotoxins and leukotoxin diols are of significant health concern (Greene, Newman, Williamson & Hammock, 2000). As the pork used for salami manufacturing in this study will contain linoleic acid and lactic acid cultures will be used for the fermentation step, it could be possible to see a meaningful increase in the end product, if any.

2.6.3 Influence of processing and storage

2.6.3.1 Cooking

Very little work has been presented where the impact of cooking on the FA profiles of CLA supplemented (dietary or direct) meat products have been evaluated (Juárez, Marco, Brunton, Lynch, Troy & Mullen, 2009). Results currently show that CLA concentration of meat seems to not be affected measurably by cooking and storage. Juárez *et al.* (2009) added CLA (6-7% of the total lipids) to sausages to study the effects of cooking on CLA enriched products. It was concluded that CLA levels from dietary supplementation and direct addition of CLA were similar to that of the raw sausages. When different raw beef steaks were compared with cooked ones broiled to 80°C internal temperature, slight increases in CLA concentration (mg/g FAME) were found. Ground beef patties that were cooked either rare (60°C) or well done (80°C) via various cooking methods such as frying, baking, broiling and microwaving resulted in CLA concentrations that did not show large differences. Higher internal temperatures did, however, result in higher CLA concentrations. It was concluded that the cooking methods used did not cause any major changes in the CLA content when concentrations were compared on a mg CLA/g of fat basis. Cooking method and degree of doneness reduced fat content and the amount of edible portion accordingly and this was found to have an effect on the CLA concentration (Shantha *et al.*, 1994).

2.6.3.2 Storage

Lipid oxidation is mainly responsible for quality deterioration of meat during storage and any successful attempt to inhibit the formation of volatile secondary oxidation products will increase shelf life (Monahan, Buckley, Gray, Morrissey, Asghar, Hanrahan & Lynch, 1990). Shantha *et al.* (1994) examined the effect of cold storage on CLA concentrations in meat. The same cooking methods were used as described above and cooking was done to the same internal temperatures. The cooked beef patties were stored at 4°C for 7 days and CLA concentration and lipid oxidation were measured by analyzing for CLA content and TBARS (Thiobarbituric Acid Reactive Substances) on days 0, 2, 4 and 7. Oxidative deterioration was found, but no changes in the CLA concentration, which suggests a greater stability of CLA versus other PUFAs. Some of these same researchers also ran storage investigations on dairy products and found no decrease in CLA concentration during a storage period of 6 months (Shantha *et al.,* 1995). This supports their earlier finding of CLA's greater stability versus other PUFAs. Beef patties prepared with 0.5% and 2% CLA inclusion also showed no change in CLA content after 14 days of storage at 4°C (Hur *et al*., 2004). When CLA levels of various hard, semi-hard, soft, mouldy and processed cheeses made from various types of milk (buffalo, cow, goat and sheep) reported in 58 publications were analysed, the cheese ripening process was found to have no effect on CLA content of any of the cheeses (El-Salam & El-Shibiny, 2012).

The oxidative stability of frozen convenience foods such as frozen pizza is often limited by ingredients like dry sausages (De las Heras, Schoch, Gibis & Fischer, 2003). In another study by Martín *et al.* (2008b) pork fat in liver pâtés was partially replaced with CLA or olive oil or a combination of the two. No changes in SFA, PUFA, and MUFA isomer content of any of the batches were identified during 71 days of storage at 4°C. There were also no changes in the content of *c*9,*t*11 or *t*10,*c*12 CLA isomers.

2.6.3.3 Addition of spices

In a quest to extend the shelf life of meat products by inhibiting the development of rancidity, various synthetic antioxidants have been used such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate. Due to the ever increasing awareness of consumers of these synthetic additives there has been increased focus on the capabilities of natural antioxidants. The inhibition of rancidity by spices has been demonstrated and synergistic effects have been identified (Madsen & Bertelsen, 1995).

Various components of garlic and extracts of garlic have been found to have antioxidant activity and this activity is concentration dependent (Yang, Yasei & Page, according to Aguirrezábal, Mateo, Domínguez & Zumalacárregui, 2000). This demonstrates the effective hydroxyl radical scavenging capability of garlic. Substances found in garlic such as alliin, diallyl sulphide, allyl sulphide and propyl sulphide are responsible for this antioxidant effect. Garlic also contains ascorbic acid, nitrates and nitrites that have antioxidant effects (Mateo, Domínquez, Aguirrezábal & Zumalacárregui, 1996; Aguirrezábal *et al.*, 2000).

The research group of Spaziani, Del Torre & Stecchini (2009) attributed low recovery of lipid oxidation products from low-acid sausages to the antioxidative activity of spices, especially garlic. The possible antioxidant effect of spices added to a meat product should, therefore, be kept in mind even if the influence is barely significant.

2.7. Salami

2.7.1 History of salami

The Italian word *salami* is the plural form of *salame* which means “spiced pork sausage”. The word originates from Vulgar Latin *salamen* or *salare* which means “to salt” (Harper, 2001-2012) and refers to the salting which is used to prepare this highly seasoned, fermented and dried sausage. The diameter of the sausage can range from thin to very thick and is often named after the city or

region of origin. The place of origin also dictates the size of the sausage, coarseness or fineness of the meat blend and seasonings used (Smith, 2007).

Dry cured sausage is a traditional cured meat product in Italy and is produced according to different methods in the different regions of the country (Casiraghi, Pompei, Dellaglio, Parolari & Virgili, 1996). Salami was first produced by rural families in the Nebrodi Mountains in north eastern Sicily at least since the 19th century. They used the best meat cuts from a local pig *Nero Siciliano*, which included the shoulder, ham, backfat, belly and neck (Moretti, Madonia, Diaferia, Mentasti, Paleari, Panseri, Pirone & Gandini, 2004). This sausage was typically made with ground pork, cubes of fat and seasoned with garlic, salt and spices according to local recipes (Smith, 2007). This type of mixture was then stuffed into natural casings from pork and ripened in a room called a traditional room which was used before controlled storage.

Those sausages that achieved distinctive and pleasing sensory attributes were called "*Sant' Angelo*" sausages. Today the *Nero Siciliano* pig population has declined due to a negative demographic trend and uncontrolled crossing with commercial breeds. The traditional and local "*Sant' Angelo*" salami is now produced using commercial breeds and little has remained of the traditional recipes (Moretti *et al.,* 2004). Salami is usually made from pork, mutton and beef, although chicken and other types of poultry have also been used in the production of fermented sausages (Todorov, Koep, Van Reenen, Hoffman, Slinde & Dicks, 2007). Pork BF plays an important role in the technological and quality characteristics of salami products and affects flavour, colour and the drying process (Severini *et al.*, 2003).

2.7.2 Classification of salami

Salami is regarded a sausage which is classified either into the dry and semi-dry group or into the cooked, smoked sausage group (Table 2.5). Salami is regarded as being unique because of its homogenous appearance and malleability and it is also an important source of proteins of high biological value (Severini *et al*., 2003). Its quality is, however, highly dependent on the quality of the raw materials used and the level of technology applied in the production thereof (Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999).

2.7.3 Fermentation of sausages

Fermented sausages is the term used to describe those meat products which partially owe their microbiological stability and organoleptic properties (properties than can be measured with the senses) to a fermentation carried out by lactic acid bacteria (LAB). The ingredients in these products are typically comminuted (minced) meat and fat, mixed with salt, curing agents, sugar and

spices, which is then filled into casings (Lücke, 1998). These sausages have a high fat content. Commercial sausages may contain up to 30% fat, which is visible upon slicing (Wirth, 1991a). The vast majority of fermented sausages undergo curing which is understood as the addition of NaCl, nitrite and/or nitrate for the proper development of texture, colour and flavour (Olesen, Meyer & Stahnke, 2004).

Table 2.5: Sausage classification (Rust, 1987).

Classification	Characteristics	Examples
Fresh sausage	Fresh meat (mainly pork); uncured, comminuted, seasoned and usually stuffed into casings; must be cooked fully before serving	Fresh pork sausage, Bratwurst Boerewors (South Africa)
Dry and semi-dry sausages	Cured meat; fermented, air dried, may be smoked before drying; served cold	Gonoa salami Pepperoni Lebanon bologna Summer sausage Droë wors or Dry wors (South Africa)
Cooked sausage	Cured or uncured meats; comminuted, seasoned, stuffed into casings, cooked and sometimes smoked; served cold	Liver sausage Braunschweiger Liver cheese
Cooked, smoked sausages	Cured meats; comminuted, seasoned, stuffed into casings smoked and fully cooked; do not require further cooking, but some are heated for serving	Frankfurters Bologna Cotto salami
Uncooked, smoked sausage	Fresh meats; cured or uncured, stuffed, smoked, but not cooked; must be fully cooked before serving	Smoked, country-style pork sausage Mettwurst Kielbasa
Cooked meat specialties	Specially prepared meat products; cured or uncured meats, cooked but rarely smoked, often made in leaves, but generally sold in sliced, package form; usually served cold	Loaves Head cheese Scrapple

The longest tradition for making fermented sausages can be found in Southern and Central Europe. The highest production and per capita consumption figures can be found in countries such as Germany, Italy, Spain and France. From data compiled in 1995 the production figures within the European Union was estimated at about 750 000 tonnes per annum (Fisher & Palmer, 1995; Lücke, 1998).

Fermented sausages may have originated from the Mediterranean. The fact that ground meat with the addition of salt, sugar and spices becomes a palatable product with a long shelf life if prepared and ripened properly was known by the Romans. The moderate temperatures and frequent rainfall of the normal winter climate of the Mediterranean countries is favourable for successful sausage ripening. The production of fermented sausages in Germany only started about 160 years ago and most fermented sausages are smoked. In contrast in France, Italy and Spain air-dried, spicy sausages dominate (Lücke, 1998).

The classification of fermented sausages varies, and may be based on attributes such as moisture content, protein content, moisture/protein ration, weight loss, etc. Fermented sausages are microbiologically best classified on the basis of a_w (water activity) and surface treatment. Lücke (1998) classified sausages with an a_w between 0.90 and 0.95 as 'semi-dry' and those with an a_w below 0.90 as 'dry'. Modern types of fermented sausages may be divided into two groups: sausages ripened more than 4 weeks (firm texture, mildly acidic and salty taste) or semi-dry sausages, ripened between 7 and 28 days (less dry, strongly acidic, salty, mild taste and softer texture) (Houben & Van 't Hooft, 2005).

The time between the filling of the casing and the time when the product is complete is called 'sausage ripening'. The ripening time is subdivided into 'fermentation' (carried out by lactic acid cultures) and 'ageing' (drying of the product, formation of aroma) (Lücke, 1998). These types of sausages are also frequently smoked (Leroy, Verluyten & De Vuyst, 2006). Weight loss is reportedly dependent on temperature and relative humidity (rH) of the ripening room, air velocity inside the room, ripening time, degree of comminution of the meat, casing material, casing width and the amount of fat present in the sausage batter (Klettner, Roedel, Ott & Ponert, according to Del Nobile *et al*., 2009).

To ensure the stability and safety of fermented sausages starter cultures are of major importance (Buckenhuskes, Hammes & Knauf, according to Samelis, Metaxopoulos, Vlassi & Pappa, 1998). These starter cultures may consist of pure or mixed strains of LAB, *Micrococcaceae*, yeasts and moulds that stabilize the fermentation process by inhibiting spoilage and pathogenic bacteria (Bacus & Brown, 1986; Çon & Gökalp, 2000; Erkkilä, Petäjä, Eerola, Lilleberg, Mattila-Sandholm & Suihko, 2001; Fadda, Oliver & Vignolo, 2002; Amézquita & Brashears, according to Rai, Zhang & Xia, 2010). A common place practice in Europe is to add a *Micrococcus* or a *Staphylococcus* species, especially *Staphylococcus carnosus*, to a lactic culture. The role of the non-lactic member is to reduce nitrates to nitrites and to produce catalase that benefits the lactic culture (Jay, Loessner & Golden, 2005).

Starter cultures also have essential biochemical activities responsible for the development of characteristics unique to the end product (Lücke & Hechelmann; Hammes & Knauf, according to Samelis *et al.,* 1998). Lactic acid bacteria are regarded as essential agents during meat fermentation by improving the hygienic and sensory quality of the final product (Fadda, López & Vignolo, 2010). Organic acids and other secondary metabolites from LAB not only enhance the aroma of fermented meat, but also extend its shelf life (De Vuyst & Vandamme, according to Todorov *et al*., 2007). *Lactobacillus plantarum* have been found to regularly predominate the microbiota of fermented dry sausages (Deibel, Wilson & Niven, 1961; Jay *et al.*, 2005).

Bacteria play a role in the oxidation of free fatty acids as well as non-enzymatic reactions that occur (Molly, Demeyer, Civera & Verplaetse, 1996; Lizaso, Chasco & Beriain, 1999). Interestingly it was found that traditional sausages in which so called 'house flora' is used are often of superior quality to those sausages made with starters produced on an industrial scale (Hammes *et al.*; Comi *et al.*, according to Samelis *et al.,* 1998). Todorov *et al.* (2007) reported an initial inhibition and 10% reduction in cell numbers of various *Lactobacillus* spp*.* strains after smoking, but that the cultures soon recovered.

Fermented sausages with their high content of animal fat are considered to have a negative impact on health (Hu & Willet, 2002; He & MacGregor, 2007; De Vuyst & Leroy, 2010). Thus, a meat product with a lower fat content may offer health benefits. Unfortunately fat as an ingredient is difficult to reduce for technological and quality reasons. Wirth (1988) regards dry fermented sausages as the most difficult type of meat product in which to reduce fat content due to the fact that it loosens up the sausage mixture. This contributes to the continuous release of moisture necessary for the fermentation and drying processes. Fat also enhances sensory properties due to the oxidation of fatty acids and its contribution to mouth feel, juiciness and texture (Leroy *et al*., 2006; Gandemer, 2002).

2.7.4 Fat quality requirements of fermented sausages

Lipid characteristics are important in terms of the technological quality of pork (Hugo & Roodt, 2007). At least 30% of the raw material of dry-cured sausages is made up out of fat and thus contributes to several quality attributes (Houben & Krol, 1983). Pork BF is the main source of fat for manufacturing salami (Severini *et al*., 2003). Pork lipids with too high a level of unsaturation would result in pork that is unsuitable for sausage production and if used, would create products which is oxidatively unstable with accelerated rancidity problems (Teye, Wood, Whittington, Stewart & Sheard, 2006).

Relevant values for fat quality parameters are subject to great variation. This is attributed to the interchangeability of contributing factors such as genotype of the pig, sex, age, feeding conditions, commercial quality grade and fatty tissue localization within the carcass (Fischer, 1989a). The content of combinations of FAs, individual FAs and ratios of these FAs has been used extensively to predict fat quality. Stearic acid is reported to be a main contributor to fat hardness (Smith, Yang, Larsen & Tume, 1998; Wood, Richardson, Nute, Fischer, Campo, Kasapidou, Shear & Enser, 2004; Chung, Lunt, Choi, Chae, Rhoades, Adams, Booren & Smith, 2006). Adipose tissue Δ^9-desaturase activity was reported to be depressed when a mixture of CLA isomers was fed to pigs. This led to a dramatic increase in the level of stearic acid (Smith *et al.*, 2002). Increased PUFA levels are associated with increased occurrence of oxidation and rancidity and coupled with

MUFAs, give a greasy, oily and soft texture to the fat (Wenk, Häuser, Vogg-Perret & Prabucki, 1990). Maximum PUFA levels proposed for good quality fat range from < 15% (Houben & Krol, 1983) to as low as < 12% (Prabucki, according to Houben & Krol, 1983). Furthermore a level of 11% linoleic acid (C18:2) is suggested for salami and fermented sausages (Fischer, 1989b).

In terms of some important FA ratios: the nutritional guideline for *n*-6/*n*-3 PUFA in the human diet is a ratio of < 4:1. Unfortunately pork is relatively unbalanced in this regard, unlike the ruminants. Luckily the ratio of total PUFA/SFA in pork is much higher than the guideline of between 0.4 and 1.0 compared to the ruminants (WHO, 2003; Scollan, Hocquette, Nuernberg, Dannenberger, Richardson & Maloney, 2006; Wood, Enser, Fischer, Nute, Shear, Richardson, Hughes & Whittington, 2008). Very high *n*-6/*n*-3 ratios are linked to cardiovascular disease, cancer and inflammatory and autoimmune diseases and the inverse situation leads to suppressive effects on these diseases (Simopoulos, 2002). The MUFA/SFA ratio is more of a “catch 22” situation. Higher ratios are beneficial to human health and nutrition (Cordain, Eaton, Sebastian, Mann, Lindeberg, Watkins, O’Keefe & Brand-Miller, 2005; Muchenje, Dzama, Chimonyo, Strydom, Hugo & Raats, 2009), but lower MUFA/SFA ratios is better in terms of fat quality (Lea, Swoboda & Gatherum, 1970).

Several problems have been identified in the use of soft fatty tissue for the manufacturing of hard raw sausages. Fat of soft consistency will smear during comminution of the meat and fat to the desired particle size. This results in a cutting surface with meat and fat particles indistinguishable from each other and poor binding with resultant unsatisfactory hardness. Another negative result of fat smearing is the formation of a fatty film around the individual meat particles and on the inside of the casing which impairs water release (Fischer, 1989b). In relation to this is the percentage extractable fat as a criterion for fat quality (Hugo & Roodt, 2007). Fat tissue should actually contain fat at around 84-90% and deposited fat should be more than 15% of the total fat content (Prabucki, 1991). In one study the percentage of extractable fat was positively correlated with fat measures and negatively correlated with all lean measures as well as with protein and water content (Babatunde *et al.*, 1966).

Except for the expected tightening effect caused by shrinkage, due to the drying process, soft fatty tissue is also responsible for fat perspiring through the casing during smoking and aging. This perspiration effect can occur from > 15 °C, when the fat tissue has an iodine value (IV) of 66 and a C18:2 content of 11% (Ten Cate, according to Fischer, 1989b). It was observed that the C18:2 content decreased with increased backfat thickness (Whittington, Prescott, Wood & Enser, 1986). A C18:0/C18:2 ratio above 1.2 results in firm backfat and a ratio below 1.2 results in soft backfat (Honkavaara, 1989). Iodine value is a widely used parameter which is used to evaluate adipose tissue softness and gives an estimate of FA unsaturation (Davenel, Riaublanc, Marchal &

Gandemer, 1999). For example, the higher the IV and thus higher the level of lipid unsaturation, the softer the fat tissue will be (Fischer, 1989a). The analytical determination of IV refers to the attachment of iodine to the double bonds of fat (Prabucki, 1991). It is defined as the amount of iodine that will react with a 100 g of fat, and pork having a high level of unsaturated fat, will have a high iodine value of around 63-65 (Smith & Smith, 2011). Various cut-off points for IV have been suggested for good fat quality. Barton-Gade (1983) and Girard, Bout & Salort (1988) suggested an IV of 70, whilst Fischer (1989b) recommended the use of back fat with an IV of no more than 60 for use in the manufacture of hard uncooked sausages to avoid problems. Immature pigs deposit mainly unsaturated lipids, which leads to softer fat, while more mature pigs deposit more saturated lipids with resultant firmer, harder fat, preferred by the butcher and meat processor (Bruwer *et al*., 1991).

The atherogenicity index is indicative of cholesterol oxidation and lipid oxidation products that may act as atherogenic agents (raising blood lipid levels) and may exhibit mutagenic, carcinogenic and cytotoxic properties (Kanner, 1994; Chizzolini, Novelli & Zanardi, 1998). Another measure of the oxidative stability of fat is the double bond index (DBI) (Alam & Alam, 1986; Affentranger, Gerwig, Seewer, Schwörer & Künzi, 1996). A double bond index of less than 80 is suggested for a good quality back fat (Prabucki, 1991). The peroxidability index (PI) can also be used as an indicator of the oxidative stability and thus quality of fats (Pamplona, Portero-Otin, Riba, Ruiz, Prat, Bellmunt & Barja, 1998). No clear criteria for the PI is currently available (Hugo & Roodt, 2007), although lower values are regarded as preferable.

Another important physical quality parameter for fat is colour. Consumers prefer pork (BF) to be white, therefore any yellow discolouration, caused by rancidity (Barton-Gade, 1983) will be rejected. Colour measurement equipment, such as the Minolta chromometer, can be used to determine backfat colour with the *CIELAB L**, *a** and *b** colour co-ordinates (Tischendorf, Schöne, Kirchheim & Jahreis, 2002).

2.7.5 The use of pre-emulsified oils in salami technology

Regular fermented sausages are known to be high fat meat products with normal recipes having fat contents of about 32% fat directly after manufacturing. This may increase to a fat content of 40-50% after the drying process is completed (Wirth, 1988). Due to the importance of fat in this type of meat product as previously discussed, it is very difficult for fat to be reduced or replaced. Growing consumer health concerns (Verbeke *et al*., 1999; Weiss *et al*., 2010) and demand for healthier food and the incorporation of health enhancing ingredients (Zhang *et al*., 2010) led to increased interest about partial fat replacement and fat lowering strategies for various meat products in the first decade of this century. According to the World Health Organization (WHO, 2003), dietary fat intake

should only account to 15-30% of dietary energy intake. Healthier formulation of meat products is regarded as one of the most current approaches to developing potential meat-based functional foods (Jiménez-Colmenero, 2007).

It is also well known that most consumers are slow or unwilling to change their eating habits, opening up an opportunity for meat product manufacturers to present them healthier and new options (Jiménez-Colmenero, 2007). Until recently, most changes in the quantity and quality of lipids in meat and meat products were brought about through animal production practices, genetic and dietary strategies (Jiménez-Colmenero, Carballo & Cofrades, 2001; Jiménez-Colmenero, Reig & Toldrá, 2006). Directly manipulating the fat content of a particular meat product through partial replacement of the fat component, usually pork BF with CLA, olive oil, fish oil or various vegetable oils, have been studied over the years with relatively high levels of success in creating stable new products (Table 2.6.). Lipid oxidation of these usually high in PUFA oils may be problematic. Fish oils are very susceptible to lipid oxidation and often have a residual fishy aroma and taste. This may be minimized by refining and deodorizing the oil and by the use of antioxidants (Garg, Wood, Singh & Moughan, 2006). Olive oil is relatively stable due to its naturally occurring vitamin E, vitamin K, carotenoids and various polyphenols acting as antioxidants (López-Miranda, Pérez-Martínez & Pérez-Jiménez, 2006). CLA was also reported in various studies as being very stable even for long periods of time (Shantha *et al*., 1994; 1995; El-Salam & El-Shibiny, 2012).

In most situations these non-meat lipids were incorporated using an oil-in-water emulsion or pre-emulsion as it is sometimes referred to. It is easy to prepare and relatively stable when a non-meat protein such as sodium caseinate, whey protein isolate or soya protein isolate is used (Bishop, Olson & Knipe, 1993; Djordjevic, McClements & Decker, 2004). In one study, no noticeable release of exudate was found even after frankfurters prepared with a pre-emulsified oil was heated (Jiménez-Colmenero, Herrero, Pintado, Solas & Ruiz-Capillas, 2010). Bloukas *et al*. (1997) reported that when oil was directly added to fermented sausages the sausages were softer, whereas when the oil was first pre-emulsified using soya protein, no differences in texture was found. They also suggested that up to 20% of pork BF can be replaced with olive oil when soya protein isolate is used. Muguerza, Gimeno, Ansorena, Bloukas & Astiasarán (2001) found that Chorizo de Pamplona with pre-emulsified olive oil was softer than the control. Severini *et al.* (2003) found that pre-emulsified olive oil did affect the texture of salami, although not at a level significant to a sensory panel.

Table 2.6: Examples of research carried out on pork backfat replacement for various sausage types.

Meat product	Primary modification	Secondary modification	Research group
Fermented sausages	Partial replacement of pork BF with olive oil (10% and 20%)	Inclusion of olive oil as liquid oil or pre-emulsified fat (PEF)	Bloukas, Panera & Fournitzis, 1997
Dry, fermented sausages	Partial replacement of pork BF with olive oil (0% and 20%)	Pork BF inclusion at different levels (30%, 20% and 10%)	Muguerza *et al*., 2002
Salami	Partial replacement of pork BF with extra-virgin olive oil (0%, 33.5%, 50% and 60%)	None	Severini *et al*., 2003
Chorizo de Pamplona	Replacement of pork BF with soya oil (15%, 20% or 25%)	None	Muguerza, Ansorena & Astiasarán, 2003
Low-fat frankfurters	Partial reduction of pork BF (25%, 12.5% or 6.75%)	Inclusion of 5% olive oil in pre-emulsion	Lurueña-Martínez, Vivar-Quintana & Revilla, 2004
Italian salami	Substitute pork BF with extra-virgin olive oil (0%, 60% and 100%)	Either whey protein-based crumb or white pan bread soaked in extra virgin olive oil	Del Nobile *et al*., 2009
Pork breakfast sausages	Partial replacement of pork BF with 2% CLA	Direct addition and dietary supplementation of CLA	Juárez *et al*., 2009
Frankfurters	Pork BF replacement with emulsified olive oil (0% and 25%)	Various protein systems for oil-in-water emulsions	Jiménez-Colmenero *et al.*, 2010
Theoretical sausage	Replacement of pork BF with chemically modified vegetable oils (100%)	Different proportions of various vegetable oils interesterified	Ospina-E, Cruz-S, Pérez-Álvarez & Fernandez-López, 2010
Dutch-style fermented sausages	Partial substitution of pork BF with fish oil (15% or 30%)	Oil added as either pure, or commercial encapsulated product; as is or in pre-emulsion	Josquin, Linssen & Houben, 2011

2.8 Development of fermented, dry sausage characteristics

2.8.1 Flavour and aroma development

Pork is considered the most important raw material source for most producers of dry sausages. The sensorial quality of dry sausages depends on the characteristics of the raw materials, the technological parameters and the formation of end products from the breakdown of carbohydrates, lipids and proteins (Ordóñez, Hierro, Bruna & de la Hoz, 1999). Both endogenous muscle enzymes and microbial enzymes (indigenous and those from added cultures) are mainly responsible for flavour development through proteolytic and lipolytic actions. Non-enzymatic reactions, may also contribute to the characteristic aroma and taste of salami (Dainty & Blom, according to Van Schalkwyk, McMillin, Booyse, Witthuhn & Hoffman, 2011). Lactic acid produced by the starter culture may even be as important as the endogenous enzymes in development of the fermented meat flavour (Blom, Hagen, Pedersen, Holck, Axelsson & Næs, 1996). In Mediterranean areas intense acid flavours from rapidly ripened sausages are rejected by European consumers (Sanz, Vila, Toldra & Flores, 1998). Curing salts (NaCl, nitrate and nitrite) are also responsible for flavour

development although nitrate has been responsible for improved flavour development versus nitrite (Wirth, 1991b).

The various flavours and aromas originate from volatile compounds from ingredients added to the sausage batter or are produced during fermentation and ripening. Most likely sources are spices, wine, lipid oxidation products, microbial metabolism products and contaminants. Microbial metabolism products can be formed from carbohydrate fermentation, amino acid catabolism, esterification activity and lipid β-oxidation (Marco, Navarro & Flores, 2004; 2006). Lactic acid bacteria have a strong influence on the composition of non-volatile and volatile compounds through the release or degradation of free amino acids and the prevention of oxidation of unsaturated free fatty acids (Talon, Leroy-Sétrin & Fadda, according to Fadda *et al.*, 2010). Staphylococci are known to contribute to the generation of the typical fermented sausage flavour (Montel, Reitz, Talon, Berdagué & Rousset-Akrim, according to Olesen *et al*., 2004). Those volatile compounds originating from lipid oxidation are thought to be very important to the development of the characteristic flavour and aroma of dry fermented sausages (Ordóñez *et al*., 1999). After isolating the volatile compounds from a dry fermented sausage, Spaziani *et al.,* (2009) found that 95-98% of the desorbed volatiles came from spices (mainly black pepper and garlic) and wine. Sniffing analysis carried out on the effluent from GC separation of salame Milano volatiles confirmed the importance of spices (up to a third was traced back to spices) and lipid oxidation products (Meynier *et al.*, 1999). Volatile flavour compounds in dry fermented sausages have been identified as several chemical families such as alkanes, alkenes, ketones, aldehydes, alcohols, carboxylic acids, esters, sulphur derivatives and terpenes (Chizzolini *et al.,* 1998). Certain aldehydes and ketones are specifically responsible for the clear dry sausages smell due to their low flavour threshold (Chizzolini *et al.*, 1998). The volatiles from other types of Italian salami were also found to contain mainly terpenes and in some cases sesquiterpenes (Meynier *et al.*, 1999; Sunesen, Dorigoni, Zanardi & Stahnke, 2001; Moretti *et al.*, 2004). Within the group of terpenoids; α-pinene, β-pinene, 3-carene, limonene, anethol and caryophyllene were the most abundant compounds reported by Moretti *et al.* (2004) with no differences found in total terpenoid content in salami ripened under different conditions.

The changes that occur to lipids are strongly linked to flavour formation (Chizzolini *et al.,* 1998). Lipid oxidation products were reported as acids, alcohols, aldehydes, ketones and lactones. Aldehydes were produced as a result of the degradation of UFAs. These aldehydes may then have been reduced to their corresponding alcohols by alcohol dehydrogenase, thus some of the alcohols found were derived from lipid oxidation (Ordóñez *et al.*, 1999). Free fatty acids are necessary to an extent; they are important precursors of volatile molecules from oxidation with and without the action of microorganisms (Molly *et al.*, 1996).

Volatile compound derivatives from carbohydrate fermentation (by microorganisms) were reported as acetic acid, butanoic acid and alcohols. Acetic acid is contributing to the dry sausage aroma and is mainly produced by homofermentative LAB and staphylococci, as well as alanine catabolism and FA oxidation (Montel, Masson & Talon, 1998). Ethyl esters give ripe and fruity flavours to sausages (Montel *et al.,* 1998; Meynier *et al.*, 1999). Lactic acid bacteria are reported to express proteolytic activity, mainly intracellular amino-, di- and tripeptidases (Montel, Seronine, Talon & Hebraud; Sanz & Toldrá, according to Fadda *et al.*, 2010). Branched chain amino acids (BCAA's) such as valine, leucine and isoleucine produce branched aldehydes, alcohols and acids, which contribute to the aroma of dry fermented sausages (Stahnke, according to Spaziani *et al.*, 2009). Nitrate is reported to increase the concentration of these compounds originating from BCAA's (Olesen *et al.*, 2004). Production of free amino acids and small peptides is considered more important for taste development in dry sausages. Their contribution to aroma appears to be limited in comparison to lipolysis and lipid oxidation (Berdagué *et al.*; Johansson *et al*., according to Mateo & Zumalacárregui, 1996).

Non-volatile components provide the basic tastes such as sweet, sour, salty and bitter as well as the recently accepted fifth taste, umami, from glutamates naturally present in protein rich foods such as meat, fish and fungi (Yamaguchi, Yoshikawa, Ikeda & Ninomiya, according to Fadda *et al.*, 2010). The smoking step applied to salami and many other types of semi-dry sausages introduces many organoleptic changes detectable in the flavour. This may be directly through the absorption of chemicals such as phenols, carbonyls and organic acids in the smoke by the salami or indirectly, through affecting the antimicrobial and antioxidant properties of the sausage (Ojeda, Bárcenas, Pérez-Elortondo, Albisu & Guillén, 2002; Maga, according to Van Schalkwyk *et al.,* 2011).

2.8.2 Colour development and measurement of colour

As a direct result of the fermentation step, the lowering of pH in fermented sausages leads to the destabilization of myoglobin and increases the rate of oxidation to metmyoglobin. The heme group is dissociated and the expected bright red colour is primarily attributed to nitrosomyoglobin formation due to the reduction of nitrates (Mendiolea, Guerrero & Taylor, 1995; Slinde & Nordal; Lawrie, according to Van Schalkwyk *et al.*, 2011). Contact of nitrosomyoglobin with air will cause oxidation to metmyoglobin and the colour will change to brown (Mendiolea *et al.*, 1995). Zeuthen (1995) regard LAB strains as contributors to desirable colour change. Lactic acid bacteria also contribute to the stabilization of colour in fermented meat (Talon, Leroy-Sétrin & Fadda, according to Fadda *et al.*, 2010).

Another lesser known contributor to colour development is the smoking step. The formation of phenolic compounds decreases lipid oxidation and the pyrolysis of the cellulose and

hemicelluloses in the casing of the salami produces carbonyls that are important in the development of the colour of the meat (Severini *et al.*, 2003; Price & Schweigert, according to Van Schalkwyk *et al.*, 2011).

In a study on the effect of CLA supplemented pork used in dry cured ham (Parma ham) it was found that CLA had no effect on the meat colour. This confirms the work of a few other research groups (Dugan, Aalhus, Jeremiah, Kramer & Schaefer, 1999; Eggert *et al.*, 2001; Joo *et al.,* 2002) who also found that meat colour was unaffected by dietary CLA. However, one research group (Wiegand *et al.*, 2002) did report that chops from CLA supplemented pork appeared to be more yellow.

The freshness of meat is perceived through its colour and this makes colour critical to the meat industry and meat science research (Mancini & Hunt, 2005). Various methods for objectively measuring the colour of foods have been developed. Earlier methods consisted of pigment extraction followed by spectrophotometric determination of the pigment concentration (Hornsey, 1956; Agulló, Centurión, Ramos & Bianchi, 1990). These initial methods were found to be tedious and time consuming and simpler methods were developed for measuring colour from food surfaces with reflected light. Numerous colour spaces were developed as standard references such as the *CIE L* a* b** colour coordinates *L*, a** and *b*.* The *L** coordinate is the measure of lightness with 0 equal to black and 100 equal to white; positive values for *a** indicates redness and negative values indicate greenness; positive values for *b** indicates yellowness and negative values indicate blueness (Pérez-Alvarez, Sayas-Barberá, Nadal & Fernández-López, 2011). Reported *a** values explained more than 90% of pigment content and form variation of pork muscles (Lindahl, Lundström & Tornberg, 2001). Hue angle describes the development of colour from red to yellow, with larger angles indicating a less red product. Chroma describes the saturation or vividness of colour with higher values indicating higher levels of colour saturation (Miltenburg, Wensing, Smulders & Breukink, 1992; Wyszecki & Styles; Renerre, according to Ripoll, Joy & Muñoz, 2011). Fat quality highly influences the optical characteristics (Irie, 2001) and this will be more pronounced in a processed meat product with highly visible pieces of fat. Added ingredients such as salt, phosphates and curing agents will affect the optical absorption of the meat product (Pérez-Alvarez *et al.*, 2011) and any variations in the amounts if these ingredients may introduce unwanted variables.

2.9 Texture and texture measurements of sausage products

Low acid dry fermented sausages are reported to have low hardness and cohesiveness even after weight loss of 30-35% which takes place through a loss in moisture content (Spaziani *et al*., 2009). Major changes in the structure of a fermented sausage takes place during the fermentation step

when the pH drops and the myofibrillar proteins aggregate to form a gel structure (Houben & Van ‘t Hooft, 2005). When the pH drops to below the isoelectric point of the myofibrillar proteins, the values for hardness and chewiness increase. After fermentation, binding and rheological properties are affected by the drying process (Houben & Van‘t Hooft, 2005; González-Fernández, Santos, Rovira & Jaime, 2006). Ruiz de Huidobro, Miguel, Blázquez & Onega (2005) found that cohesiveness, representing the strength of the internal bonds making up the sample, correlates negatively with pH values below 5.0.

When considering adhesiveness, the adhesion force can be described as a combination of an adhesive force and a cohesive force. The adhesive force is high and the cohesive force is low when a foodstuff is found to be sticky (Hoseney & Smewing, 1999). Dry matter content and pH of a sausage have been found to play a significant role in adhesiveness (Herrero, Ordóñez, De Avila, Herranz, De la Hoz & Cambero, 2007). Parameters such as product formulation, ionic strength, functionality of meat proteins and the content and characteristics of fat may influence the textural properties of meat products (Cavestany *et al*., according to Martín *et al.*, 2008b).

In the process of selection and consumption of food products, texture is an important factor for consumers (Moskowitz & Jacobs, 1987; Szczesniak & Kleyn, according to Guerrero, Gou & Arnau, 1999; Houben & Van‘t Hooft, 2005). Textural properties such as tenderness and juiciness are very important in consumer quality perception and may influence acceptability (Szczesniak, according to Guerrero *et al.*, 1999).

Although dry-cured products, such as ham do not experience drip loss, some moisture losses can be produced during storage that could affect the textural properties of the product. Córdoba (according to García-Estaban, Ansorena & Astiasarán, 2004) observed higher shear force values in dry-cured ham with significantly lower moisture content. The resulting greater hardness of drier samples is partially due to the fact that shrinkage is proportional to water loss in meat products (Spaziani *et al.*, 2009). The consistency of fermented sausages is largely determined by the pH value as well as the a_w level of the product (Van Schalkwyk *et al.*, 2011). The consistency changes from a soft batter to hard, sliceable salami (Todorov *et al.*, 2007). Lactic acid bacteria are reported to facilitate cohesion of the meat particles in fermented meat products (Zeuthen, 1995).

With regard to consumer acceptance of food, textural characteristics are regarded as important aspects. Quite a few instrumental methods have been developed in the determination of food textural properties, with special reference from Herrero *et al.* (2007) to those of Bourne (1978). The compression method of texture profile analysis (TPA) which mimics conditions to which food is subjected to during mastication (chewing) is the most commonly used instrumental method. It was first developed for the General Foods Texturometer (Szczesniak, Brandt & Friedman, 1963) and

then later adapted for use with the Instron Universal Testing Machine (Bourne, 1978). Many authors (cited by Herrero *et al.,* 2007) have used the compression parameters obtained from TPA in their evaluation of meat products such as dry fermented sausages to establish finished product quality and for the selection of the best functional ingredients. Another widely used test is the Warner-Bratzler shear force test which is used with a Warner-Bratzler apparatus to measure the maximum shear force. However, the results obtained usually show tenderness discrepancies after estimation with trained sensory panels or other objective measurements (Lepetit & Culioli, 1994; Shackelford, Wheeler & Koohmaraie, 1995; Timm, Unruh, Dikeman, Hunt, Lawrence, Boyer Jr. & Marsden, 2003).

Textural measurements can be based on the resistance of the sample to the force of deformation such as puncture, cutting, shear and tensile tests. Dependent variables such as adhesiveness, breaking strength, cohesiveness, fat particle size, hardness, springiness and non-dependent variables such as a_w, dry matter, fat content and pH may be considered. These methods are reportedly simple and have the added advantage of being correlatable with sensory analysis of texture, meaning results from these methods may support those from sensory analysis. Spaziani *et al.* (2009) reported using TPA with an Instron UTM (Universal Testing Machine) on sample cylinders (25 x 30 mm diameter) for the measurement of hardness, cohesiveness and adhesiveness. Van Schalkwyk *et al.* (2011) also reported using TPA with the Instron UTM on cylindrical cores (15 x 22 mm diameter) for the measurement of hardness, cohesiveness and gumminess. Casiraghi *et al.* (1996) reported using the Instron UTM on cylindrical cores (15 x 25 mm diameter) for measurement of hardness. For shear force determinations on Pariza salami, Raphaelides, Grigoropoulou & Petridis (1998) used an Instron UTM with a Warner-Bratzler attachment on cylindrical samples (50 x 21 mm diameter). They also determined compression force at 10% deformation of the initial sample height. The cylindrical samples were taken from the centre of the salami, perpendicular to the long axis, using a metal borer with 21 mm inner diameter.

Breaking strength gives information about the binding force between the fat and meat particles inside dry fermented sausages. Data could be generated about how these products behave when they are sliced, vacuum packaged and then opened at a later stage during consumption. For example, after opening the vacuum packaging and the sausage slices are taken out, they tend to break. This happens because the product breaking strength is less than the superficial adhesion force between the product surfaces. It would be useful to know if sausages are suitable for slicing and vacuum packaging beforehand (Herrero *et al.,* 2007).

2.10 Sensory characteristics of sausages

In sensory analysis the aim is to determine the probable product acceptance by consumers in the early stages of development. Reliable results are obtained from studies carried out with the target population and these results are then widely used in developing new products (Ruiz, Villanueva & Contreras-Castillo, 2010). Examples of sensory analysis in literature were found to have mainly made use of trained panels although there are some examples of consumer panels. The nine point hedonic scale (where 1=dislike extremely and 9=like extremely) was preferred by most research groups. Samples were coded with random three digit codes and served in a randomized order (Moretti *et al.,* 2004; Herrero *et al.*, 2007; Todorov *et al.*, 2007; Del Nobile *et al.,* 2009; Spaziani *et al.*, 2009; Ruiz *et al.*, 2010; Van Schalkwyk *et al.*, 2011). Discriminant sensory attributes reported include: appearance as colour intensity, colour non-conformity, dullness, redness, compactness; texture as elasticity, fat/lean ratio, fat/lean demarcation, hardness, cohesiveness, chewiness, fatty mouth feel, juiciness, overall acceptability; aroma as garlic, black pepper, game, salami, wine, sour, rancid or sweet, and flavours as salty, level of saltiness, acid, level of acidity, salami, game, smoky, sweet or mouldy (Moretti *et al.*, 2004; Ruiz Pérez-Cacho, Galán-Soldevilla, Crespo & Recio, 2005; Todorov *et al*., 2007; Spaziani *et al.*, 2009; Van Schalkwyk *et al.*, 2011). Sausage slice thickness was reported as 0.15-0.5 cm and served at 6-18°C or room temperature with water and unsalted crackers as palate cleansers and cling wrapped prior to each session (Ruiz Pérez-Cacho *et al*., 2005; Del Nobile *et al.*, 2009; Spaziani *et al.*, 2009; Van Schalkwyk *et al*., 2011).

A few researchers (Dugan *et al.*, 1999; Wiegand *et al.*, 2002) reported that CLA incorporated in the finishing diets of pigs had no significant effect on tenderness, juiciness or flavour intensity of cooked pork. No effect on sensory characteristics or Warner-Bratzler shear force values has been reported (Larsen *et al.*, according to Corino *et al*., 2003).

2.11 Functional foods

2.11.1 The functionality of foods

There is still no clear and precise definition of what a functional food is. Roberfroid (2000) mentioned that a functional food should “contain a component with a selective effect on one or various functions of the organism whose positive effects can be justified as functional (physiological) or even healthy”. Jiménez-Colmenero *et al*. (2001) stated that three basic requirements should be considered for a functional food. This includes: 1) It should be derived from naturally occurring ingredients; 2) be consumed as part of a daily diet; and 3) be involved in regulating specific processes for humans including delaying the aging process, preventing the risk of disease and improving immunological ability.

Acceptability of functional foods by consumers varies widely and is dependent on their social, economical, geographical, political, cultural and ethnic backgrounds (Jiménez-Colmenero *et al*., 2001). The first country to develop the idea of functional food was Japan, where between 1988 and 1998 more than 1700 functional foods have been introduced to the market (Hardy, 2000; Kwak & Jukes, 2001; Menrad, 2003). The most dynamic market reported is the United States where the market share of functional foods in the total food market was estimated at 4-6% in 2008 (Benkouider, according to Zhang *et al.*, 2010). In European countries the market for functional foods has been increasing steadily, but a divide in consumer acceptance exists. Central and Northern European countries are reportedly more favourable to functional foods than Mediterranean countries where fresh and natural foods are preferred (Menrad, 2003).

The meat industry is shifting its focus towards products with high organoleptic standards, longer shelf-life and the inclusion of specific nutrients to cover special consumer demands. Consumers on the other hand, demand pathogen free food together with less processing, preservatives and additives. This opens the door for biopreservation where natural means are used to control the shelf-life and safety of meats. Lactic acid bacteria is regarded as one of the best candidates because of their GRAS (Generally Regarded As Safe) status and prevalence during storage (Fadda *et al*., 2010). Conjugated linoleic acid with its positive effects on the composition and stability of lipids (Dugan *et al.,* 1999) may prove to be another good candidate.

2.11.2 Creation of a novel functional food

The peculiarity of a product can be better preserved through the identification and quantification of those properties that better describe the characteristics of the product. This may be accomplished through microbiological, chemical and sensory determinations of these properties (Moretti *et al*., 2004). A probiotic is defined as a culture of living microorganisms, mainly LAB or bifidobacteria, which positively affect the health of the host when ingested at certain levels. This is done by preventing growth of pathogenic bacteria through competitive exclusion and the generation of organic acids and antimicrobial compounds in the colon (Salminen *et al.,* according to Zhang *et al.*, 2010). The therapeutic use of LAB to cure some types of gastro-intestinal disorders has been supported for almost a century (Cohendy; Hawley *et al*., according to Incze, 1998). The use of probiotics in the meat industry appears most promising in raw fermented products that are consumed without prior heating, which would cause the death of the beneficial microorganisms (Scheid, according to Ruiz *et al.*, 2010). As salami is a fermented food containing LAB that does not undergo a heat treatment and may enhance the survival of probiotic bacteria in the digestive system, it may be viewed as a probiotic food (Arihara, 2006; De Vuyst *et al*., 2008).

The minimum level of the probiotic bacterial population should be at least 6 log cfu/g. It should be established if the cultures used in salami have the following properties: resistance to acid and bile toxicity; adherence to human intestine cells; colonization in human guts; antagonism against pathogenic bacteria; production of antimicrobial substances and immune modulation properties (Incze, 1998; Brassart & Schiffrin, 2000). The development of fermented meat products with probiotics is challenging as the viability of the bacteria is affected by the high content of the curing salt, low pH due to acidification and low a_w due to drying (De Vuyst *et al.*, 2008).

Conjugated linoleic acid supplementation may also contribute to the creation of novel functional foods. This may be achieved by the CLA's ability to cause changes in fatty acid composition which may result in improvements of the technological quality of lipids (Corino *et al*., 2003).

2.12 Conclusions

This review explored the composition of fat in meat and the reasons for the need to modify the composition thereof. The levels of CLA currently ingested by humans as well as the level needed for physiological or therapeutic effects as well as ways of increasing these levels were discussed. The biosynthesis of CLA in monogastric animals and ruminants differ completely and this will be reflected in the CLA concentration of different meat and meat products. Factors affecting CLA concentration pre mortem as well as post mortem were discussed. The fermented dried sausage, salami, is discussed as well as its history, classification and the technology involved since this product will be used as a model in this study. This review also provided an introduction to functional foods and the growth of this food product type. The development and importance of flavour and colour in a fermented, dried sausage was also explored. Lastly, texture and the importance of textural measurements and sensory measurements to product quality were discussed.

The majority of available literature focuses on CLA and ruminants such as cattle as a source of CLA. There is also plenty of information available regarding the biosynthesis and biohydrogenation of CLA in ruminants as well as concentration of CLA in ruminant products. A difference was observed regarding literature that covers the subject of CLA and monogastric animals and humans. From this the deduction is made that CLA and ruminants have been given a lot more thought than CLA in non-ruminant sources. This is probably because CLA was first identified from ruminant meat. The more recent research however, is starting to cover non-ruminants, especially pigs and chickens as well as human interactions with CLA supplementation.

Pigs are seen as a great candidate for CLA supplementation as a way of improving the quality of pork and changing the fat content and fat composition to create a healthier consumer image of this

undervalued meat source in the human diet. It may even lead to the creation of novel functional foods with physiological or therapeutic effects. It is clear that CLA supplementation of pigs does have a positive effect on the meat and fat, but its effect on a fermented, dried sausage product needs to be evaluated.

CHAPTER 3

THE EFFECT OF DIETARY CLA SUPPLEMENTATION AND SLAUGHTER WEIGHT OF PIGS ON THE MICROBIAL AND LIPID STABILITY OF A CURED, FERMENTED SAUSAGE

ABSTRACT

Fourty, Duroc X Landrace gilts weighing on average 35 kg were randomly divided into two groups fed either a diet containing 0.5% sunflower oil (SFO) or a diet containing 0.5% conjugated linoleic acid (Luta-CLA® 60, BASF). These groups were further divided into two slaughter weight groups of ±70 and ±90 kg. After slaughter the lean meat and BF from the loins of these animals were pooled by treatment group and utilized to manufacture salami. The aim was to determine if salami quality is influenced by slaughter weight and dietary supplementation of CLA. Both variables had major effects on the FA composition and fatty acid ratios of the muscle and fat raw material as well as salami. The FAs and FA ratios of technological importance were mostly positively influenced while the FAs and FA ratios of nutritional and health concern were mostly negatively influenced by increased slaughter weight and dietary CLA supplementation. The microbial, physical, sensory and lipid stability parameters of salami were unaffected or inconsistently affected by both variables. Although dietary CLA was deposited successfully in muscle and fat, the deposition level was low. Consumption of a 28 g portion of salami manufactured from CLA enriched pork could only supply in 1 % of the RDA for CLA. It could be concluded that although dietary supplementation of pork with CLA improved the technological properties of fat tissue it could not be considered a very successful approach to increase human consumption of CLA.

Keywords: Conjugated linoleic acid; dietary supplementation; pork; salami; sunflower oil; slaughter weight; fatty acid composition

3.1 INTRODUCTION

Currently there is a worldwide drive to create more functional foods and also healthier foods as consumers have become more aware of what they eat. Red meat consumption is associated with being high in fat and contributing to cancer (Valsta, Tapanainen & Männistö, 2005). Processed meat is also associated with high levels of sodium chloride which has been linked to hypertension and various other lifestyle diseases (Ruusunen & Puolanne, 2005). Pork for example has a negative consumer image and is associated with being an unhealthy source of meat due to its high SFA content (Verbeke *et al*., 1999). In some European countries consumers regard pork as inferior

to beef, and it is not used for “special” occasions (Ngapo, Dransfield, Martin, Magnusson & Bredahl, 2003). The meat industry has reacted in using various strategies to create meat products with more beneficial ingredient profiles and by doing this, add value to their products.

In one attempt to produce healthier pork with less SFAs the use of leaner pigs has been suggested. One way of producing leaner pigs is to slaughter pigs at a lower slaughter weight. In leaner pigs a thinner and less saturated BF layer results in an increase in PUFAs with an inverse reduction in SFAs. This strategy is, however, plagued with the deteriorating effects of PUFAs on fat quality (Warnants *et al*., 1998). On the other hand, with increasing slaughter weight feed is converted with less efficiency and pigs produce carcasses with increased subcutaneous and intramuscular fat (IMF) content (García-Macias *et al*., 1996; Gispert *et al*., 1996; Candek-Potokar *et al*., 1998). Another result of increased slaughter weight is the increased degree of saturation of the BF and muscle lipids (Babatunde *et al*., 1966; Allen *et al*., 1967; Staun, 1972; Martin *et al*., 1972). Increased slaughter weight improves fat quality (Garcia-Macias *et al*., 1996) and heavier carcasses with more saturated and thus harder fat are seen as more desirable to the meat processor (Bruwer *et al.*, 1991). It is clear that an inverse relationship between nutritional and technological qualities exists (Hugo & Roodt, 2007). Fat quality is especially important in high value products like fermented sausages (Hugo & Roodt, 2007).

Another strategy to produce healthier pork with less SFAs is the incorporation of more functional lipids in existing meat products to create healthier versions of already available meat products (Juárez *et al.*, 2009). These products are regarded as meat-based functional foods and may improve the “image” of meat products as well as address the changing needs of customers. Lipids are the bioactive compounds in food that have received the most quantitative and qualitative attention in terms of development of healthier meat products (Jiménez-Colmenero, 2007). Quality of the fat tissue in pork is highly influenced by the amount of dietary FAs and the composition thereof (St. John *et al.*, 1987; Cardenia *et al.,* 2011). In an attempt to improve the image of pork a lot of research has been done on dietary supplementation with CLA. Conjugated linoleic acid introduces various positive effects on human health as well as technological improvements to pork (Corino *et al.*, 2003; Lauridsen *et al.*, 2005; Hah *et al.*, 2006; Schmid *et al*., 2006; Martin *et al.,* 2008; Martín *et al.*, 2008b; Marco *et al.*, 2009; Toomik *et al*., 2012). The current RDA for CLA for humans is 3.5g of CLA per day (USDA, 2011). No information could be found in literature on the effect of dietary CLA supplementation of pigs on the quality of cured fermented sausages like salami.

In this study dietary CLA supplemented pork was utilized to create a novel, cured and fermented pork sausage (salami) and it was studied throughout the whole production cycle to determine if

quality was influenced by dietary CLA supplementation and subcutaneous lipid maturation as induced by two slaughter weights.

3.2 MATERIALS AND METHODS

Animal feeding and slaughter

Fourty, Duroc X Landrace gilts weighing on average 35 kg were divided into two groups. Of these, 20 gilts were fed on a diet containing 0.5% sunflower oil (SFO) and the other 20 gilts were fed on a diet containing 0.5% CLA (Luta-CLA® 60, BASF). LUTA-CLA® 60 is registered for usage in pig feeding (Fernández, 2004) and inclusion of CLA at 0.5% reportedly has no impact on the measured slaughter and meat quality responses. It also decreases the amount of feed needed in the finishing diet of pigs (Lauridsen *et al*., 2005). The CLA preparation consisted of a 50:50 ratio of the C18:2*c*9,*t*11 and C18:2*t*10,*c*12 isomers. Each of these groups was further divided into two slaughter weight groups. Ten gilts from each group were slaughtered at ±70 kg and the other 10 gilts were slaughtered at ±90 kg. In effect there were four treatments groups with 10 pigs in each group (n=10).

3.2.1 Determination of BF and IMF quality

Tissue sampling

After slaughter the BF thickness was measured using a Hennessey Grading Probe. The carcasses were hanged in a cold room at approximately 1°C for 24h. Afterwards the heads were removed and the carcasses were split. Fat hardness was measured on the cross sectional surface at the position between the second and third last rib after the fat was shaved and smoothed using a fat hardness meter MK2 (FHM). The left loin including the last three ribs of each carcass was removed. The loins were deboned, deskinned and the BF and lean meat was separated. The backfat and lean meat of each dietary treatment was then pooled and minced through an Okto mincer (13 mm plate size). The lean meat and BF was then vacuum packaged as ±1 kg (lean meat) and ±0.375 kg (BF) quantities and stored at -18°C until used.

Total lipid extraction

A 10.00 g sample from each of three salamis from each batch at the same intervals as for microbial analysis was used for quantitative extraction of total lipids, according to the method of Folch, Lees, & Sloane-Stanley (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001% to the chloroform:methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were further dried overnight in a vacuum oven at 50°C, using phosphorous pentoxide as moisture adsorbent. Total extractable fat was determined gravimetrically from the

salami and expressed as % (w/w) per 100 g salami. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for the Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100g salami. The moisture content of the muscle and BF was determined by subtraction (100% - (% lipid + % FFDM)) and expressed as % moisture (w/w) per 100 g salami. The fat extracted from the salami was stored in a polytop (glass vial with push-in plastic top) under a blanket of nitrogen (Air Liquide, SA) and frozen at -20°C pending fatty acid analyses.

Iodine value determination (actual)

A lipid sample of 0.5 g, extracted using the Folch method on the BF was used to determine the IV which was expressed as the number of gram iodine absorbed by 100 g of fat, indicating the level of unsaturation of the fat (AOAC nr. 920.158, 2000). The IV of the IMF was determined through calculations described under GC analysis.

Fatty Acid Methyl Ester (FAME) preparation for GC analysis

A lipid aliquot (20-30 mg) of salami lipid were converted to methyl esters by base-catalysed transesterification, in order to avoid CLA isomerisation, with sodium methoxide (CH_3ONa; 0.5 M solution in anhydrous methanol) during 2 h at 30 °C, as proposed by Park, Albright, Cai & Pariza (2001), Kramer, Blackadar & Zhou (2002) and Alfaia, Castro, Martins, Portugal, Alves, Fontes, Bessa & Prates (2007).

GC analysis

Fatty acid methyl esters from muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Analysis was performed using an initial isothermic period (40°C for 2 min). Thereafter, temperature was increased at a rate of 4°C/minute to 230°C. Finally an isothermic period of 230°C for 10 min 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, while nitrogen was employed as the makeup gas. Galaxy chromatography software (V.1.9.302.530) recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the 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). Conjugated linoleic acid standards were obtained from Matreya Inc. (Pleasant Gap, United States). These standards included: *cis*-9,*trans*-11-18:2 and *trans*-10,*cis*-12-18:2 isomers. All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid present in

the sample. The following fatty acid combinations were calculated: omega-3 (*n*-3) fatty acids, omega-6 (*n*-6) fatty acids, *n*-6/*n*-3 ratio, total MUFAs, total PUFAs, total SFAs, MUFA/SFA ratio, PUFA/SFA ratio. The IV, DBI and PI values were also calculated from this data. The DBI was calculated as: DBI = Σ% of UFA x number of double bonds of each UFA (Alam & Alam, 1986). Peroxidizability index was calculated as: PI = [(% Monoenoic x 0.0025) + (% Dienoic x 1) + (% Trienoic x 4) + (Pentaenoic x 6) + (% Hexaenoic x 8)] (Pamplona *et al*., 1998). The IV for IMF was calculated from fatty acid data according to Ham, Shelton, Butler & Thionville (1998).

Conjugated linoleic acid isomers were quantitatively determined by using nonadecanoic acid (C19:0) as internal standard. The *cis*-9,*trans*-11-18:2 and *trans*-10,*cis*-12-18:2 isomers could then be expressed as mg CLA/g fat. The areas of the CLA isomers were expressed against the area of the internal standard. Correction factors for different CLA isomers were also calculated.

3.2.2 Preparation of the salami models

Salami formulation

Salami models from different treatments were prepared according to Tables 3.1 – 3.3.

Table 3.1. Ingredients used for salami manufacturing.

Ingredient:	Weight (g):
Pork 90/10 (lean meat / backfat)	10000.00
Pork 90/10	993.81
Pork backfat	375.00
Curing salt	76.15
Spice mixture	54.41
Starter culture	0.63
TOTAL	**2500.00**

Table 3.2. The spice mixture used for salami manufacturing.

Ingredient or spice:	Weight (g):
Dextrose	25.00
Sucrose	25.00
White pepper	2.50
Garlic powder	1.50
Nutmeg	0.41
TOTAL	**54.41**

Table 3.3. The curing salt mixture used for salami manufacturing.

Additive:	Weight (g):
Sodium chloride	75.62
Sodium nitrate (formulated to 120 ppm in end product)	0.30
Sodium nitrite (formulated to 92 ppm in end product)	0.23
TOTAL	**76.15**

A freeze-dried mixed starter culture, Bactoferm™ T-D-66 from CHR HANSEN (Lake Foods, Sandton, Johannesburg) was used. It consists 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). The starter culture was stored in its original packaging at -18°C until used and each batch of salami was prepared with starter culture with the same unique batch number. All spices were supplied by Crown National, Bloemfontein. The dextrose, sodium chloride and sucrose were supplied by Merck (Wadeville, Gauteng) and the sodium nitrate and sodium nitrite was supplied by Sigma-Aldrich (Johannesburg).

Method for salami preparation

1. The first 1000 g of frozen lean pork was chopped (OKTO 20L Bowl Cutter) to a 10 mm particle size.
2. The starter culture and spice mixture was added and the meat was chopped to a fine consistency.
3. The 998.31 g of frozen pork was added and chopped to a 20 mm particle size.
4. The backfat and curing salt was added and chopped at low speed.
5. The mixture was further chopped at high speed to reach a particle size of 4-5 mm.
6. The mixture was filled into wetted Colpak Fibrous Bak 65/50 casings (Crown National, Bloemfontein) to produce salamis of ±200 g each.
7. The individually labelled salamis were fermented for 48 h at 22°C with an rH of 90%.
8. After fermentation the salamis were smoked with a Crown Mills smoking cabinet for 10 min at 18 - 22°C.
9. After smoking the salamis were ripened at 12°C at an rH of 75%.
10. 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 20% weight loss was reached which indicated the end of the ripening process.
11. After the ripening period the salamis still present were vacuum packaged (SAFARIVAC Model T.M.-400 SERIES; S.A.) for a one month storage period at 4°C.

The salamis were manufactured in three separate rounds with intervals of three months between the rounds during which the salamis from the previous round were analysed. This was done to negate any variation in processing conditions between rounds.

3.2.3 Salami sampling

Each of the four salami batches produced 12 salamis per batch with an average weight of ±200 g. After manufacturing three salamis from each batch were selected for microbial and chemical analysis.

3.2.4 Microbial analysis

The microbial analysis of the salami models were performed on three salamis from each of the four treatments after manufacturing, after fermentation, after drying and after one month storage. A 10 g sample from each salami was aseptically weighed and placed into a Nasco Whirlpak® bag, 90 mL of a sterile 0.1 M phosphate buffer was added and it was stomached (AME Stomacher Lab-Blender 400, JHB) for 1 minute. For each of these a dilution series (10^{-2} to 10^{-8}) was prepared using McCartney bottles containing 9 mL of the same sterile 0.1 M phosphate buffer (Harrigan, 1998). These samples were then plated out using the pour plate method on various media. MRS (deMan, Rogosa & Sharpe Agar; Oxoid CM0361) media was used for total LAB counts which were then incubated at 32°C for 48 h. Violet Red Bile Lactose agar + 4-Methylumbelliferyl-β-D-glucuronide (VRBA + MUG; Oxoid CM0978) was used for total coliform counts and detection of *Escherichia coli* (*E. coli*) which was incubated at 37°C for 48 h. Lastly Rose-Bengal Chloramphenicol agar (RBCA; Oxoid CM0549) with Chloramphenicol supplement (SR0078) was used for yeast and mould enumeration and incubated at 25°C for 4 days. Coliform, *E. coli*, yeast and mould counts were selected as common potential microbial pathogenic and spoilage parameters associated with this type of product. The presence of *E. coli* on VRBA plates was confirmed by fluorescence under ultraviolet light (366 nm; CAMAG Universal UV Lamp) (Harrigan, 1998).

3.2.5 Chemical analysis

Total lipid extraction

The total lipid extraction was conducted as described in this chapter on pp. 37-38.

Free Fatty Acid (FFA) analysis

Free fatty acid analysis was performed to determine the levels of lipid hydrolysis. This was carried out using the method by Pearson (1968) on a 0.50 g lipid sample obtained from the Folch extraction.

Peroxide Value (PV) analysis

Peroxide value analysis was performed to determine the levels of primary lipid oxidation products according to AOAC nr. 965.33 (2000) on 0.50 g of extracted lipid.

Fatty Acid Methyl Ester (FAME) preparation for GC analysis

Fatty acid methyl ester preparation was conducted as described in this chapter on p. 38.

GC analysis

GC analysis was conducted as described in this chapter, on p. 38-39.

TBARS analysis

Another 5.00 g of each of the models (at the same intervals as for the microbial analysis) were used in the analysis of thiobarbituric acid reactive substances (TBARS) which were used as an indication of the level of formation of secondary oxidation products (Raharjo, Sofos & Schmidt, 1993).

Total Acidity

A 10.00 g sample of each of the models (same intervals as mentioned previously) was used in the analysis of the total acidity of the models through a titration technique for meat and meat products using NaOH (Konieko, 1985).

pH

The pH of three models of each dietary treatment group was also determined at the same intervals as for the weight loss determinations. The measurements for pH 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 each day before measurement with pH 4.00 and 7.00 buffer solutions from Merck (uniVAR®, Wadeville, Gauteng, SA).

Determination of actual CLA content of the salami

For this study salamis were created from pigs fed on either CLA or SFO and slaughtered at two different slaughter weights (70 or 90 kg). The goal of this was to determine if the fatty acid composition could be improved and if CLA would be supplemented to a level that would grant neutraceutical status to a 28 g portion of salami. A portion size of 28 g for salami is recommended by the United States Department of Agriculture's National Nutrient Database for Standard Reference (Release 23, USDA 2011). Therefore it was important to determine the actual content of CLA as well as the content of each particular isomer for this portion size.

Determination of CLA as a percentage of the RDA for CLA

The percentage of the suggested (USDA, 2011) RDA for CLA of 3.5g/day, was calculated from the amount of CLA (mg Total CLA/28g salami) quantified in the salami through the GC analysis previously carried out.

3.2.6 Physical analysis

Weight loss

Weight loss through a loss in moisture due to the drying process was determined at time 0, 12h, 24h, 36h, 48h and every 48h until a 20% loss in weight was reached. This was used to determine the end of the ripening period.

Water activity (a_w)

The a_w of each sample was measured after sample collection, simultaneously with the microbial analysis, using a Novasina Thermoconstanter a_w meter (model No. TH200). Measurements were carried out at room temperature (25°C) after the machine was allowed to reach equilibrium with deionised distilled water. Readings given as percentage relative humidity (% rH) was later converted to a_w values.

Colour analysis

For colour analysis, the finished salamis at the end of manufacturing was sliced through and left for 30 min before measurements were taken. The colour of each sample was then measured six times (one second for each measurement) using a Minolta CR 400 chromometer (8 mm measuring area). The *CIE L*a*b** colour scale was used for comparison where *L** represents lightness, *a** represents redness and *b** represents yellowness. Chroma (*C**) as well as hue angle (*H**) was also measured by using *a** and *b** the chroma ($\sqrt{a*^2 + b*^2}$) and hue angle ($\tan^{-1}(b*/a*)$) was calculated (Ripoll *et al.,* 2011; Tapp, Yancey & Apple, 2011).

Texture analysis

For texture analysis six salamis from each of the four batches at the end of manufacturing were used and six samples were taken from each salami. Samples were left for one hour at room temperature of 23°C. Samples of 10 mm^3 were analysed for compression force using an Instron Universal Testing Machine (UTM, Model 430) with a compression head at 300.00 mm/min crosshead speed. Sample compression data was generated simultaneously by the Instron Series IX AMTS 8.34.00 software. A 12.5 mm diameter core sample (also six for each of six salamis per batch) were analysed for shear force using the same Instron UTM with a Warner-Bratzler attachment while shear force data was simultaneously generated by the previously mentioned software.

3.2.7 Sensory analysis

Sensory analysis was carried out on all four batches of the salamis after manufacturing, using a 75 member consumer panel consisting of 52 females and 23 males with an average age of 33 years. A nine-point hedonic scale ranging from 1 for dislike extremely up to 9 for like extremely was used to score aroma, taste, firmness and overall liking as attributes (Table 3.4). The samples were encoded with a randomized, 3-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 35 mm diameter. Samples were removed from refrigerated storage, allowed to reach room temperature and then served at 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.

Table 3.4 Simplified example of the hedonic scale used for sensory analysis.

Nine-point hedonic scale for aroma, taste firmness and overall liking								
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

3.2.8 Statistical analysis

This experiment was a 2 x 2 x 3 factorial design representing the two slaughter weights, two dietary treatments, and three batches with three replicates per treatment. An analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of slaughter weight, dietary treatment and processing stage on proximate composition, microbial stability, chemical stability and fatty acid composition of salami. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007). The same statistical methods were also used for the pork BF and pork IMF.

3.3. RESULTS AND DISCUSSION

3.3.1 Fat quality of the BF and IMF used for salami manufacturing

3.3.1.1 Backfat quality

Physical properties of the BF

The BF thickness was found to be significantly affected ($p < 0.01$) by slaughter weight (Table 3.5). The 90SFO and 90CLA groups had significantly thicker BF layers than the 70SFO group. The optimum range of BF thickness for good fat quality is regarded as being between 17.5 mm and 20.0 mm (Cannon, Morgan, McKeith, Smith, Sonka, Heavner & Meeker, 1996) and the range of the four treatment groups was much lower at between 11.32 ± 1.87 and 15.32 ± 3.51 mm. It should however be kept in mind that these gilts were slaughtered at lower live weights than the more than 100 kg normally considered as the ideal pig weight by most international markets (Anonymous, 1996). Backfat thickness can thus be regarded as a function of slaughter weight with higher weight resulting in thicker BF (Nürnberg, Wegner & Ender, 1998) and lower slaughter weight resulting in thinner BF (Santoro, 1983). What makes BF thickness important is its influence on juiciness and to a lesser extent, flavour, tenderness and overall acceptability (Wood, Jones, Francombe & Whelehan, 1986). Fat hardness was affected significantly ($p < 0.001$) by both slaughter weight and diet (Table 3.5). The 70SFO group had the lowest fat hardness, the 90CLA group had the highest fat hardness and the 70CLA and 90SFO groups were found somewhere in the middle with no significant difference between them. The results for the latter two groups indicated that CLA dietary

supplementation and increased slaughter weight contributed almost equally to increasing fat hardness. In terms of the colour characteristics of the BF of the four treatment groups, the greatest number of significant differences was found between different slaughter weight groups (Table 3.5). The lower slaughter weight groups were significantly more red ($p < 0.05$) and more yellow ($p < 0.001$) than the higher slaughter weight groups. The chroma (colour intensity) of both 70SFO and 70CLA was significantly ($p < 0.001$) higher than that of the 90SFO group.

Table 3.5 Physical and chemical parameters and FA and FA ratios of importance to BF as raw material for salami manufacturing.

Slaughter weight (kg)	**70**		**90**		**Sign. level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
Physical properties:					
Backfat thickness (mm)	11.32 ± 1.87^{a}	12.92 ± 2.25^{ab}	15.32 ± 3.51^{b}	15.12 ± 1.60^{b}	$p < 0.01$
Fat Hardness	600.34 ± 66.48^{a}	710.96 ± 80.96^{b}	700.64 ± 69.69^{b}	807.31 ± 46.70^{c}	$p < 0.001$
Colour *L** - Value	74.05 ± 2.32^{ab}	72.61 ± 2.02^{a}	75.92 ± 1.58^{b}	74.39 ± 2.44^{ab}	$p < 0.05$
Colour *a** - Value	5.49 ± 1.29^{b}	5.54 ± 0.79^{b}	4.22 ± 0.76^{a}	5.15 ± 0.78^{a}	$p < 0.05$
Colour *b** - Value	7.75 ± 0.78^{b}	7.57 ± 0.75^{b}	6.21 ± 0.74^{a}	6.60 ± 0.75^{a}	$p < 0.001$
Chroma	9.52 ± 1.33^{b}	9.39 ± 1.00^{b}	7.52 ± 1.00^{a}	8.38 ± 0.98^{ab}	$p < 0.001$
Hue Angle	55.13 ± 4.37	53.87 ± 2.49	55.94 ± 2.71	52.10 ± 3.03	NS
Chemical properties:					
Extractable Fat (%)	68.26 ± 8.11^{a}	69.82 ± 5.79^{ab}	75.70 ± 2.38^{bc}	76.55 ± 2.65^{c}	$p < 0.01$
FFDM (%)	12.45 ± 1.74	11.63 ± 1.35	11.13 ± 1.44	11.17 ± 1.63	NS
Moisture (%)	19.30 ± 8.83^{b}	18.55 ± 6.06^{ab}	13.17 ± 1.92^{b}	12.27 ± 2.07^{a}	$p < 0.05$
Iodine value (IV)	72.82 ± 3.57^{c}	67.53 ± 4.29^{ab}	71.62 ± 2.93^{bc}	63.49 ± 3.54^{a}	$p < 0.001$
FAME (% of total fatty acids)					
C14:0	1.43 ± 0.13^{a}	2.12 ± 0.25^{b}	1.40 ± 0.07^{a}	2.26 ± 0.22^{b}	$p < 0.001$
C15:0	0.01 ± 0.02^{a}	0.05 ± 0.03^{b}	0.01 ± 0.02^{a}	0.04 ± 0.04^{ab}	$p < 0.01$
C16:0	25.71 ± 1.06^{a}	28.59 ± 1.37^{b}	26.01 ± 0.54^{a}	29.11 ± 1.11^{b}	$p < 0.001$
C16:1c9	2.15 ± 0.20^{ab}	2.35 ± 0.25^{b}	2.02 ± 0.21^{a}	2.34 ± 0.25^{b}	$p < 0.01$
C17:0	0.36 ± 0.07^{a}	0.51 ± 0.10^{b}	0.37 ± 0.08^{a}	0.51 ± 0.15^{b}	$p < 0.01$
C18:0	10.54 ± 0.77^{a}	13.45 ± 0.66^{b}	11.07 ± 0.76^{a}	14.58 ± 1.38^{b}	$p < 0.001$
C18:1t9	0.02 ± 0.01^{a}	0.06 ± 0.02^{b}	0.02 ± 0.02^{a}	0.07 ± 0.02^{b}	$p < 0.001$
C18:1c9	38.27 ± 1.48^{b}	31.85 ± 1.48^{a}	39.61 ± 1.51^{b}	32.49 ± 0.89^{a}	$p < 0.001$
C18:1c7	2.49 ± 0.15^{b}	2.06 ± 0.09^{a}	2.52 ± 0.13^{b}	2.16 ± 0.18^{a}	$p < 0.001$
C18:2c9,12(n-6)	17.00 ± 1.73^{b}	16.52 ± 2.19^{b}	15.23 ± 1.62^{ab}	14.12 ± 1.06^{a}	$p < 0.01$
C18:2c9,t11(n-6)(CLA)	0.02 ± 0.03^{a}	0.45 ± 0.04^{b}	0.01 ± 0.02^{a}	0.52 ± 0.03^{c}	$p < 0.001$
C18:2t10,c12(n-6)(CLA)	ND	0.19 ± 0.02	ND	0.20 ± 0.02	NS
C18:3c9,12,15(n-3)	0.57 ± 0.21^{b}	0.53 ± 0.29^{ab}	0.28 ± 0.25^{a}	0.43 ± 0.16^{ab}	$p < 0.05$
C20:0	0.16 ± 0.02	0.16 ± 0.03	0.18 ± 0.01	0.17 ± 0.02	NS
C20:1c11	0.49 ± 0.09	0.45 ± 0.10	0.52 ± 0.09	0.47 ± 0.06	NS
C20:2c11,14(n-6)	0.49 ± 0.07^{b}	0.41 ± 0.05^{a}	0.49 ± 0.02^{b}	0.40 ± 0.04^{a}	$p < 0.001$
C20:3c11,14,17(n-3)	0.05 ± 0.02	0.03 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	NS
C20:4c5,8,11,14(n-6)	0.16 ± 0.03^{b}	0.14 ± 0.04^{ab}	0.16 ± 0.02^{b}	0.11 ± 0.02^{a}	$p < 0.001$
C22:6c4,7,10(n-3)	0.09 ± 0.05^{b}	0.07 ± 0.05^{ab}	0.06 ± 0.05^{ab}	0.02 ± 0.04^{a}	$p < 0.05$

Fatty acid ratios:					
MUFA (%)	43.42 ± 1.65^{b}	36.77 ± 1.52^{a}	44.69 ± 1.45^{b}	37.52 ± 1.03^{a}	p < 0.001
Dienoic (%)	17.51 ± 1.74^{b}	17.57 ± 2.23^{b}	15.73 ± 1.64^{ab}	15.23 ± 1.11^{a}	p < 0.01
Trienoic (%)	0.62 ± 0.21^{b}	0.56 ± 0.31^{ab}	0.33 ± 0.25^{a}	0.46 ± 0.16^{ab}	p < 0.05
Tetraenoic (%)	0.16 ± 0.03^{b}	0.14 ± 0.04^{ab}	0.16 ± 0.02^{b}	0.11 ± 0.02^{a}	p < 0.001
Hexaenoic (%)	0.09 ± 0.05^{b}	0.07 ± 0.05^{ab}	0.06 ± 0.05^{ab}	0.02 ± 0.04^{a}	p < 0.05
Penta + Hexaenoic (%)	0.09 ± 0.05^{b}	0.07 ± 0.05^{ab}	0.06 ± 0.05^{ab}	0.02 ± 0.04^{a}	p < 0.05
SFA (%)	38.20 ± 1.77^{a}	44.89 ± 1.74^{b}	39.04 ± 1.09^{a}	46.66 ± 1.71^{b}	p < 0.001
UFA (%)	61.80 ± 1.77^{b}	55.11 ± 1.74^{a}	60.96 ± 1.09^{b}	53.34 ± 1.71^{a}	p < 0.001
MUFA/SFA	1.14 ± 0.08^{b}	0.82 ± 0.05^{a}	1.15 ± 0.05^{b}	0.81 ± 0.05^{a}	p < 0.001
Slaughter weight (kg)	**70**		**90**		**Sign. level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
DBI	81.48 ± 3.51^{c}	74.58 ± 4.15^{b}	78.11 ± 2.95^{bc}	69.90 ± 2.91^{a}	p < 0.001
PI	21.19 ± 2.35^{b}	20.74 ± 2.83^{b}	18.59 ± 2.26^{ab}	17.66 ± 1.44^{a}	p < 0.01
PUFA (%)	18.38 ± 1.91^{b}	18.34 ± 2.36^{b}	16.27 ± 1.83^{ab}	15.82 ± 1.18^{a}	p < 0.01
Atherogenicity Index	0.51 ± 0.04^{a}	0.67 ± 0.06^{b}	0.52 ± 0.02^{a}	0.72 ± 0.05^{b}	p < 0.001
PUFA/SFA	0.48 ± 0.07^{c}	0.41 ± 0.07^{b}	0.42 ± 0.06^{bc}	0.34 ± 0.04^{a}	p < 0.001
n-6 (%)	17.67 ± 1.76^{b}	17.71 ± 2.26^{b}	15.88 ± 1.66^{ab}	15.34 ± 1.12^{a}	p < 0.01
n-3 (%)	0.71 ± 0.26^{b}	0.63 ± 0.33^{ab}	0.39 ± 0.27^{a}	0.48 ± 0.17^{ab}	p < 0.05
n-6/*n*-3	45.40 ± 70.73	69.36 ± 98.07	103.17 ± 116.98	60.27 ± 97.83	NS

Means with different superscripts in the same row differ significantly ND = Not detected NS = Not significant

Chemical properties of the BF

The amount of extractable fat was significantly ($p < 0.01$) influenced by slaughter weight (Table 3.5). The percentage of extractable fat was significantly higher in the higher slaughter weight groups (90SFO and 90CLA) compared to the 70SFO group indicating a more mature subcutaneous fat in heavier animals. An extractable fat content of 84% from BF is considered as the minimum for good fat quality. Backfat samples from all treatments in this study were below the minimum value of 84% lipid in the fatty tissue for good technological quality of BF as proposed by Prabucki (1991). This variation in extractable fat content relates to the percentage moisture where the group with the highest level of extractable fat (90CLA) had the significantly ($p < 0.05$) lowest percentage moisture (Babatunde, 1966) which may influence drying rates. The moisture content of the 90CLA BF group (12.27 ± 2.07) was also significantly ($p < 0.05$) lower than that of the 90SFO (13.17 ± 1.92) group. In literature a similar result was reported where CLA dietary treatment resulted in a product with lower moisture content (Marco *et al*., 2009). The percentage FFDM of the BF was completely unaffected by differences in slaughter weight and diet.

The backfat IV was also found to be significantly ($p < 0.001$) affected by dietary CLA supplementation. The 70SFO group had a significantly ($p < 0.001$) higher IV than both the 70CLA and 90CLA groups and the 90SFO group had a significantly ($p < 0.001$) higher IV than the 90CLA group. This dietary effect can be explained by CLA's ability to increase SFA levels (Cordero,

Isabel, Menoyo, Daza, Morales, Piñeiro & Lopez-Bote, 2010). A higher IV value indicates a higher degree of lipid unsaturation and thus more oxidizable and softer fat (Davenel *et al*., 1999). Although not statistically significant, BF from the heavier slaughter weights had lower IV compared to BF from the lighter slaughter weights (Table 3.5). This can be explained by the normally expected increase in fatty tissue saturation with animal maturation (Bruwer *et al*., 1991; Banskalieva, Sahlu & Goetsch, 2000). In literature a thinner BF layer was shown to correspond to a lower percentage of extractable fat content, higher moisture and IV content and more unsaturated FA (Barton-Gade, 1983). For salami type products an iodine value of less than 60 is recommended (Fischer, 1989a) Thus in terms of IV, the 90CLA group with the lowest IV had the best quality fat for use in the manufacturing of salami even though it is still above the recommended threshold. This research indicates that dietary supplementation of CLA may improve the fat quality of pork at a relatively low slaughter weight.

Fatty acid composition of the BF of technological and nutritional importance

Both CLA dietary treatment groups (CLA70 and CLA90) contained significantly higher levels of SFAs such as C14:0 ($p < 0.001$), C15:0 ($p < 0.01$), C16:0 ($p < 0.001$), C17:0 ($p < 0.01$) and C18:0 ($p < 0.001$) compared to the two SFO dietary treatment groups (70SFO and 90SFO) (Table 3.5). This is also reflected in the total SFA percentages of the CLA dietary treatment groups being significantly higher ($p < 0.001$) than the SFA percentages of the SFO dietary treatment groups. This indicates that CLA dietary supplementation led to an increase in SFAs which is explained by CLA's ability to inhibit the Δ^9-desaturase enzyme (Smith *et al*., 2002). The only SFA that was not significantly affected by dietary treatment was C20:0 with no significant differences between any of the treatment groups. Changes in MUFAs were observed for: C16:1*c*9 which was found to increase significantly ($p < 0.01$) with dietary CLA supplementation; C18:1*c7* which was found to decrease significantly ($p < 0.001$) with dietary CLA supplementation; C18:1*c*9 which was found to decrease significantly ($p < 0.001$) with CLA dietary treatment; and C20:1*c*11 which was not significantly affected by dietary treatment. The percentage total MUFAs (Table 3.5) was significantly ($p < 0.001$) influenced by dietary treatment and not by slaughter weight. The two CLA dietary treatment groups had significantly lower percentages of MUFAs compared to the two SFO dietary treatment groups. In terms of TFAs, C18:1*t*9 was observed as representative fatty acid. This TFA were present at extremely low levels in all four treatment groups and was affected significantly ($p < 0.001$) by dietary treatment in that CLA dietary supplementation led to significantly higher levels of this TFA.

Linoleic acid (C18:2*c*9,12) levels were found to be lower in the higher slaughter weight groups (Table 3.5). This was caused by replacement of linoleic acid by more SFAs due to animal maturation in the case of the 90SFO group and also due to CLA dietary supplementation in the 90CLA group. In the case of the CLA group this difference was significant ($p < 0.01$). All four

treatment groups were found to contain C18:2*c*9,12 at an excess of 11% of the total FA which was in line with the suggested maximum value for salami and fermented sausages (Fischer, 1989b). Various other PUFAs such as C18:3*c*9,12,15, C20:2*c*11,14, C20:4*c*5,8,11,14 and C22:6c4,7,10 exhibited a trend similar as was found for C18:2c9,12. The only PUFA found not to be affected by dietary treatment was C20:3*c*11,14,17. The total percentage of PUFAs in the BF (Table 3.5) was only slightly affected by the differences in slaughter weight with a significant difference ($p < 0.01$) between 70CLA and 90CLA, but not between 70SFO and 90SFO. The total percentage of UFA in the BF (Table 3.5) is the sum of MUFAs and PUFAs representing all the UFAs. This ratio was significantly ($p < 0.001$), affected by the differences in dietary treatment with the two SFO dietary treatment groups having the highest levels of UFAs with no effect on slaughter weight.

No CLA were detected in the SFO dietary treatment groups (Table 3.5). This confirms that monogastric animals cannot synthesize meaningful amounts of CLA (Schmid *et al.*, 2006). Conjugated linoleic acid supplementation led to CLA deposition in the fat reserves of the supplemented animals and an accumulative effect was found after comparing the 70CLA dietary treatment with the 90CLA dietary treatment. It is reported in literature that the incorporation level of CLA into adipose tissues and the accumulation of CLA is dependent upon the amount of CLA in the diet and the length of dietary exposure (Banni *et al.*, 2001). A higher level of the C18:2*c*9,*t*11 isomer than the C18:2*t*10,*c*12 isomer was found between the two CLA groups. The C18:2*c*9,*t*11 isomer is reported as being the more effectively deposited isomer (Chin *et al.*, 1992).

Fatty acid ratios of BF of technological and nutritional importance

Dietary CLA inclusion significantly ($p < 0.001$) increased the SFA and CLA levels and reduced the MUFA/SFA ratios ($p < 0.001$) between dietary treatments (Table 3.5). An effect on PUFA levels (Table 3.5) was only seen between the 70CLA and 90CLA groups which indicated a significant ($p < 0.01$) effect of slaughter weight. Cordero *et al.* (2010) also found no effect of dietary CLA supplementation on PUFA levels. The PUFA/SFA ratio differed significantly ($p < 0.001$) between the 70CLA and 90CLA slaughter weight groups and in the case of the 90CLA group was below the recommended PUFA/SFA ratio of 0.4 (Scollan *et al*., 2006; Wood *et al.,* 2008). All three other treatment groups had PUFA/SFA ratios above this cut-off point. Dietary CLA supplementation had a significant ($p < 0.001$) effect on DBI (Table 3.5). Only the 70CLA, 90SFO and 90CLA groups had DBI below the cut-off point of 80 (Prabucki, according to Hugo & Roodt, 2007), indicating acceptable quality in terms of lipid quality.

The only significant ($p < 0.001$) difference for peroxidizability index (PI) was found between different slaughter weights for the two CLA groups with the 90CLA group having the lower value. That is another indication that with animal maturation the fatty tissue composition becomes more saturated and therefore more stable. Although not significant, it seemed as if CLA dietary

supplementation contributed to slightly lower PI values for the 70CLA and 90CLA groups compared to the 70SFO and 90SFO groups (Table 3.5). Atherogenicity index was significantly ($p < 0.001$) influenced by dietary CLA supplementation, indicating that the BF from animals receiving dietary CLA supplementation may have cholesterol raising properties (Table 3.5). Neither slaughter weight, nor dietary treatment had an effect on *n*-6/*n*-3 ratio of BF (Table 3.5).

3.3.1.2 Physical and chemical characteristics of muscle of importance for salami manufacturing

The pH of the meat 24 h after slaughter did not differ significantly between treatments (Table 3.6). The final pH level of ±5.4 indicates that the muscle was not dark, firm and dry (DFD) pork and had acceptable water holding capacity (Arias, 2012). Significant ($p < 0.001$) differences in colour characteristics were observed between muscle from the 70SFO and 70CLA treatment groups indicating a dietary effect at low slaughter weight. The 70CLA treatment group showed significantly ($p < 0.01$) lighter, redder, yellower as well as more saturated colour characteristics than 70SFO. Moisture content was also of importance as the amount of moisture present in the meat as raw material will affect the chemical, microbial and physical parameters of salami. Dietary treatment had a significant effect in the higher slaughter weight group with the 90CLA group containing significantly ($p < 0.05$) less moisture than the 90SFO group. As expected from modern lean pork the muscle contained a very low percentage of IMF, between 1.45 ± 0.27 and 1.56 ± 0.15%. Since salami usually contains more than 15% fat it means that the contribution of muscle to the total fat content of salami is very small. The fat in salami is mostly originating from the BF used in the formulation. The FA profile of muscle was therefore also not discussed.

Table 3.6. Physical and chemical characteristics of muscle of importance for salami manufacturing.

Slaughter weight (kg)	**70**		**90**		**Sign. Level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
$pH_{24hours}$	5.38 ± 0.05	5.38 ± 0.09	5.38 ± 0.08	5.40 ± 0.06	NS
Colour *L - Value**	52.59 ± 1.31^{a}	56.48 ± 1.65^{b}	54.98 ± 2.96^{ab}	55.14 ± 2.64^{ab}	p < 0.01
Colour *a - Value**	7.33 ± 0.88^{a}	9.51 ± 1.17^{c}	8.02 ± =1.26^{ab}	8.82 ± 1.35^{bc}	p < 0.01
Colour *b - Value**	6.39 ± 0.73^{a}	8.17 ± 1.06^{b}	7.37 ± 1.30^{ab}	8.43 ± 1.53^{b}	p < 0.01
Chroma	9.73 ± 1.08^{a}	12.55 ± 1.45^{b}	10.91 ± 1.72^{ab}	12.22 ± 1.92^{b}	p < 0.001
Hue Angle	41.07 ± 2.33	40.63 ± 2.96	42.50 ± 3.27	43.52 ± 3.15	NS
Moisture (%)	74.91 ± 0.43^{c}	74.41 ± 0.63^{bc}	74.22 ± 0.41^{b}	73.60 ± 0.42^{a}	p < 0.001
Extractable fat (%)	1.45 ± 0.27	1.51 ± 0.19	1.56 ± 0.35	1.52 ± 0.15	NS

Means with different superscripts in the same row differ significantly. NS = Not significant.

3.3.2 Actual CLA content of the BF and muscle as raw materials for salami manufacturing

Actual total CLA content of BF was significantly ($p < 0.001$) influenced by dietary treatment (Table 3.7). The 70SFO and 90SFO groups contained very low levels of CLA. That is in agreement with literature (Chin *et al*., 1992) that ascribed this observation to limited biohydrogenation of linoleic acid (Dugan *et al.,* 2004). The 90SFO group consisted of animals slaughtered much later than the 70SFO group and contained even less CLA deposited in the BF (Table 3.7). With increased slaughter weight there is an increase in the deposition of SFAs and a decrease in the deposition of PUFAs (Wood, 1984; Kouba, Enser, Whittington, Nute & Wood, 2003). This offer a possible explanation for the lower CLA level in the 90SFO group compared to the 70SFO group. The CLA levels for both CLA dietary treatment groups were clearly much higher with CLA90 having a significantly ($p < 0.001$) higher level of CLA than CLA70. Conjugated linoleic acid is effectively incorporated into the adipose and muscle tissues of pigs with high efficiency (Kramer, Sehai, Dugan, Mossagba, Jurawecz, Roach, Eulitz, Aalhus, Schaefer & Ku, 1998; Dugan *et al.,* 2004; Corino *et al*., 2003; Lauridsen *et al*., 2005). The C18:2*t*10,*c*12 isomer was not detected in the SFO dietary groups and detected at a much lower level in the CLA dietary groups compared to the C18:2*c*9,*t*11 isomer. This is due to the fact that the C18:2*c*9,*t*11 isomer is more readily accumulated in adipose tissues than the C18:2*t*10,*c*12 isomer (Thiel-Cooper *et al*., 2001; Lauridsen *et al*., 2005).

Table 3.7. Actual CLA content (mg/100 g BF) of BF of gilts from the different treatments.

Weight	**70**		**90**		**Sign. Level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
C18:2*c*9,*t*11(n-6)CLA	26.70 ± 37.88[a]	526.39 ± 74.95[b]	12.33 ± 26.00[a]	647.52 ± 90.41[c]	$p < 0.001$
C18:2*t*10,*c*12(n-6)CLA	ND	216.99 ± 35.75	ND	231.11 ± 31.65	NS
mg total CLA	26.70 ± 37.88[a]	743.38 ± 108.01[b]	12.33 ± 26.00[a]	878.64 ± 119.61[c]	$p < 0.001$

Means with different superscripts in the same row differ significantly ND = Not detected NS = Not significant

Although the IMF contributed very little to the fatty acid composition of the salami, similar deposition trends were found for the actual CLA content as to that from the BF. Although total CLA content was significantly ($p < 0.001$) higher in the two CLA groups compared to the two SFO groups, it was less than 2 mg CLA/100 g of muscle (Table 3.8). It can be considered as negligible compared to the contribution made by the BF tissue (Table 3.7).

Table 3.8. Actual CLA content (mg/100 g meat) of muscle of gilts from the different treatments.

Weight (kg)	**70**		**90**		**Sign. Level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
C18:2*c*9,*t*11(n-6) CLA	0.38 ± 0.18[a]	1.46 ± 0.40[b]	0.49 ± 0.23[a]	1.44 ± 0.31[b]	p < 0.001
C18:2*t*10,*c*12(n-6) CLA	ND	0.52 ± 0.17	ND	0.45 ± 0.14	NS
mg total CLA	0.38 ± 0.18[a]	1.98 ± 0.55[b]	0.49 ± 0.23[a]	1.89 ± 0.42[b]	p < 0.001

Means with different superscripts in the same row differ significantly ND = Not detected NS = Not significant

3.3.3 Salami quality

Drying time and final moisture loss

The number of days recorded for each treatment group of salamis to reach a 20% loss in moisture content was significantly ($p < 0.001$) influenced by dietary treatment (Figure 3.1). The 70SFO and 90SFO groups needed significantly ($p < 0.001$) more time to dry than the 70CLA group and 70SFO needed significantly ($p < 0.001$) more time to dry than the 90CLA group (Figure 3.1). This is indicative of faster moisture release by salami manufactured from CLA supplemented pork. This observation did not support the finding of (Joo *et al.,* 2002) that dietary supplementation of CLA reportedly increases the water-holding capacity of pork which would affect the drying time of a fermented, dried sausage negatively. The number of days needed for reaching a 20% loss in moisture varied from 15.00 ± 0.84 days for the 70CLA group to 16.33 ± 1.28 days for the 70SFO groups (Figure 3.1) Consistently shorter drying times for salamis supplemented with CLA through dietary manipulation could prove to be profitable as more salamis could then be finished in a given amount of time. Slaughter weight of pigs had no effect on the number of days to reach 20% moisture loss within the two dietary treatments (Figure 3.1). The final weight loss (±20%) by the salamis (Table 3.9) were not significantly influenced by slaughter weight or dietary treatment. This can be ascribed to the precise monitoring of the final weight loss. This was also reflected in the ±10-12% loss in moisture from after manufacturing to after drying (Table 3.10). It should also be mentioned that the end of the drying period was calculated on the basis of a 20% loss in weight from the total salami weight and not as a 20% loss in moisture of the total moisture content.

pH and total acidity

The pH and total acidity of salami (Figures 3.2 and 3.3) was significantly ($p < 0.001$) influenced by processing stage. After manufacturing the pH of the four treatment groups were at ±5.74, after fermentation at ±5.28 and after drying at ±4.99. There were no significant differences in the pH values of any of the treatment groups for a given processing step. Only the set of pH values for one processing step differed significantly from the set of pH values of another processing step (see superscripts c, b & a on Figure 3.2). Total acidity increased from ±0.9 mL 0.1 N NaOH after manufacturing to ±1.1 mL 0.1 N NaOH after fermentation to ±1.5 mL 0.1 N NaOH after drying. This

increase in total acidity and decline in pH during processing can be ascribed to an increase in the lactic acid bacteria (LAB) count and lactic acid formation during fermentation, resulting in a

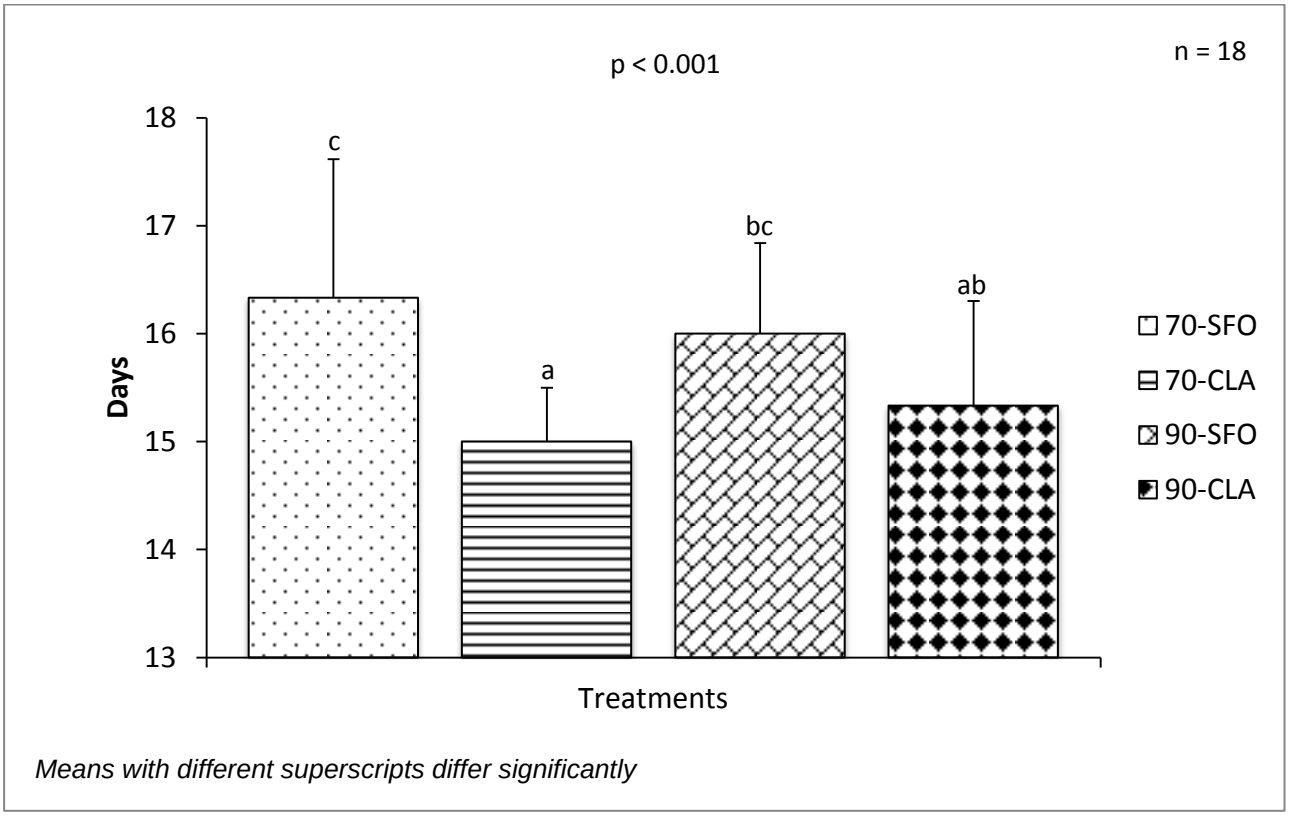


Figure 3.1. The number of days needed for each treatment group to reach a 20% loss in moisture.

Table 3.9. Final moisture loss of salami.

Treatment	70-SFO (n = 18)	70-CLA (n = 18)	90-SFO (n=18)	90-CLA (n =18)	Sign. level
Final weight loss (%)	20.44 ± 0.81	20.32 ± 0.71	20.39 ±0.67	20.86 ± 0.90	NS

Means with different superscripts in the same column differ significantly. NS = Not significant

decrease in salami pH. Slaughter weight and diet had no significant effect on total acidity or pH of salami (Figure 3.2). The lack of difference in total acidity and salami pH between dietary treatment groups was surprising since the dietary supplementation of CLA in pigs reportedly increased the number and diameter of white muscle fibres with simultaneous reduction in the number and diameter of red muscle fibres (Migdał, Paściak, Wojtysiak, Barowicz, Pieszka & Pietras, 2004). Porcine muscles, with a high degree of white muscle fibres which is also dependent on factors such as breed, race, sex and age (Karlsson, Klont & Fernandez, 1999) have a better buffering capacity than dark muscle types (Puolanne & Kavikari, 2000). Increase in buffering capacity, is defined as the ability of meat to resist change in pH (Kylä-Puhju, Ruusunen, Kivikari & Puolanne, 2004). The effect of dietary treatment on total acidity and pH patterns can therefore not be used to explain the more rapid drying rate of 70CLA salami compared to 70SFO salami.

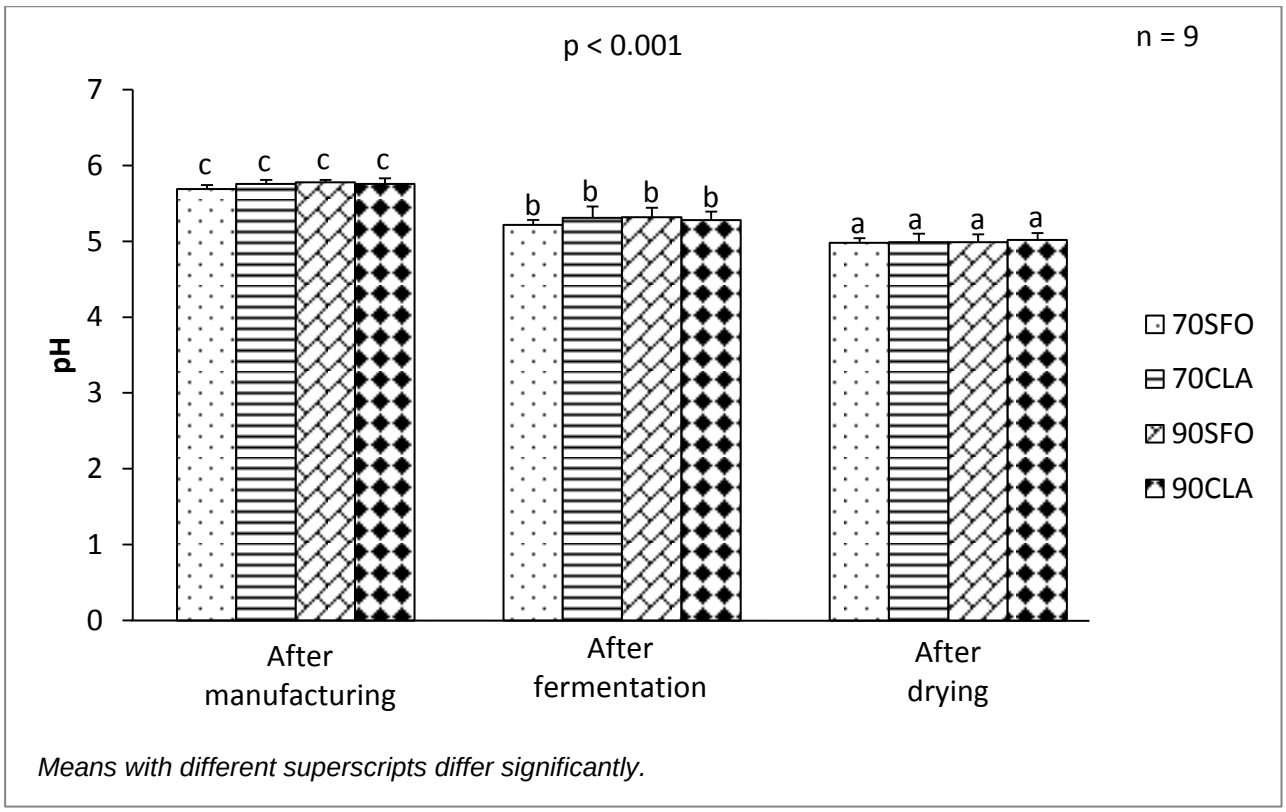


Figure 3.2. The pH of the four dietary treatment groups during processing.

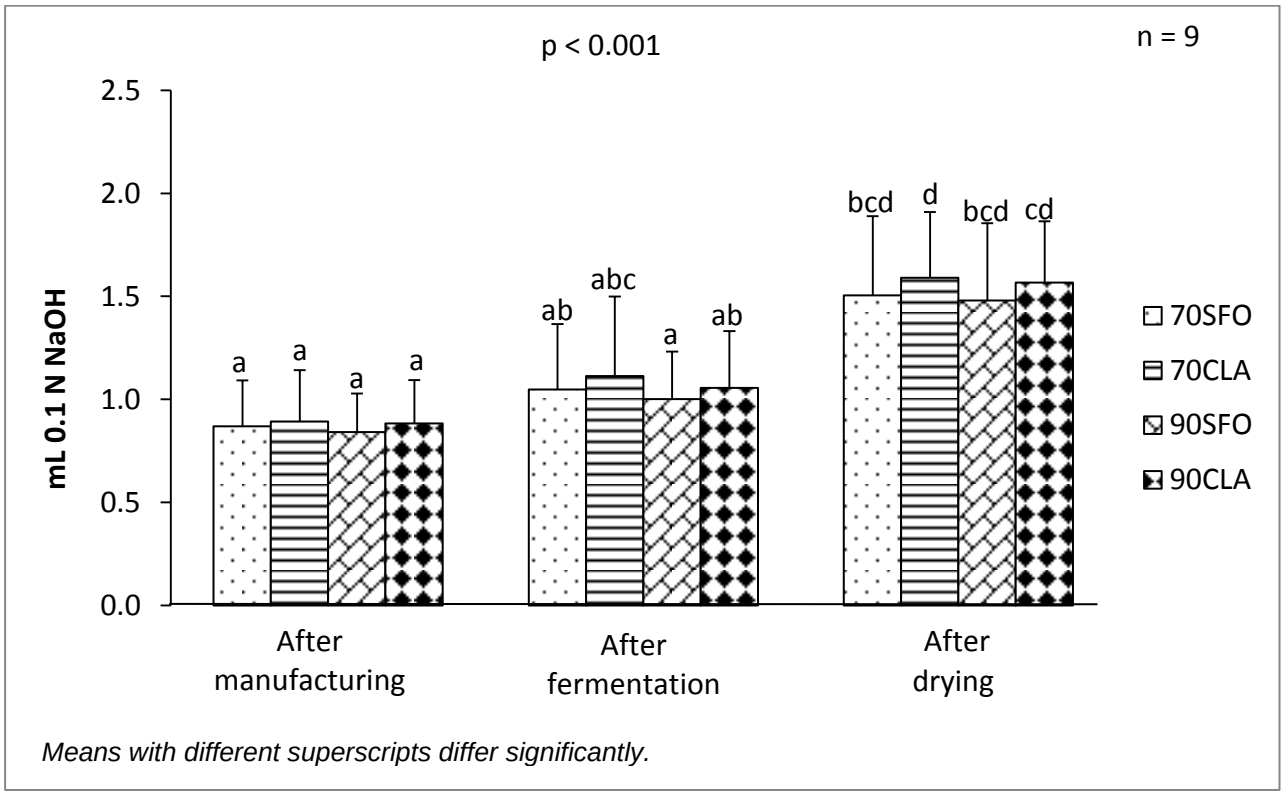


Figure 3.3. Total acidity of the four treatment groups during processing.

3.3.4 Proximate composition of salami

Moisture content

Moisture content of salami was significantly ($p < 0.001$) influenced by processing stage (Table 3.10). From directly after manufacturing, throughout the ripening and drying process the salamis continually released moisture at different rates during each processing stage. Moisture content decreased from between 62.05 ± 1.16% and 64.04 ± 0.62% after manufacturing to between 51.64 ± 1.59% and 52.68 ± 2.30% after drying. This can be ascribed to an increase in the LAB count during fermentation of salami, resulting in an increase in total acidity and a decrease in salami pH. As the pH of salami decrease meat proteins move towards its isoelectric point where it release water. No statistically significant effect for moisture content was observed for slaughter weight and dietary treatment (Table 3.10). Conjugated linoleic acid is reportedly capable of decreasing the moisture content of pork, even at low dietary supplementation levels (Marco *et al.,* 2009). That finding was however not confirmed in this study.

Fat content and fat free dry matter content

As a result of the decrease in moisture content during processing, a significant ($p < 0.001$) increase in fat content of salami was observed from directly after manufacture to after drying (Table 3.10). As the salami dried the fat to total mass ratio increased. Slaughter weight and dietary treatment had no significant effect on the fat content of salami. There was however a trend towards reduced fat content of salami manufactured from CLA containing BF and muscle towards the end of drying compared to salami manufactured from pigs receiving sunflower oil in their diet. Salami manufactured from CLA supplemented pork typically had about 1% lower fat content than salami manufactured from pigs receiving sunflower oil in their diet. This diet effect may be explained by the observation that dietary intake of CLA reduces body fat content of pigs (Keim, 2003). This effect was particularly visible in the 70 kg slaughter weight group where the 70SFO salami had 19.02% fat compared to the 17.46% fat of the 70CLA salami.

Only processing stage had a statistically significant ($p < 0.001$) effect on FFDM content of salami (Table 3.10). Slaughter weight and dietary treatment had no effect on FFDM content. Fat free dry matter content increased from between 23.34 ± 0.70% and 24.00 ± 0.94% after manufacturing to between 29.34 ± 1.24% and 29.92 ± 1.22% after drying. Changes in FFDM content during processing can also be ascribed to decrease in moisture content during processing as in the case for increased fat content.

3.3.5 Microbial stability parameters

The a_w of salami was not affected by differences in slaughter weight and diet (Figure 3.4).

Table 3.10. The proximate composition of salami of importance in ripening.

Processing stage	Slaughter weight	Diet	Moisture (%)	Fat (%)	FFDM (%)
After manufacture	70	SFO (n=9)	62.42 ± 1.44^{bc}	14.24 ± 1.17^{abc}	23.34 ± 0.70^{a}
		CLA (n=9)	64.04 ± 0.62^{c}	14.24 ± 1.17^{abc}	24.00 ± 0.94^{ab}
	90	SFO (n=9)	62.05 ± 1.16^{bc}	14.61 ± 1.87^{bc}	23.34 ± 1.25^{a}
		CLA (n=9)	62.23 ± 1.18^{bc}	13.80 ± 0.96^{ab}	23.98 ± 0.74^{ab}
After fermentation	70	SFO (n=9)	59.15 ± 3.77^{b}	14.71 ± 0.95^{bc}	25.19 ± 1.66^{ab}
		CLA (n=9)	61.47 ± 1.91^{bc}	13.32 ± 1.49^{ab}	25.21 ± 1.58^{ab}
	90	SFO (n=9)	58.70 ± 4.50^{b}	16.37 ± 2.63^{cde}	24.92 ± 2.30^{ab}
		CLA (n=9)	59.68 ± 3.52^{b}	15.22 ± 1.78^{bcd}	25.84 ± 1.68^{b}
After drying	70	SFO (n=9)	51.64 ± 1.59^{a}	19.02 ± 1.24^{f}	29.34 ± 1.24^{c}
		CLA (n=9)	52.68 ± 2.30^{a}	17.46 ± 1.76^{def}	29.86 ± 1.43^{c}
	90	SFO (n=9)	52.48 ± 2.85^{a}	17.95 ± 1.24^{ef}	29.57 ± 1.92^{c}
		CLA (n=9)	52.08 ± 2.80^{a}	17.99 ± 1.96^{ef}	29.92 ± 1.22^{c}
Significance level			p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly.

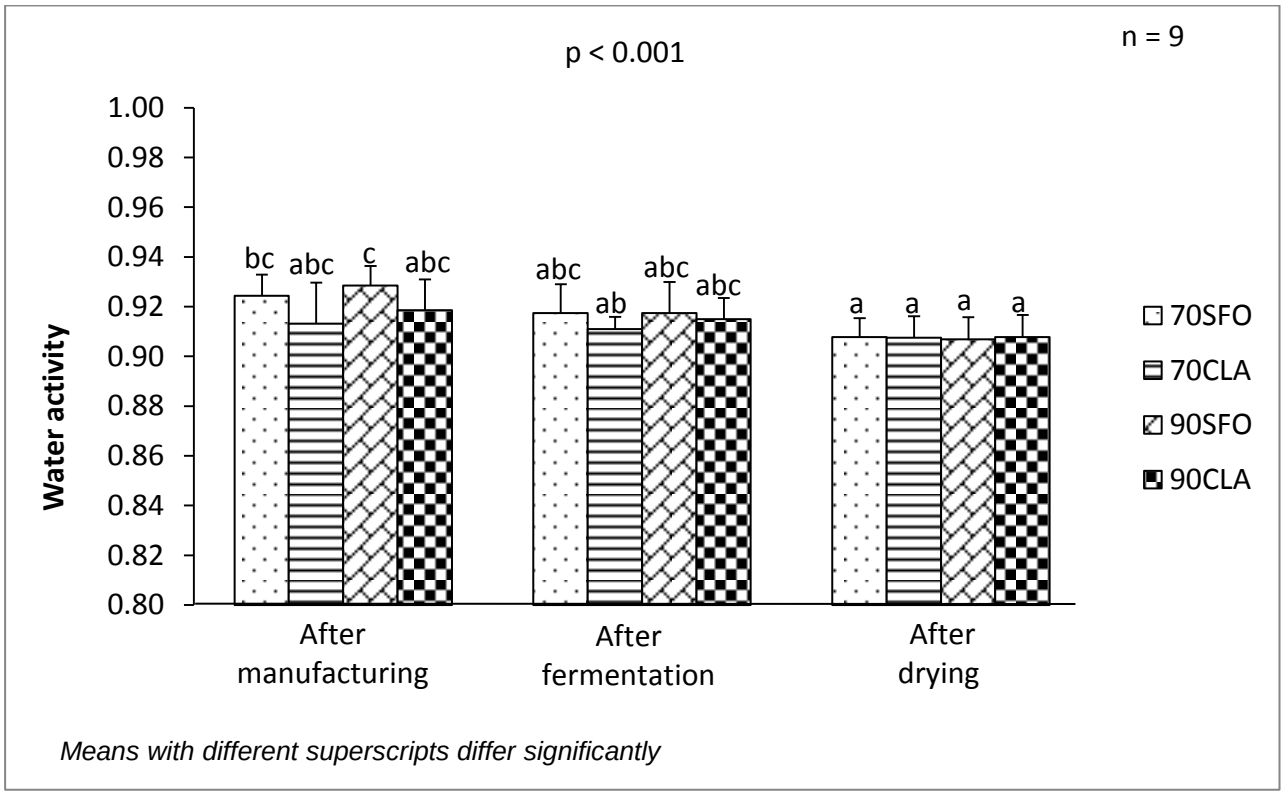


Figure 3.4. Water activity of the four treatment groups during processing.

Processing stage was the only parameter with a significant ($p < 0.001$) effect on a_w. A steady decline in a_w was observed from ±0.92 after manufacturing to ±0.90 after drying. The lack of statistical differences in a_w between slaughter weight and dietary treatment at specific processing stages implies that a_w cannot be used to explain possible differences between microbial parameters at specific processing stages.

Results of the microbial parameters chosen for quantification of the mixed starter culture and common pathogenic and spoilage microorganisms associated with this type of fermented meat product are shown in Table 3.11. For the effects on microbial parameters the following results were obtained. The slaughter weight and diet only affected the yeast and mould counts of the treatment groups significantly ($p < 0.001$). Processing stage was found to affect every microbial parameter significantly ($p < 0.001$) due to the fact that this model system was highly dynamic in terms of microbial growth during different processing stages.

Table 3.11. Microbial stability of salami as affected by slaughter weight, dietary treatment and processing stage.

Processing Stage	Slaughter weight	Diet	Lactic acid bacteria (log cfu/g)	Coliforms (log cfu/g)	*E. coli* (presence)	Yeasts and moulds (log cfu/g)
After manufacture	**70**	**SFO** (n=9)	5.72 ± 0.26^{a}	2.14 ± 0.26^{e}	Present	3.50 ± 0.24^{e}
		CLA (n=9)	5.69 ± 0.36^{a}	1.85 ± 0.50^{e}	Present	2.91 ± 0.21^{cde}
	90	**SFO** (n=9)	5.50 ± 0.24^{a}	1.78 ± 0.28^{de}	Present	2.84 ± 0.19^{bcd}
		CLA (n=9)	5.60 ± 0.43^{a}	1.64 ± 0.67^{cde}	Present	2.72 ± 0.22^{bcd}
After Fermentation	**70**	**SFO** (n=9)	7.31 ± 0.29^{b}	1.78 ± 0.13^{de}	Present	3.25 ± 0.36^{de}
		CLA (n=9)	7.28 ± 0.18^{b}	1.52 ± 0.40^{cde}	Present	2.67 ± 0.21^{bcd}
	90	**SFO** (n=9)	7.23 ± 0.10^{b}	0.97 ± 0.50^{bc}	Present	2.62 ± 0.13^{abcd}
		CLA (n=9)	7.20 ± 0.14^{b}	1.06 ± 0.55^{bcd}	Present	2.52 ± 0.12^{abc}
After drying	**70**	**SFO** (n=9)	7.49 ± 0.09^{bc}	0.21 ± 0.28^{a}	Present	2.94 ± 0.51^{cde}
		CLA (n=9)	7.72 ± 0.67^{bc}	1.08 ± 0.53^{bcd}	Present	1.99 ± 0.79^{a}
	90	**SFO** (n=9)	7.95 ± 0.46^{c}	0.36 ± 0.47^{ab}	Present	2.69 ± 0.56^{bcd}
		CLA (n=9)	7.46 ± 0.37^{bc}	0.90 ± 0.73^{abc}	Present	2.21 ± 0.63^{ab}
Significance level			$p < 0.001$	$p < 0.001$	n.a.	$p < 0.001$

Means with different superscripts in the same column differ significantly.

Lactic acid bacteria

After manufacturing the counts for all four treatment groups were already in excess of log 5 cfu per gram (Table 3.11). This was due to the high inclusion level (estimated at log 6 cfu/g) of the mixed lactic starter culture added to the batter during manufacturing. Counts were significantly ($p < 0.001$) higher after fermentation at log ±7.3 cfu/g and were found to increase a little more through the drying process to log ±7.6 cfu/g. These high counts for LAB also confirmed their dominance over the microbiological population. Processing stage was the only parameter for which significant ($p <$ 0001) differences were found. This is attributed to the growth of the starter culture which increased at each processing step with no significant differences between any slaughter weight and dietary treatment groups within any particular processing stage. No negative effects on the LAB in especially the CLA supplemented salami, such as a slowdown in growth or limited growth, were regarded as a very positive result. Any slowdowns or inhibitions in LAB or their metabolic activities would have negatively affected the quality and stability of the salamis which would have been unacceptable (Fadda *et al*., 2010).

Coliforms

The coliform counts were found to decrease as processing of the salamis progressed (Table 3.11). This is due to their reduced competitiveness at low oxygen tension, pH and due to the presence of sodium chloride, sodium nitrate and sodium nitrite. The decrease in moisture due to drying concentrated the salts and lactic acid, driving the a_w down, further inhibiting coliform growth (Lücke, 1998). The decrease in counts proved not to be as linear as expected with some variations between treatment groups for a particular processing step. For example, after fermentation the 90SFO group had the lowest coliform count, significantly ($p < 0.001$) lower than the 70SFO coliform which had the highest coliform count. After drying the 70SFO group had the lowest coliform count, significantly ($p < 0.001$) lower when compared to the 70CLA coliform count.

E. coli

As an example of a type of coliform bacteria, the presence or absence of *E.coli* was evaluated (Table 3.11). Although the coliform counts were found to decrease over time, probably due to the reasons stated in the previous paragraph, this type of coliform bacteria were able to survive increasingly unfavourable conditions This explained why *E. coli* was found to be present in all four treatment groups throughout processing, from after manufacturing to after drying. Due to the fact that *E.coli* was evaluated qualitatively and not and not quantitatively, and all four treatment groups as well as processing steps had the same results, no accurate deductions could be made in terms of these parameters.

Yeasts and moulds

The microbial environment of the meat processing plant where the salamis were manufactured over the time period stated on page 38 was not regarded to contribute significantly to the yeast and mould counts of the salami. This was due to the fact that the environment was constant throughout this timeframe and more importantly, the influences from the environment were not regarded as parameters in this study. The general trend was for salami from SFO supplemented groups to have higher yeast and mould counts than salami from CLA supplemented groups (Table 3.11). This effect was only significant ($p < 0.001$) between 70CLA and 70SFO after drying (Table 3.11). This may be an indication of an inhibitory effect of CLA on yeasts and/or moulds. Various fatty acids are known to have antifungal activity (Kabara, Sweiczkowski, Conley & Truant, 1972; Bergsson, Arnfinnsson, Steingromsson & Thormar, 2001) which may involve inhibition of FA biosynthesis and inhibition of SFAs and UFA elongation for cell membrane biosynthesis (Wood & Lee, 1981). The salami manufactured from meat from the 70 kg slaughter weight groups tended to have higher yeast and mould counts than salami manufactured from the 90 kg slaughter weight groups (Table 3.11). That can possibly also be attributed to an inhibitory effect of CLA on yeasts and/or moulds since BF from the 90 kg slaughter weight groups usually contain higher CLA levels than BF from the 70 kg slaughter weight groups (Tables 3.7 and 3.8).

Although not as clear as in the case of coliforms and *E. coli*, a statistically significant ($p < 0.001$) decrease was observed in yeast and mould counts as processing of the salamis progressed. Certain LAB such as *Lactobacillus plantarum* (in the mixed starter culture) have been reported to produce antifungal compounds (Magnusson, Ström, Roos, Sjögren & Schnürer, 2003) of which lactic acid is an example (Lind, Jonsson & Schnürer, 2005). This may have contributed to the effects seen for the processing stages. The possible inhibitory effect of CLA on yeasts and/or mould counts discussed above may also play a role in the inhibition of yeasts and moulds with progression in salami processing since the moisture loss during processing will result in an increase in CLA concentration.

3.3.6 Lipid stability parameters

The pro-oxidative factors involved in salami manufacturing is the addition of pork muscle (Monahan, Crackel, Gray, Buckley & Morrissey, 1993) and BF rich in PUFAs and very conducive to lipid oxidation (Gray *et al*., 1996; Bryhni *et al*., 2002), the addition of salt which is a known pro-oxidant working in synergy with the iron in muscle foods (Decker & Xu, 1998) and relatively high processing temperatures which are also conducive to lipid oxidation (Jin, He, Zhang, Yu, Wang & Huang, 2012). Some bacterial starter cultures may also have lipolytic properties (Molly *et al*., 1996; Lizaso *et al*., 1999). The added nitrate can be reduced to nitrite by *Lactobacillus plantarum.* Nitrite is also added and is known to have antioxidant effects on meat lipids (Gray & Pearson, 1984).

Free fatty acids

In the degradation of lipids the first products to form is FFA through lipid hydrolysis (Fritsch, 1981). An increase in FFA leads to a decrease in lipid quality (Atinafu & Bedemo, 2011). The amount of FFAs formed was not significantly affected by slaughter weight or dietary treatment (Figure 3.5). Processing stage had a significant ($p < 0.001$) effect on free fatty acids formed during salami manufacturing (Figure 3.5). After drying, all the treatment groups had significantly ($p < 0.001$) higher levels of FFA than before. Although not statistically significant, the 70 kg slaughter weight groups with higher levels of UFA had higher levels of FFA than the 90 kg slaughter weight groups at all processing stages (Figure 3.5). No trends regarding the effect of dietary treatment on FFA formation could be observed.

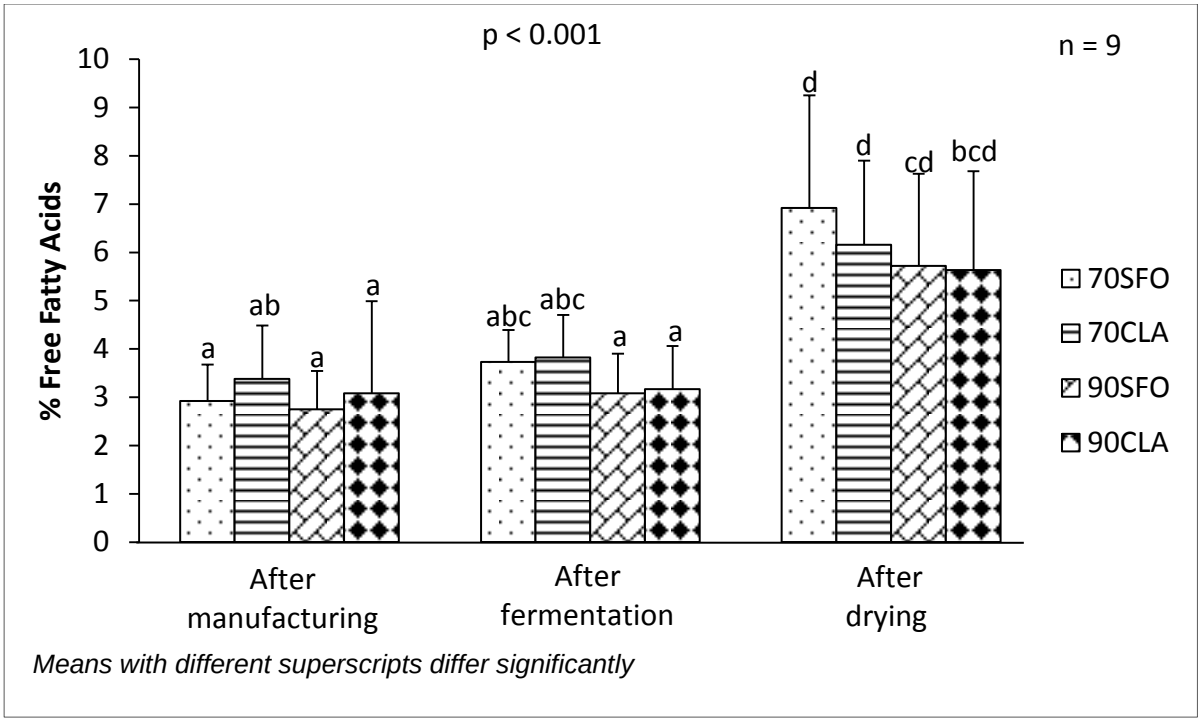


Figure 3.5. Free fatty acid development of the four treatment groups during salami processing.

Peroxide Value

In one of many possible degradation reactions that can affect FA, FFA are oxidised to some form of primary oxidation products known as peroxides (Fritsch, 1981). Peroxides are formed in the early stages of oxidation and are in themselves unstable and highly susceptible to further degradation to secondary oxidation products (Farhoosh & Moosavi, 2009). Peroxide values were not affected by slaughter weight or diet (Figure 3.6). Although not significant, there was a trend for peroxides to increase during ripening (Figure 3.6). Even the 90CLA group with the highest PV of 12.45 ± 3.74 milliequivalents (mEq) peroxide per kg of fat was far below the acceptable limit of 25 mEq peroxide per kg of fat for fatty foods (Narasimham, Raghuver, Arumngham, Bhat & Sen, 1989). As previously discussed, peroxides are unstable and after drying all the treatment groups had lower PVs, although this proved to be insignificant. This decreasing trend in peroxides may

also be attributed to the observation that some LAB from naturally fermented salami have even been shown to significantly decrease the concentration of peroxides (Sawitzki, Fiorentini, Junior, Bertol & Sant Anna, 2008).

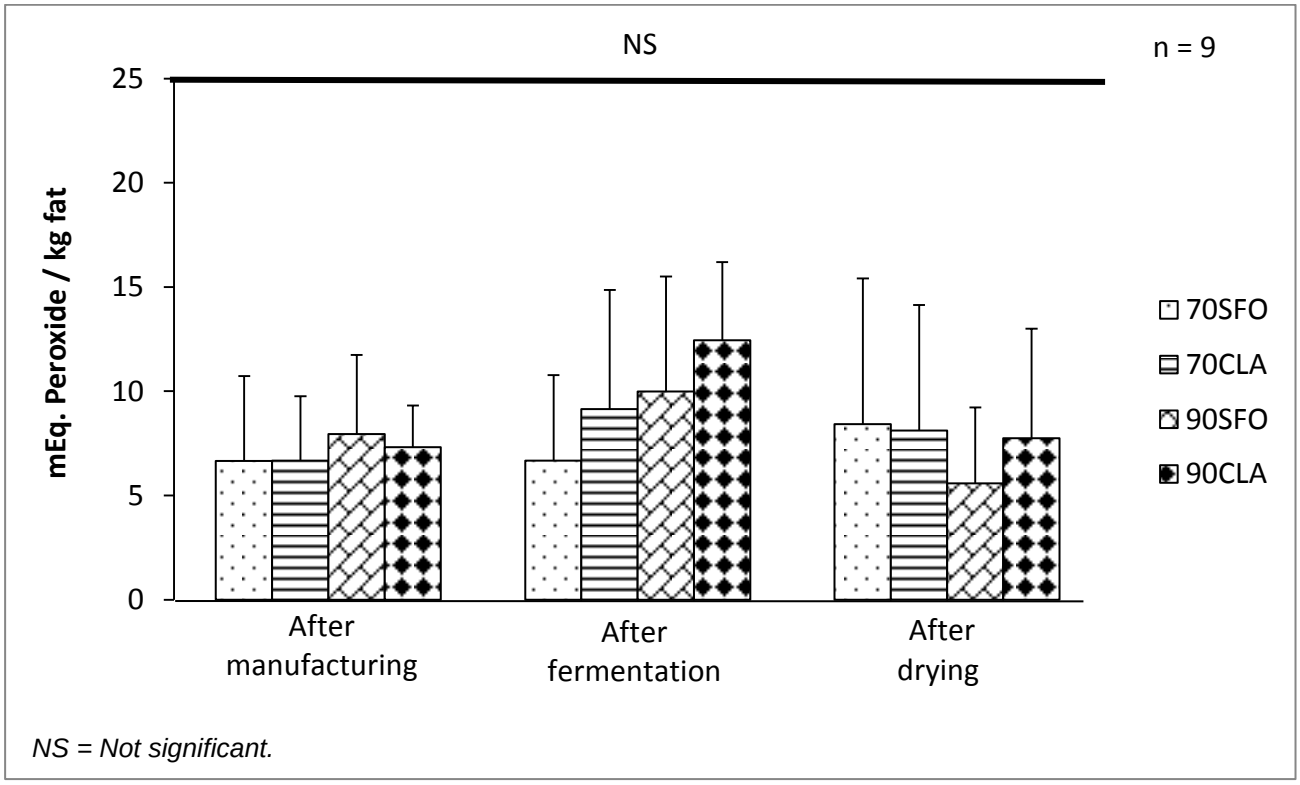


Figure 3.6. Peroxide formation of the four treatment groups during salami processing with the 25 mEq peroxide per kg of fat limit indicated as a solid line.

TBARS

Throughout salami processing secondary oxidation products for all four treatment groups were detected (Figure 3.7), indicating the further oxidation of primary oxidation products. These products known as aldehydes were quantified as TBARS. Slaughter weight and diet had no significant effects on the formation of TBARS (Figure 3.7). After manufacturing the TBARS for all four treatment groups were in a very narrow range, this range increased after ripening and the range for TBARS after drying was found to be spread over a very wide range. This is attributed to the sensitivity of TBARS analyses to the interference from amino acids, sugars and nitrite (Janero, 1990). Salami from the 70SFO group with a TBARS value of 0.64 ± 0.61 mg malonaldehyde/kg salami after drying was the only group with a level of lipid oxidation capable of producing a rancid odour and taste which may be detectable by consumers. Values for TBARS above 0.5 are considered to have a negative effect on the sensory properties of meat products (Wood *et al*., 2008).

3.3.7 Fatty acid content, composition and ratios

Fatty acid content and composition

The fatty acid composition of the four treatment groups were compared on the finished product at the end of drying. The two CLA dietary treatment groups were found to contain significantly ($p < 0.001$) higher levels of the following SFAs than the two SFO groups: C14:0; C15:0; C16:0 and C17:0 (Table 3.12). The concentrations of these fatty acids were not affected by the different slaughter weights. This confirmed the findings of various authors (Smith *et al*., 2002; Wiegand *et al*., 2002; Lauridsen *et al*., 2005) who reported similar results where higher amounts of SFAs were present in tissue lipids from animals receiving CLA supplemented diets compared to tissue lipids from animals receiving SFO supplemented diets at the same dietary inclusion level. Slaughter weights and dietary treatment significantly ($p < 0.001$) affected the concentration of C18:0 with higher slaughter weights and dietary CLA supplementation resulting in significantly higher levels of C18:0. A higher slaughter weight also led to a significantly higher ($p < 0.001$) level of C20:0 in the 90SFO group compared to the 70SFO group.

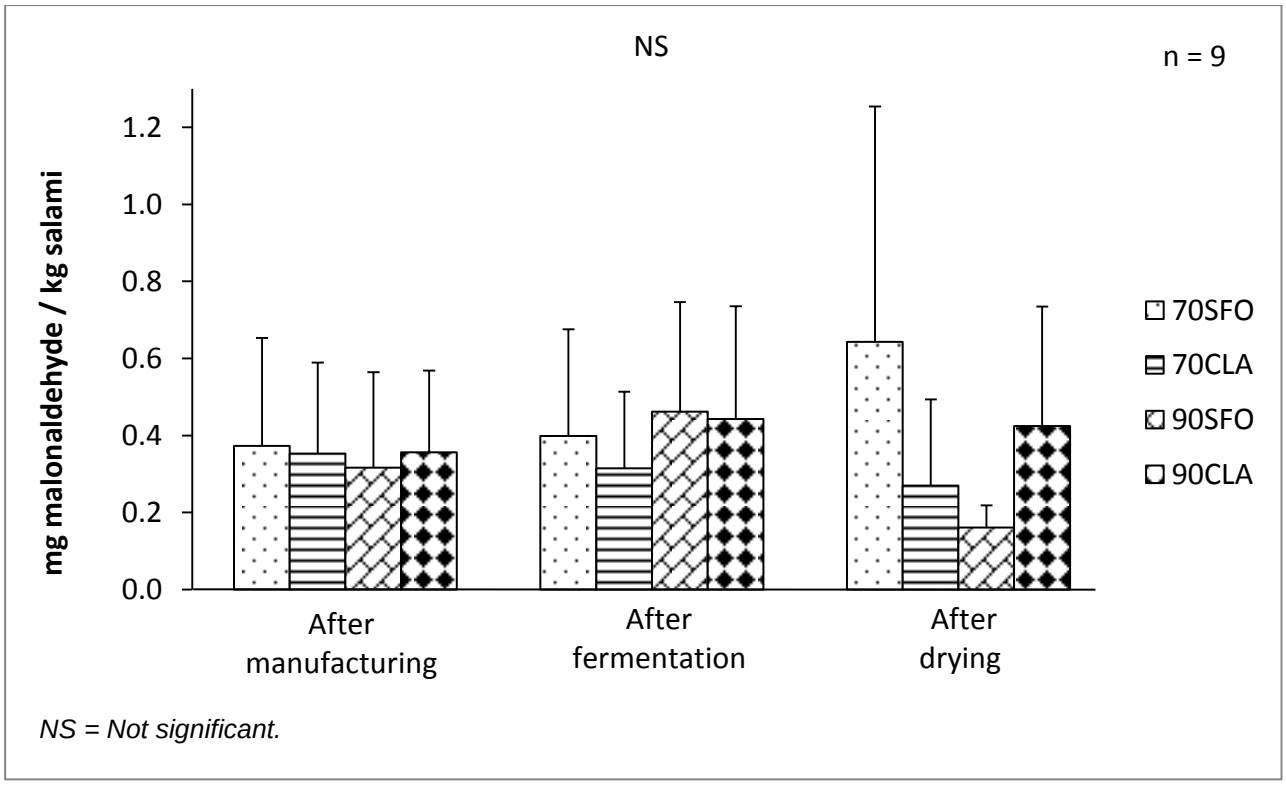


Figure 3.7. Thiobarbituric acid reactive substance analysis of the four treatment groups during salami processing.

As far as MUFAs were concerned, the concentration of C16:1*c*9 was significantly ($p < 0.001$) affected by both slaughter weight and dietary treatment (Table 3.12). The 70SFO group had a significantly higher concentration of C16:1*c*9 compared to the 90SFO group. This effect of decreasing the level of some MUFAs (C16:1*c*9, C18:1c7) with increased slaughter weight

appeared to have been inhibited in the CLA dietary treatment groups to below significant levels (Table 3.12). Dietary treatment and slaughter weight had similar effects on C18:1*c*9, except that here it led to a significant increase in C18:1*c*9 for the 90SFO group compared to the 70SFO group (Table 3.12). For C18:1*t*9 present at very low levels in all four treatment groups, significant ($p < 0.001$) differences were found between dietary treatments with lower concentrations for the two SFO treatment groups compared to the higher concentrations of the two CLA treatment groups (Table 3.12). Slaughter weight also contributed to C18:1*t*9 content, although this effect was only significant between the two SFO groups with the 70SFO group having the lower concentration and the 90SFO group having the higher concentration of C18:1*t*9 (Table 3.12). The concentration of C20:1*c*11 was significantly ($p < 0.001$) affected by dietary treatment with the SFO dietary treatment groups having significantly higher levels of C20:1*c*11 than the two CLA dietary treatment groups.

Table 3.12. The FA composition (%) of salami as affected by slaughter weight and diet at the end of processing.

Slaughter weight (kg)	**70**		**90**		**Sign. level**
Diet	**SFO** (n=9)	**CLA** (n=9)	**SFO** (n=9)	**CLA** (n=9)	
C14:0	1.36 ± 0.20^{a}	1.92 ± 0.10^{b}	1.30 ± 0.06^{a}	1.97 ± 0.13^{b}	$p < 0.001$
C15:0	0.02 ± 0.02^{a}	0.04 ± 0.03^{b}	0.01 ± 0.02^{a}	0.04 ± 0.03^{b}	$p < 0.001$
C16:0	25.02 ± 1.33^{a}	27.80 ± 1.40^{b}	25.30 ± 1.27^{a}	28.44 ± 1.70^{b}	$p < 0.001$
C16:1*c*9	2.07 ± 0.08^{b}	2.37 ± 0.08^{c}	1.81 ± 0.07^{a}	2.41 ± 0.07^{c}	$p < 0.001$
C17:0	0.38 ± 0.03^{a}	0.49 ± 0.03^{b}	0.37 ± 0.02^{a}	0.48 ± 0.03^{b}	$p < 0.001$
C18:0	12.09 ± 0.43^{a}	14.64 ± 0.67^{c}	13.38 ± 0.54^{b}	15.62 ± 0.57^{d}	$p < 0.001$
C18:1*t*9	0.02 ± 0.03^{a}	0.07 ± 0.03^{c}	0.04 ± 0.03^{b}	0.08 ± 0.03^{c}	$p < 0.001$
C18:1*c*9	38.15 ± 0.39^{b}	32.33 ± 0.41^{a}	39.08 ± 0.34^{c}	32.38 ± 0.37^{a}	$p < 0.001$
C18:1*c*7	2.43 ± 0.09^{c}	2.13 ± 0.06^{a}	2.33 ± 0.07^{b}	2.15 ± 0.04^{a}	$p < 0.001$
C18:2*c*9,12 (*n*-6)	15.86 ± 0.44^{c}	15.28 ± 0.28^{b}	13.99 ± 0.19^{a}	13.74 ± 0.87^{a}	$p < 0.001$
C20:0	0.17 ± 0.03^{a}	0.18 ± 0.02^{a}	0.21 ± 0.02^{b}	0.18 ± 0.03^{a}	$p < 0.001$
C20:1*c*11	0.59 ± 0.05^{b}	0.46 ± 0.07^{a}	0.63 ± 0.11^{b}	0.49 ± 0.05^{a}	$p < 0.001$
C18:3*c*9,12,15 (*n*-3)	0.38 ± 0.28	0.39 ± 0.28	0.27 ± 0.20	0.33 ± 0.24	NS
C18:2*c*9,*t*11 (*n*-6) (CLA)	0.03 ± 0.03^{a}	0.43 ± 0.03^{b}	0.01 ± 0.02^{a}	0.48 ± 0.04^{c}	$p < 0.001$
C18:2*t*10,*c*12 (*n*-6) (CLA)	ND	0.18 ± 0.01^{a}	ND	0.19 ± 0.04^{b}	$p < 0.001$
C20:2*c*11,14 (*n*-6)	0.51 ± 0.04^{b}	0.41 ± 0.03^{a}	0.50 ± 0.04^{b}	0.38 ± 0.03^{a}	$p < 0.001$
C20:3*c*11,14,17 (*n*-3)	0.09 ± 0.02^{b}	0.09 ± 0.02^{ab}	0.08 ± 0.01^{ab}	0.08 ± 0.02^{a}	$p < 0.05$
C20:4*c*5,8,11,14 (*n*-6)	0.34 ± 0.06^{b}	0.32 ± 0.05^{b}	0.32 ± 0.03^{b}	0.26 ± 0.04^{a}	$p < 0.001$
C22:6*c*4,7,10,13,16,19 (*n*-3)	0.23 ± 0.05^{b}	0.21 ± 0.04^{ab}	0.18 ± 0.04^{a}	0.19 ± 0.04^{a}	$p < 0.001$

Means with different superscripts in the same row differ significantly. NS = Not significant

A significantly ($p < 0.001$) lower level of C18:2*c*9,12 was found for 70CLA compared to 70SFO as a result of CLA dietary supplementation (Table 3.12). No such effect was found between 90SFO and 90CLA. The amount of C18:2*c*9,12 were significantly ($p < 0.001$) lower for both 90 kg slaughter weight groups compared to the 70 kg slaughter weight groups with no significant difference in concentration between the two 90 kg groups. The C18:3*c*9,12,15 level of salami was not significantly influenced by slaughter weight or dietary CLA supplementation. The significant difference observed in the BF between the 70SFO and 90SFO groups for this fatty acid (Table 3.5) completely disappeared between the four treatment groups after salami processing (Table 3.12). The C18:2*c*9,*t*11 isomer of CLA occurred at levels in salami after manufacturing comparable to

levels found in BF before processing (Tables 3.5 and 3.12). Both SFO treatment groups had very low levels of C18:2*c*9,*t*11 as opposed to the much higher concentrations for 70CLA and 90CLA. The concentration of the C18:2*t*10,*c*12 isomer of CLA remained undetected in both SFO groups after salami processing. Both CLA isomers were found at significantly ($p < 0.001$) higher concentrations in salami from the 90CLA group compared to salami from the 70CLA group (Table 3.12). This was in contrast with the concentrations of both CLA isomers in the BF raw material (Table 3.5) where only the C18:2*c*9,*t*11 isomer had a significantly ($p < 0.001$) higher concentration in the 90CLA group. It was reported that increases in the CLA concentrations occurred in dairy products over time due to the metabolic action of LAB (Ha *et al*., 1989; Shantha *et al*., 1995; Jiang, *et al.*, 1998). This study did not confirm these findings for a fermented meat product.

A dietary treatment effect was reported for C20:2*c*11,14 which occurred in the salami at levels similar to the concentrations found in BF (Table 3.5). The concentration of C20:2*c*11,14 was significantly ($p < 0.001$) higher for 70SFO and 90SFO compared to 70CLA and 90CLA (Table 3.12). No slaughter weight effect was observed for this FA (Table 3.12). Although it occurred at very low levels in salami, the concentration of C20:3*c*11,14,17 differed significantly ($p < 0.05$) between the 70SFO and 90CLA groups (Table 3.12). For C20:4*c*5,8,11,14 three of the treatment groups (70SFO, 70CLA & 90SFO) basically had the same concentration while the 90CLA group had a significantly ($p < 0.001$) lower concentration compared to the other three treatments (Table 3.12) The 70SFO group had a concentration of C22:6*c*4,7,10,13,16,19 of 0.23 ± 0.05% which was significantly ($p < 0.001$) higher than that of the 90SFO and 90CLA groups at 0.18 ± 0.04% and 0.19 ± 0.04% respectively. The lower content of PUFAs in salami in the 90 kg CLA group may be attributed to the more saturated profile of more mature fat tissue in heavier pigs and also to the fact that the longer exposure time to dietary CLA in the heavier pigs will also lead to increased lipid saturation (Migdał *et al*., 2004). The only TFA detected in salami was C18:1*t*9 that occurred at very low levels in all four treatment groups. The two SFO groups had significantly ($p < 0.001$) lower C18:1*t*9 content than the two CLA treatment groups. Slaughter weight also contributed since the 90SFO group had significantly higher C18:1*t*9 than the 70SFO group.

Fatty acid ratios

The fatty acid ratios of the salamis at the end of processing (Table 3.13) more or less mirrored the results found for the BF (Table 3.5) as the main contributor of lipids to the salamis. The percentages of MUFAs in the two CLA dietary treatment groups were significantly ($p < 0.001$) lower than the percentages found for the two SFO dietary treatment groups (Table 3.13). The salami from the 90SFO group also had significantly ($p < 0.001$) higher MUFAs than the salami from the 70SFO group. The percentages of PUFAs present in the four treatment groups were only significantly affected by slaughter weight; diet had no effect on PUFA content (Table 3.13). As in the case of BF, salami from the 90 kg slaughter weight groups had significantly ($p<0.001$) lower

PUFA content than salami from the 70 kg slaughter weight groups (Tables 3.5 and 3.13). The total UFA content of salami were significantly ($p < 0.001$) influenced by both slaughter weight and dietary treatment (Table 3.13). The main effect was however brought about by diet as the two SFO groups, SFO70 and SFO90, had UFA levels of 60.90 ± 1.10% and 59.40 ± 0.78% respectively, compared to the significantly ($p < 0.001$) lower levels of UFA for 70CLA and 90CLA of 54.85 ± 0.93% and 53.22 ± 1.62% respectively (Table 3.13). The percentage of SFAs in the salamis after processing was found to follow a similar trend to the UFA with slaughter weight and diet having a significant ($p < 0.001$) effect on SFA content (Table 3.13). The SFA content of 70SFO and 70CLA salamis was 39.10 ± 1.10% and 45.15 ± 0.93% and differed significantly ($p < 0.001$) from each other and were also significantly ($p < 0.001$) lower in SFA content than their corresponding higher slaughter weight groups at 40.60 ± 0.78% and 46.78 ± 1.62% respectively (Table 3.13). In the BF raw material the slaughter weight effect on SFA content was not statistically significant (Table 3.5).

Table 3.13. Fatty acid ratios of nutritional and technological importance of the four dietary treatment groups at the end of salami processing.

Slaughter weight (kg)	**70**		**90**		**Sign. level**
Diet	**SFO** (n=9)	**CLA** (n=9)	**SFO** (n=9)	**CLA** (n=9)	
MUFA (%)	43.39 ± 0.48^{b}	37.49 ± 0.47^{a}	44.00 ± 0.41^{c}	37.58 ± 0.44^{a}	$p < 0.001$
PUFA (%)	17.51 ± 0.85^{b}	17.36 ± 0.68^{b}	15.41 ± 0.43^{a}	15.63 ± 1.25^{a}	$p < 0.001$
UFA (%)	60.90 ± 1.10^{d}	54.85 ± 0.93^{b}	59.40 ± =0.78^{c}	53.22 ± 1.62^{a}	$p < 0.001$
SFA (%)	39.10 ± 1.10^{a}	45.15 ± 0.93^{c}	40.60 ± 0.78^{b}	46.78 ± 1.62^{d}	$p < 0.001$
MUFA/SFA	1.11 ± 0.04^{d}	0.83 ± 0.03^{b}	1.08 ± 0.03^{c}	0.80 ± 0.04^{a}	$p < 0.001$
PUFA/SFA	0.45 ± 0.03^{c}	0.38 ± 0.02^{b}	0.38 ± 0.02^{b}	0.34 ± 0.04^{a}	$p < 0.001$
IV (Calculated)	69.11 ± 2.08^{d}	63.69 ± 1.82^{b}	65.75 ± 1.34^{c}	60.73 ± 2.75^{a}	$p < 0.001$
DBI	80.60 ± 2.57^{d}	74.33 ± 2.20^{b}	76.62 ± 1.67^{c}	70.51 ± 3.54^{a}	$p < 0.001$
PI	21.88 ± 1.85^{b}	21.46 ± 1.58^{b}	19.23 ± 1.15^{a}	19.19 ± 2.11^{a}	$p < 0.001$
Atherogenicity Index	30.52 ± 1.72^{a}	35.51 ± 1.76^{b}	30.53 ± 1.46^{a}	36.38 ± 2.14^{b}	$p < 0.001$
***n*-6 (%)**	16.77 ± 0.53^{b}	16.64 ± 0.37^{b}	14.85 ± 0.22^{a}	15.05 ± 0.92^{a}	$p < 0.001$
***n*-3 (%)**	0.74 ± 0.39	0.72 ± 0.36	0.56 ± 0.28	0.58 ± 0.36	NS
***n*-6/*n*-3**	33.82 ± 25.97	36.55 ± 27.94	36.43 ± 25.21	34.66 ± 33.88	NS

Means with different superscripts in the same row differ significantly. NS = Not significant.

The MUFA/SFA ratios of salami from the four dietary treatment groups were found to be significantly ($p < 0.001$) affected by both slaughter weight and diet (Table 3.13). The 70SFO group had the highest ratio of MUFA/SFA which made it from a human nutrition and health point of view, the best group (Cordain *et al*., 2005; Muchenje *et al*., 2009), but the worst group from a fat quality point of view (Lea *et al*., 1970). The salami from the 70SFO group had a significantly ($p < 0.001$) higher ratio of MUFA/SFA than the 90SFO group at 1.11 ± 0.04 and 1.08 ± 0.03 respectively. The values for these two SFO groups were both significantly ($p < 0.001$) higher than the corresponding CLA dietary treatment groups of 70CLA and 90CLA at 0.83 ± 0.03 and 0.80 ± 0.04 respectively. For the PUFA/SFA ratio, the 70SFO group was the only group that managed to fall within the dietary guideline of 0.4 to 1.0 (WHO, 2003; Scollan *et al*., 2006; Wood *et al*., 2008) with a PUFA/SFA of 0.45 ± 0.03 (Table 3.13). The low values of the other three groups (all below a

PUFA/SFA ratio of 0.4) are ascribed to the SFAs increasing effects as a result of dietary CLA supplementation and increased slaughter weight respectively, or in combination with each other, depending on the particular treatment group. With an IV of between 60 (Fischer, 1989b) and 70 (Barton-Gade, 1983; Girard *et al*., 1988) suggested for good quality fat, the calculated IV of all four treatment groups fall within this range (Table 3.13). The 90 CLA group with a high slaughter weight, the highest SFA content and the lowest UFA content had the lowest calculated IV of 60.73 ± 2.75. On the other side of the spectrum, 70SFO with a low slaughter weight, lowest SFA and highest UFA content had a calculated IV of 69.11 ± 2.08 that approached the upper border of acceptable fat quality.

The DBI of three of the four treatment groups were below the recommended DBI of 80 for a good quality BF (Prabucki, 1991). The DBI of 70SFO was found to be slightly above the cut-off point at 80.60 ± 2.57 (Table 3.13). Dietary treatment and slaughter weight both affected the DBI significantly ($p < 0.001$) with higher slaughter weights and dietary CLA supplementation resulting in lower DBI values (Table 3.13). Peroxidizability index was only significantly ($p < 0.001$) influenced by slaughter weight (Table 3.13). Salami from the two higher slaughter weight groups of 90SFO and 90CLA had PI of 19.23 ± 1.15 and 19.19 ± 2.11 respectively, which was significantly ($p < 0.001$) lower than the PI of salami from the 70SFO and 70CLA groups at 21.88 ± 1.85 and 21.46 ± 1.58 respectively (Table 3.13). That means that salamis manufactured from meat from higher slaughter weight pigs will be more resistant to oxidation than salami manufactured from meat from pigs of lighter slaughter weight groups. The atherogenicity index of salami which is very important in terms of human health (Kanner, 1994; Chizzolini *et al*., 1998) was only significantly ($p < 0.001$) influenced by dietary treatment ($p < 0.001$). It was found that salami from the SFO supplemented diets in both the 70SFO and 90SFO groups, gave significantly ($p < 0.001$) lower values at 30.52 ± 1.72 and 30.53 ± 1.46, compared to the much higher atherogenicity indices of salami from both the 70CLA and 90CLA groups at 35.51 ± 1.76 and 36.38 ± 2.4 respectively. The higher atherogenicity index of salami from the CLA supplemented groups compared to the SFO supplemented groups may imply that the lipids of salami manufactured from dietary CLA supplemented meat may have cholesterol raising properties. Lower values for this index are preferred for good human health. It should be kept in mind that CLA on its own also has cholesterol lowering, therapeutic and anti-oxidant effects (Pariza *et al.,* 1985; Lee *et al*., 1994; Belury *et al*., 1996; Park *et al*., 1999; Du *et al.*, 2000; Miller *et al*., 2001; Smedman & Vessby, 2001; Hur *et al*., 2004).

The percentage *n*-6 FA was significantly ($p < 0.001$) affected only by slaughter weight. The *n*-6 values of salami from the two higher slaughter weight groups (90SFO and 90CLA) at 14.85 ± 0.22% and 15.05 ± 0.92% being significantly ($p < 0.001$) lower than that of the lower slaughter weight groups (70SFO and 70CLA) at 16.77 ± 0.53% and 16.64 ± 0.37%. The percentage of *n*-3 present in each treatment group was not affected by slaughter weight or diet. The higher slaughter

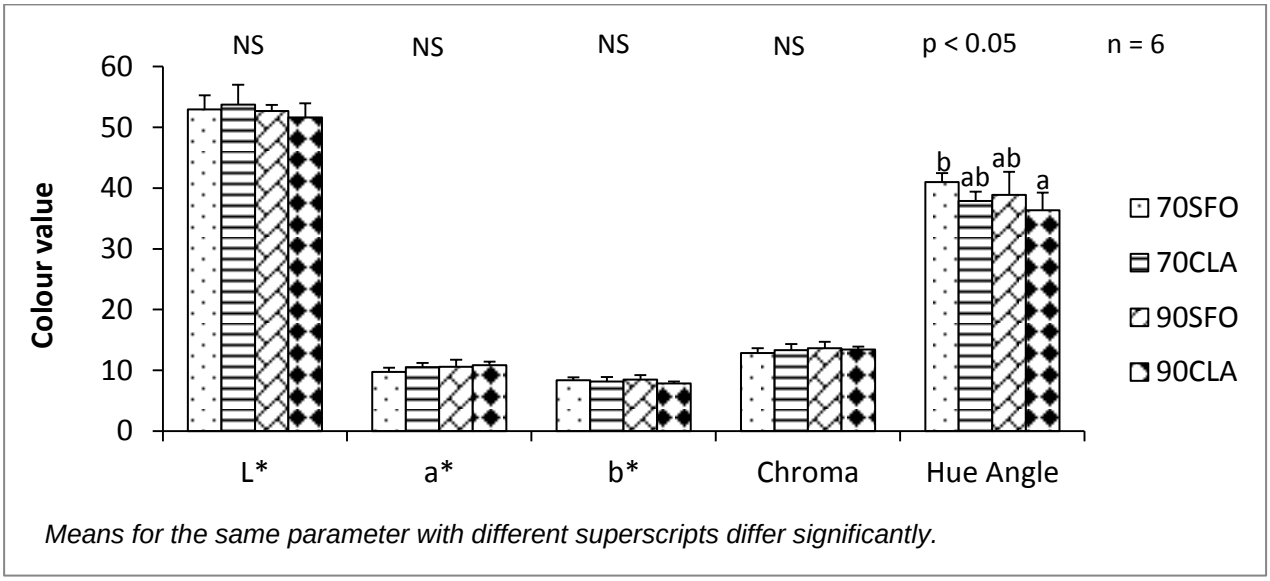
weight groups did however show lower values for the percentage *n*-3 fatty acids (Table 3.13). Diet and slaughter weight had no significant effect on the *n*-6/*n*-3 ratio which is a nutritionally important guideline with a ratio of < 4:1 recommended for the human diet (Table 3.13). The *n*-6/*n*-3 ratios of all four treatment groups clearly overstepped this guideline, negatively affecting the nutritional profile of all four treatment groups and proving that pork lipids are unbalanced in this regard (Scollan *et al*., 2006; Wood *et al*., 2008). The inclusion of lipid sources rich in *n*-3 fatty acids in pig diets will improve the *n*-6/*n*-3 ratio of pork products by increasing the amount of *n*-3 in adipose tissues (Romans, Wulf, Johnson, Libal & Costello, 1995).

3.3.8 Physical parameters

Colour and texture (shear and compression) was selected as criteria to monitor physical quality of salami from different treatments. Analysis was only performed on the finished salamis at the end of processing (after drying).

Colour

No significant differences were observed between any of the dietary treatment groups for the following colour parameters (Figure 3.8): *L** (lightness); *a** (redness); *b** (yellowness) or Chroma (vividness of colour). Joo *et al.* (2002), Tischendorf *et al.* (2002) and Intarapichet, Maikhunthod & Thungmanee (2008) also reported that dietary supplementation of CLA in pigs did not affect *L*, a** or *b** values. Hue angle was the only colour parameter for which a significant ($p < 0.05$) difference was identified. The 90CLA group had a significantly smaller Hue angle compared to the 70SFO group, indicative of a less red product.



Means for the same parameter with different superscripts differ significantly.

Figure 3.8. Comparison of the four treatment groups for the main colour parameters at the end of processing.

Texture measurements

Significant differences were identified between treatment groups for both shear force ($p < 0.05$) and compression force ($p < 0.01$) of salami (Figure 3.9). For shear force the 70SFO group needed significantly more force to be sliced through than the 70CLA group. No clear explanation for this could be found. Shear force is reportedly not affected by dietary CLA supplementation (Tischendorf *et al.*, 2002; Intarapichet *et al.*, 2008). The significantly higher shear force needed for 70SFO can also not be explained by increased connective tissue as the animals were in the lowest slaughter weight group. Peri-mortem stress would have resulted in dark, firm and dry meat (DFD) with a high initial pH and effectively decreased tenderness (Essén-Gustavsson, 1992). This was not the case as pH_{24} (Table 3.6) was relatively the same for all four treatment groups. If DFD had occurred, it would also have been reflected in the compression force results (Figure 3.6). For compression force the 90CLA group needed significantly more force to be compressed to 10% of the original sample height compared to all other treatment groups (Figure 3.3). This can be described as an effect of dietary CLA supplementation which increases fat hardness due to increases in SFA with corresponding decreases in UFA (Joo *et al*., 2002; Wiegand *et al*., 2002; Lo Fiego, Macchioni, Santoro, Pastorelli & Corino, 2005; Marco *et al*., 2009).

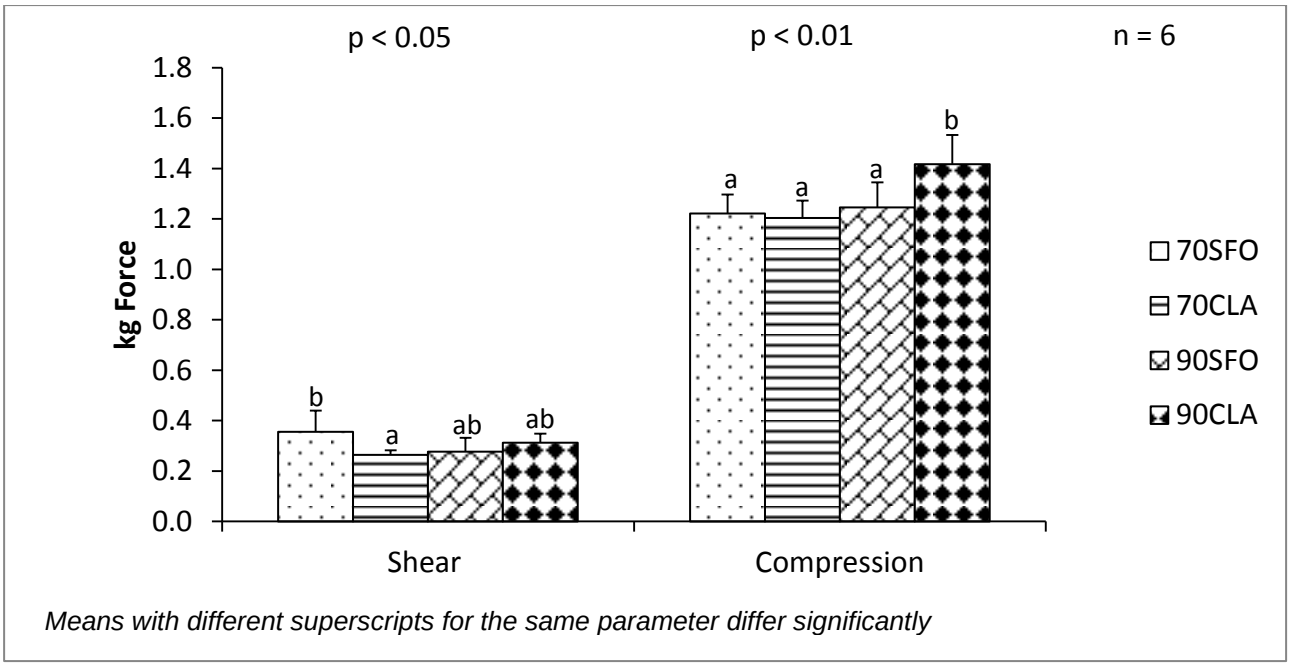


Figure 3.9. Comparison of the four treatment groups for compression force and shear force at the end of processing.

3.3.9 Sensory analysis

The sensory analysis was performed on the salamis after drying at the end of processing using a 75 member consumer panel. The panel did not identify any significant differences between any of the four treatment groups for aroma, taste, firmness or overall acceptability (Figure 3.10). This indicates that dietary supplementation and slaughter weight as variables led to a situation where

the liking of the sensory properties of the four treatment groups did not differ. This is in agreement with the findings of Alonso, Najes, Provincial, Guillén, Gil, Roncalés & Beltrán (2012) who found that dietary fat supplementation did not significantly affect sensory attributes of pork; and is in agreement with the findings of Migdał *et al*. (2004) who found that CLA dietary supplementation had no overall effect on the sensory attributes of pork. The scores for all four attributes fell between "neither like nor dislike" and just under "like moderately" indicating no clear liking to the salamis in general. This could be a result of the low intensity smoking or relatively basic spice mixture used to minimise the introduction of any extra variables.

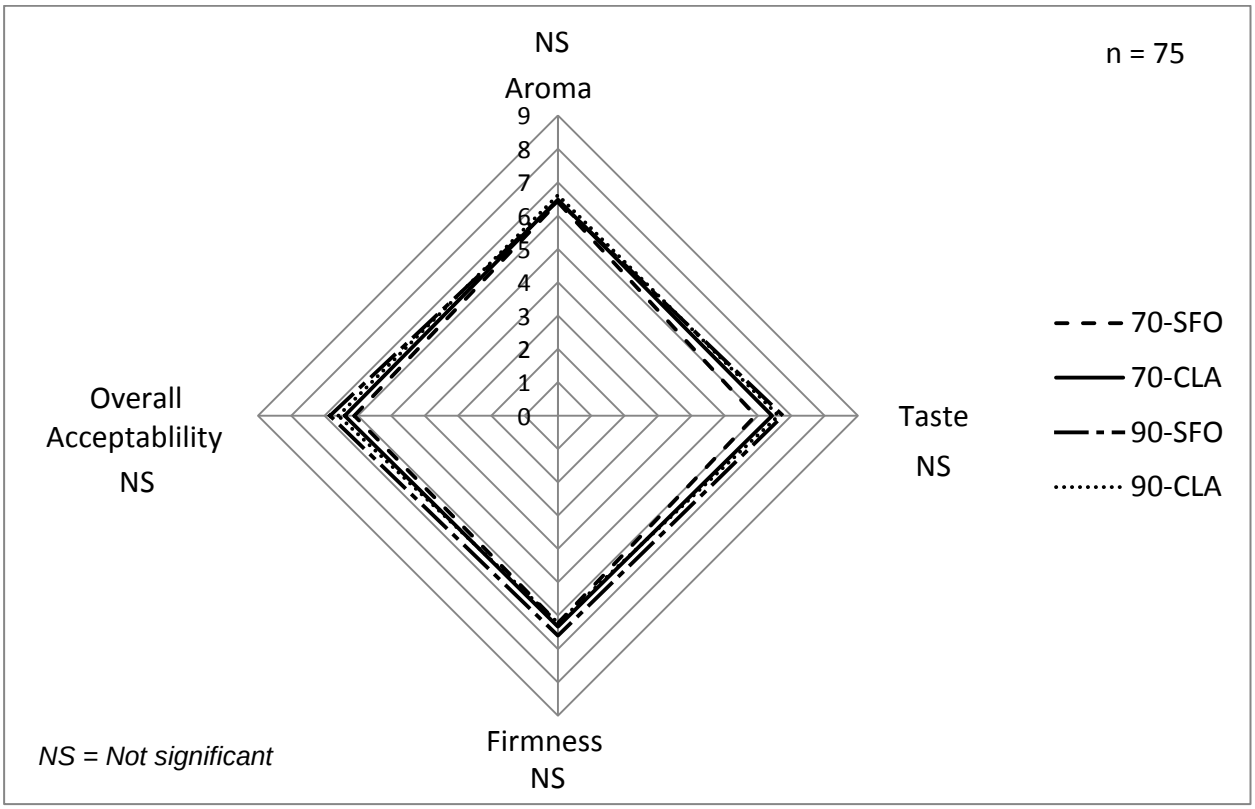


Figure 3.10. A spider plot of the sensory scores of four attributes for each of the four treatment groups.

3.3.10 Actual CLA content of the salami

Concentration of each CLA isomer per 28 g portion of salami

The CLA levels of each of the two relevant isomers in the salamis at the end of processing (Table 3.14) revealed the same trends as that of the BF raw material (Table 3.7). This is due to the fact that pork BF was the main contributor of CLA to the salamis. Actual CLA content of salami was significantly ($p < 0.001$) influenced by dietary treatment and slaughter weight. The total amount of CLA per 28 g portion of salami varied from extremely low levels at 2.11 ± 1.20 mg CLA/28 g salami for the 70SFO group tot 42.63 ± 5.79 mg CLA/28 g salami for the 90CLA group.

Table 3.14. Conjugated linoleic acid content of salami per 28 g portion and per 100 g as affected by diet at the end of processing.

Slaughter weight (kg)	**70**		**90**		**Sign. Level**
Diet	**SFO** (n=10)	**CLA** (n=10)	**SFO** (n=10)	**CLA** (n=10)	
C18:2c9,t11 (mg/28 g salami)	2.11 ± 1.20[a]	27.65 ± 3.57[b]	3.58 ± 1.05[a]	31.05 ± 4.23[c]	p < 0.001
C18:2c9,t11 (mg/100 g salami)	7.52 ± 4.27[a]	98.73 ± 12.75[b]	12.79 ± 3.75[a]	110.90 ± 15.12[c]	p < 0.001
C18:2t10,c12 (mg/28 g salami)	ND	9.84 ± 2.80[a]	ND	11.58 ± 1.58[b]	p < 0.001
C18:2t10,c12 (mg/100 g salami)	ND	35.13 ± 9.98[a]	ND	41.35 ± 5.66[b]	p < 0.001
mg Total CLA /28 g salami	2.11 ± 1.20[a]	37.48 ± 5.39[b]	3.58 ± 1.05[a]	42.63 ± 5.79[c]	p < 0.001
% of RDA	0.06 ± 0.03[a]	1.07 ± 0.15[b]	0.10 ± 0.03[a]	1.22 ± 0.17[c]	p < 0.001

Means with different superscripts in the same row differ significantly ND = Not detected

Percentage of the RDA of each of the four treatment groups

When the CLA concentrations of the four treatment groups were expressed as percentages of the RDA for CLA it became clear that dietary CLA supplementation of pigs did not succeed in increasing the CLA content of meat to very high levels (Figure 3.11). Even the two CLA dietary supplemented groups, 70CLA and 90CLA, only managed to reach the 1.07 ± 0.15% and 1.22 ± 0.17% RDA level (Table 3.14). Roughly translated into practical terms this means that for a person to receive 100% of the RDA, about 3.5 kg salami would have to be consumed per day! With a 1% contribution to RDA, dietary supplementation of pork with CLA cannot be considered a very successful approach to increase human consumption of CLA.

3.3.11 Effects of CLA dietary supplementation and slaughter weight on extended cold storage of salami

Three salamis from each of the four treatment groups at the end of salami processing was subjected to vacuum packaging and cold storage at 4°C for one month in an attempt to simulate a real life retail-type situation where the salamis would have to be able to remain stable for a number of weeks before being purchased. Various chemical and microbial parameters expected to be affected by slaughter weight and diet with storage over time were measured.

The final pH of the salamis remained stable at a pH between 4.98 ± 0.10 for 90SFO and 5.08 ± 0.08 for 90CLA with no significant differences between any of the treatment groups (Table 3.15). These values seem to have been largely unaffected over time and were in close proximity to those pH values observed after drying (Figure 3.2). The a_w of the four treatment groups were also found in very close range to each other with an a_w of 0.890 ± 0.006 for 90CLA as the lowest and 0.893 ± 0.010 for 90SFO at the highest, close to the a_w observed after drying. The total acidity of the SFO

treatment groups increased slightly from after drying (Figure 3.3) to after one month cold storage (Table 3.15). On the other hand, both CLA treatment groups experienced a slight decrease in acidity after one month cold storage. These variations in acidity between the treatment groups were however not significant.

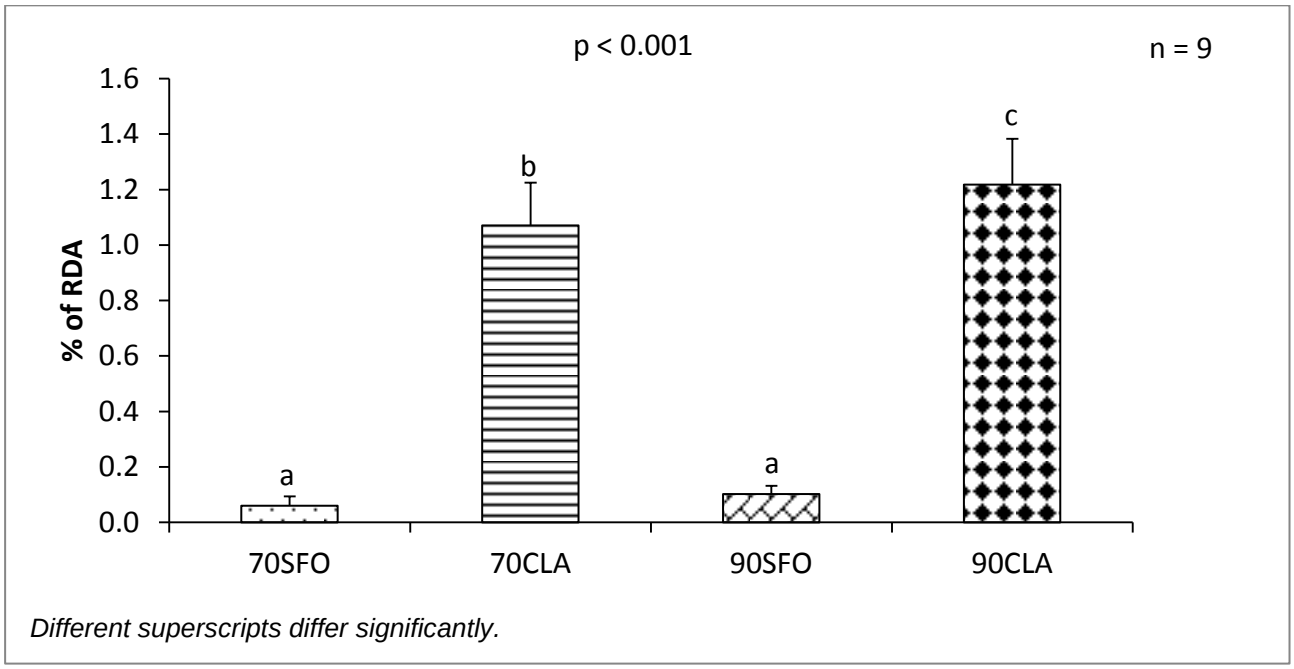


Figure 3.11. The concentration of CLA for each of the treatment groups expressed as percentages of the RDA.

The four treatment groups demonstrated no significant differences in FFA, PV or TBARS implicating no effects by slaughter weight or diet after one month cold storage, although various minor changes were identified for these parameters from after drying to after the cold storage period (Table 3.15). The amount of FFA of all four treatment groups increased similarly from after drying (Figure 3.5) to after cold storage (Table 3.15). The amount of peroxides of the two lower slaughter weight groups decreased slightly while those of the two higher slaughter weight groups increased slightly (Table 3.15). For the TBARS there was absolutely no possible pattern following slaughter weight or diet (Table 3.15). The TBARS of 70SFO decreased, being just at the critical level for detection of rancid odour and taste; those of 70CLA and 90SFO increased and lastly the TBARS of 90CLA decreased (Table 3.15). None of the chemical parameters of any of the four treatment groups were affected in any significant way by slaughter weight or diet after one month cold storage at 4°C.

Table 3.15. Chemical and microbial stability of salami as affected by slaughter weight and diet after 30 days of extended cold storage.

Treatment	70SFO (n=9)	70CLA (n=9)	90SFO (n=9)	90CLA (n=9)	Sign. level
Chemical parameters:					
Final pH	5.02 ± 0.13	5.01 ± 0.07	4.98 ± 0.10	5.08 ± 0.08	NS
a_w	0.8902 ± 0.0072	0.8920 ± 0.0099	0.8929 ± 0.0097	0.8900 ± 0.0055	NS
Total acidity	1.53 ± 0.43	1.51 ± 0.42	1.51 ± 0.44	1.51 ± 0.36	NS
% Free Fatty Acids	7.64 ± 1.51	7.52 ± 1.68	6.98 ± 1.98	6.53 ± 1.37	NS
Peroxide value	7.76 ± 6.30	7.56 ± 6.00	6.97 ± 4.40	7.83 ± 4.54	NS
TBARS value	0.50 ± 0.60	0.47 ± 0.47	0.27 ± 0.27	0.34 ± 0.40	NS
Microbial parameters:					
Lactic acid bacteria (log cfu/g)	6.85 ± 0.38	7.06 ± 0.34	6.94 ± 0.37	7.02 ± 0.52	NS
Coliforms (log cfu/g)	0.15 ± 0.33	0.11 ± 0.23	0.29 ± 0.43	0.12 ± 0.26	NS
***E. coli* (present)**	present	present	present	present	NS
Yeasts and moulds (log cfu/g)	2.32 ± 0.24^{c}	1.26 ± 0.20^{a}	1.36 ± 0.18^{a}	1.83 ± 0.39^{b}	$p < 0.001$

Different superscripts in the same row differ significantly. NS = Not significant

None of the microbial parameters of any of the groups differed after one month cold storage, except that of the yeast and mould counts (Table 3.15). The LAB population of the salamis were possibly already in the early death phase of the growth curve as their counts decreased slightly from after drying (Table 3.11) to after cold storage (Table 3.15). At more than log 6 cfu/g these salamis were still considered to be probiotic in nature. The counts for coliforms which were already below the National limit (SANS 885) after drying (Table 3.11) were found to be even lower after one month cold storage. *E. coli* was found to still be present after one month cold storage. It was not expected that any coliforms counts or confirmation of *E. coli* presence would be made after such a long time at such a low temperature low pH and acidic environment. The yeast and mould count were found to have decreased for all four treatment groups from after drying (Table 3.11) to until after one month cold storage (Table 3.15). These counts were the only parameter of all of those monitored after cold storage that had a significant difference ($p < 0.001$) between treatment groups (Table 3.15). The 70SFO group with a count of log 2.32 ± 0.24 cfu/g had significantly ($p < 0.001$) higher counts than the other three treatment groups. This was followed by a significantly ($p < 0.001$) higher count of the 90CLA group at log 1.83 ± 0.39 cfu/g compared to the log 1.26 ± 0.20 cfu/g count of the 70CLA group and log 1.36 ± 0.18 cfu/g count of the 90SFO groups. This is basically the same trend as was observed after drying.

3.4 Conclusions

Dietary CLA supplementation of pigs resulted in a significant ($p < 0.001$) increase in the CLA content of BF and IMF. It also resulted in increased lipid saturation of fat tissue in agreement with what was reported in literature (Smith *et al*., 2002). Increased slaughter weight resulted in more mature fat tissue with increased lipid saturation and decreased unsaturation of fat tissue which is

also in agreement with previous findings (Babatunde *et al.*, 1966; Allen *et al*., 1967; Staun, 1972; Martin *et al*., 1972). Fat tissue with a higher SFA content is regarded as being of superior quality from a meat technology point of view since it is firmer, more resistant to lipid oxidation (Wenk *et al*., 1990; Boselli *et al*., 2005) with superior sensory properties (Jiménez-Colmenero, 2000). Fat quality makes out a significant proportion of the totality of meat quality (St. John *et al*., 1987; Cardenia *et al*., 2011). The salami manufactured from the meat from the two slaughter weight (70 kg vs. 90 kg) and dietary treatment (SFO vs. CLA) groups were subjected to various microbial, chemical, physical and sensory quality evaluations.

None of the parameters used to monitor the ripening process of salami for different treatments were significantly influenced by slaughter weight and only the number of days it took to reach a 20% loss in moisture was significantly influenced by dietary treatment. Salami manufactured from CLA supplemented pork needed significantly ($p < 001$) less time to reach a 20% moisture loss compared to salami manufactured from SFO supplemented pork. Chemical and microbial analyses performed in this experiment failed to offer an explanation for this observation. Consistently shorter drying times for salamis supplemented with CLA through dietary manipulation could prove to be profitable as more salamis could then be finished in a given amount of time. Effects of slaughter weight and dietary treatment on the microbial parameters (LAB, coliforms and *E. coli*) were also limited. Only yeast and mould counts were somewhat affected by CLA dietary supplementation leading to a lower yeast and mould count of salami at the lower slaughter weight with CLA supplementation. The LAB of salami were found not be negatively affected in any way for any by of the four treatment groups.

Salami manufactured from the SFO supplemented pigs were found to contain very little CLA while salami manufactured from CLA fed animals showed successful incorporation of CLA, dependent on length of exposure due to different slaughter weights (Banni *et al*., 2001; Schmid *et al.*, 2006). Salami manufactured from meat originating from heavier slaughter weight pigs and CLA supplemented pigs also demonstrated improved fat quality as was observed in the raw materials. This improvement in fat quality was confirmed by FA ratios such as MUFA/SFA ratio and DBI (Lea *et al*., 1970; Prabucki, according to Hugo & Roodt, 2007). The improvement in fat quality of salami manufactured from pork originating from meat from pigs of heavier slaughter weight or dietary CLA supplementation demonstrated a significant decrease in nutritional and health properties as demonstrated by fatty acid ratios such as MUFA/SFA ratio (Cordain *et al*., 2005; Muchenje *et al.,* 2009), PUFA/SFA ratio (Scollan *et al.*, 2006; Wood *et al.,* 2008) and atherogenicity index (Kanner, 1994; Chizzolini *et al*., 1998).

Physical and sensory parameters measured at the end of salami processing (after drying) were also largely unaffected by dietary treatment or slaughter weight. The only colour parameter

affected, Hue angle, indicated that the 90CLA group had a significantly smaller Hue angle compared to the 70SFO group, indicative of a less red product. In terms of texture, only salami from the highest slaughter weight group supplemented with CLA had experienced an increase in force needed to be compressed. Sensory analysis revealed that a 75 member consumer panel was unable to detect any difference in firmness or any difference between any of the other sensory attributes between salami from the different treatment groups.

The lipid stability data generated by monitoring the salamis throughout processing showed no significant effects by slaughter weight or dietary treatment on any of the treatment groups. This was not expected since mention is made in literature about the antioxidant effects of CLA in meat products (Ip *et al*., 1999; Du *et al*., 2001; Joo *et al*., 2002; Hur *et al.,* 2004). The lack of expression of antioxidant properties of CLA in CLA supplemented salamis may be explained by either the very low concentration of CLA found in the lipids or by an overpowering antioxidant effect of nitrite added to salami (Gray & Pearson, 1984). Even after the finished salamis were subjected to one month cold storage under vacuum no significant effects on any of the stability parameters were introduced. These results indicated that all the treatment groups consisted of very stable meat products regardless of slaughter weight or dietary treatment.

Increased slaughter weight of pigs proved to be a successful method to improve the technological properties of fat tissue intended for use in fermented sausages. Unfortunately increased slaughter weight resulted in deterioration in the health and nutritional properties of salami manufactured from such meat. Similarly, dietary CLA supplementation also improved the technological quality of the fat component with a concurrent deterioration in the health and nutritional properties of the lipid component of salami as demonstrated by PUFA/SFA ratios and atherogenicity index. The higher atherogenicity index of salami from the CLA supplemented groups compared to the SFO supplemented groups may imply that the lipids of salami manufactured from dietary CLA supplemented meat may have cholesterol raising properties. It should however be kept in mind that CLA on its own also has cholesterol lowering, therapeutic and antioxidant effects (Pariza *et al.,* 1985; Lee *et al*., 1994; Belury *et al*., 1996; Park *et al*., 1999; Du *et al.*, 2000; Miller *et al*., 2001; Smedman & Vessby, 2001; Hur *et al*., 2004)

With a 1% contribution to RDA, dietary supplementation of pork with CLA cannot be considered a very successful approach to increase human consumption of CLA. Direct addition of CLA to salami formulations may therefore be an option worth considering.

CHAPTER 4

THE EFFECT OF DIRECT ADDITION OF CLA ON THE MICROBIAL AND LIPID STABILITY OF A CURED, FERMENTED PORK SAUSAGE

ABSTRACT

Lean pork and pork BF procured fresh from a local butchery was utilized in the manufacturing of four distinct groups of novel cured and fermented pork sausages (salami). The aim of this study was to increase the CLA content of salami to three different percentages (25%, 50% and 100%) of the Recommended Dietary Allowance for conjugated linoleic acid per 28 g portion of salami. This was accomplished through the direct addition of CLA (Tonalin® TG 80) in a pre-emulsified form with proportional decreases in the normally used pork BF content of the salamis. The salamis from these three treatment groups were then compared to a 100% pork BF control group for any possible effects on the microbial, physical, and lipid stability parameters as well as FA composition and FA ratios. Microbial and sensory parameters were largely unaffected with varying effects on the physical and lipid stability parameters. Major effects on the FA composition and FA ratios of the salamis were observed. The partial replacement of pork BF and direct addition of CLA to salami proved to be an effective method of increasing CLA levels in salami in an attempt to improve the health aspects of salami to the point where it could be regarded as a functional food.

Key words: Conjugated linoleic acid; Tonalin®; salami; pre-emulsified oil; direct addition; pork backfat; partial replacement

4.1 INTRODUCTION

Meat and especially meat products are associated with nutrients and nutritional profiles such as high levels of SFAs, cholesterol and sodium that are often regarded as negative from a human health point of view (Aida *et al*., 2005; Valsta *et al*., 2005; Ruusunen & Puolanne, 2005). Saturated fatty acids are considered the main cause of hypercholesterolemia (Mattson & Grundy, 1985). Methods are available to improve these negative anti-nutritional factors. Leaner meat may be used, adipose fat tissue removed or dietary manipulation can be used to alter the FA profile of meat (Decker & Park, 2010). In an effort to improve the health image of meat and meat products as well as to create functional meat and meat products, methods of direct manipulation should also not be overlooked. Direct manipulation refers to the addition of certain food components such as healthier fatty acids, and or the reduction of others such as SFAs.

In contrast to manipulating FA composition through changes in the animal diet, direct methods involve replacing SFAs in the form of pork BF for example with PUFA rich oils such as linseed oil, rapeseed oil, fish oils and olive oil (Josquin *et al.,* 2011). Reduction of fat or replacement with less saturated fat is not always a simple process and has been reported to have negative effects on the technological and sensory properties of meat products (Jiménez-Colemenero, 2000). Pork BF is especially known to be a very important determinant of processing and quality characteristics of salami-type meat products as it affects the colour, flavour, texture, and drying process (Severini *et al*., 2003).

The use of olive oil with its high levels of health promoting MUFAs in the partial replacement of pork BF in salami has been reported (Severini *et al*., 2003). These authors indicated that the health features of this type of product could be improved without compromising taste. Bloukas *et al*., (1997) and Muguerza *et al.* (2002) found that not more than 20 to 25% BF could be replaced with oils. When pre-emulsified olive oil was used at 30% replacement, significant drip loss of fat was found during ripening. No increase in rancidity was detected through sensory analysis and a decrease in TBA values was found compared to the Control. This was thought to be due to a possible antioxidant effect imparted by the soya protein isolate used in the emulsion (Muguerza *et al*., 2001).

Partial replacement of pork BF with olive oil and CLA in liver pâtés did not lead to increased lipid oxidation, due to the use of nitrite as antioxidant in this type of product. A much weaker consistency and poorer emulsion stability was however observed in oil supplemented liver pâtés (Martín *et al*., 2008b). The addition of these types of bioactives should therefore be accompanied with considerations over their stability in the food product, their possible impact on sensory attributes (Decker & Park, 2010) and an increased risk of oxidation where more unsaturated fatty acids are used (Martín *et al*., 2008b). Therefore, Josquin *et al.* (2011) suggested using oils that have been pre-emulsified or encapsulated to contribute to a more stable emulsion in the product. No evidence of salami being fortified with direct addition of CLA could be found in literature.

In this study direct incremental addition of CLA was utilized to create a novel, cured and fermented pork sausage (salami). The product was studied throughout the whole production cycle to determine if quality was influenced by the partial or complete replacement of pork BF with CLA compared to a pork BF control.

4.2 MATERIALS AND METHODS

4.2.1 Preparation of the salami models

Calculations for the formulation of the salami models

Four salami formulations were used in this study. Salami formulations were based on the CLA content of the final product. The aim for the upper limit was 3.5 g CLA per 28 g portion of salami which is regarded as the RDA for CLA (USDA, 2011). This is considered as the minimum amount to be consumed daily to obtain a therapeutic effect for CLA in humans (Ha *et al*., 1989). Twenty eight grams is a dietary guideline recommended portion of salami (USDA National Nutrient Database for Standard Reference, 2011). The salami formulas contained incremental amounts CLA with corresponding decreases in pork BF content. The final formula contained only CLA as fat source with no pork BF added. A control formula was also devised containing only pork BF as fat source. The four salami formulations were named: Control, CLA25, CLA50 and CLA100. The Control salami was formulated to contain no CLA in a 28 g portion (0% of RDA), The CLA25 salami was formulated to contain ± 0.875 g CLA in a 28 g portion (25% of RDA). The CLA50 salami was formulated to contain ± 1.75 g CLA in a 28 g portion (50% of RDA). The CLA100 salami was formulated to contain ± 3.5 g CLA in a 28 g portion (100% of RDA).

A 1:8:10 (soya isolate:water:BF and/or Tonalin®) pre-emulsion was used as carrier for the BF and Tonalin® into the salami. The emulsion was introduced to the salami in a frozen form to prevent fat smearing during filling of the casings. All four formulations were formulated so as to share in the closest possible way, the same fat content, moisture content, moisture to meat protein ratio, and moisture to total protein ratio. In formulating the products, the following common assumptions were made for meat formulating purposes: all salamis were dried to 30% moisture loss; the pork BF contained 85% fat, 10% moisture, and 5% protein; muscle contained 75% water and 20% protein; soya protein isolate contained 90% protein.

The formulations for the salami and 1:8:10 (soya isolate:water: BF and/or Tonalin®) pre-emulsion are shown in Tables 4.1 and 4.2. The calculations for the formulation of the salami and pre-emulsions included estimates for: amount of fat in the final product; moisture content after manufacturing; moisture to meat protein ratio and moisture to total protein ratio (consisting of the estimated protein contribution from the lean pork, the pork BF and the soya used in the emulsions). The commercial CLA product Tonalin® TG 80 was used as the source of CLA and from here on referred to as just Tonalin®, typically contains 80% CLA according to the Product Datasheet (BASF Group). In Figure 4.1 a more complete make-up of Tonalin® TG 80 is given. Laboratory analysis indicated that the Tonalin® used in this experiment contained 77% total CLA. It was decided to include emulsions at 25% of total salami formulation. The exception were the 100CLA treatment where emulsion inclusion level was reduced to 22% to make sure that the fat content, moisture content, protein content, moisture:meat protein ratio and moisture:total protein ratio remained comparable to other treatments.

Table 4.1. Salami formulation.

	Control	CLA25	CLA50	CLA100
Pork 90/10 (lean meat/backfat) (g)	1000.00	1000.00	1000.00	1000.00
Pork 90/10 (g)	743.81	743.81	743.81	818.81
Backfat and/or Tonalin® emulsion (g)	625.00	625.00	625.00	550.00
Curing salt (g)	76.15	76.15	76.15	76.15
Spice mixture (g)	54.41	54.41	54.41	54.41
Starter culture (g)	0.63	0.63	0.63	0.63
TOTAL (g)	**2500.00**	**2500.00**	**2500.00**	**2500.00**

Table 4.2. Formulation for the manufacture of 625 and/or 550 g of the 1:8:10 emulsion.

Ingredient (g)	Control (100% BF)	CLA25 (25% RDA)	CLA50 (50% RDA)	CLA100 (100% RDA)
BF	328.95	256.59	184.22	0.00
Tonalin®	0.00	72.36	144.73	289.47
Water	263.16	263.16.	263.16	231.59
Soya protein isolate	32.89	32.89	32.89	28.94
Total	625.00	625.00	625.00	550.00

The CLA content of the proposed four salami formulations were verified in an attempt to make sure that the salamis comply as much as possible to the proposed 0%, 25%, 50% and 100% of RDA after manufacturing (Table 4.3). According to Table 4.3 the theoretical % of the RDA were 0, 25.43%, 50.86%, and 102% for the Control, CLA25, CLA50 an CLA100 formulations.

Table 4.3. Verification of theoretical CLA content and contribution to CLA RDA for different salami formulations.

	Origin of information or formula used	Control	CLA25	CLA50	CLA100
Weight of salami after manufacture (g)	Table 4.1	2500	2500	2500	2500
Weight loss of salami during manufacturing (%)	Salamis were dried to a 30% weight loss	30	30	30	30
Weight of salami after drying (g)	$\frac{70}{100} \times \frac{\text{Wet weight of salami}}{100}$	1750	1750	1750	1750
Weight of Tonalin® in salami (g)	Table 4.2	0.00	72.36	144.73	289.47
Actual CLA content of Tonalin® (%)	GC analysis indicated a 77% total CLA content of Tonalin®	77	77	77	77
Actual CLA content in batch of salami after drying (g)	$\frac{77}{100} \times \frac{\text{Weight of Tonalin®}}{1}$	0.00	55.72	111.44	222.89
Actual CLA content of 28 g of dried salami (g)	$\frac{\text{Actual CLA content}}{1750} \times 28$	0.00	0.89	1.78	3.57
% of RDA for CLA	$\frac{\text{Actual CLA content of 28 salami}}{3.5} \times 100$	0.00	25.43	50.86	102

In Table 4.4 the theoretical moisture content, fat content, moisture:meat protein ratio and moisture:total protein ratio of salami formulations were compared. That was done to ensure that possible differences observed between manufactured salamis can be ascribed to treatment

Table 4.4. Verification of theoretical moisture content, fat content, moisture to meat protein and moisture to total protein ratios for different salami formulations.

	Origin of information or formula used	Control	CLA25	CLA50	CLA100
Weight of salami after manufacture (g)	Table 4.3	2500	2500	2500	2500
Weight of salami after drying (g)	Table 4.3	1750	1750	1750	1750
Verification of theoretical fat content of different salami formulations:					
Tonalin® added to salami (g)	Table 4.2	0	72.36	144.73	289.47
BF added to salami (g)	Table 4.2	328.95	256.59	184.22	0.00
Total fat content of salami (g)	$\left(\frac{85}{100} \times \text{BF added (g)}\right) + \text{Tonalin added (g)}$	279.61	290.46	301.32	289.47
Fat content of salami after manufacturing (%)	$\frac{\text{Total fat content of salami (g)}}{2500\text{ g}} \times 100$	11.18	11.62	12.05	11.58
Fat content of salami after 30% drying	$\frac{\text{Total fat content of salami (g)}}{1750\text{ g}} \times 100$	15.97	16.60	17.22	16.54
Verification of theoretical moisture content of different salami formulations:					
Moisture content of lean pork (90/10) in salami (g)	$\frac{75}{100} \times \frac{\text{Weight of lean pork (g)}}{1}$	1307.86	1307.86	1307.86	1364.11
Moisture content of backfat of salami (g)	$\frac{10}{100} \times \frac{\text{Weight of backfat (g)}}{1}$	33.00	25.66	18.42	0.00
Water added to emulsion (g)	Table 4.2	263.16	263.16	263.16	231.59
Total water content of 2500 g batch of salami (g)	Moisture content of lean meat (g) + Moisture content of backfat (g) + Added water (g)	1604.02	1596.68	1589.44	1595.70
Moisture content of salami after manufacturing (%)	$\frac{\text{Total water content of 2500 g batch of salami (g)}}{2500} \times \frac{100}{1}$	64.15	63.87	63.58	63.83
Verification of theoretical moisture:meat protein ratios of different salami formulations:					
Protein content of lean pork (90/10) in salami (g)	$\frac{20}{100} \times \frac{\text{Weight of lean pork (g)}}{1}$	348.76	348.76	348.76	363.76
Protein content of backfat in salami (g)	$\frac{5}{100} \times \frac{\text{Weight of backfat (g)}}{1}$	16.45	12.83	9.21	0.00
Total meat protein content of 2500 g batch of salami (g)	Protein content of lean pork (g) + Protein content of backfat (g)	365.21	361.59	357.97	363.76
Moisture: Meat protein ratio of salami after manufacturing	$\frac{\text{Total meat protein content of 2500 batch of salami (g)}}{\text{Total water content of 2500 g batch of salami (g)}}$	1:0.23	1:0.23	1:0.23	1:0.23
Verification of theoretical moisture:total protein ratios of different salami formulations:					
Protein content of soya protein isolate in 2500 g salami (g)	$\frac{90}{100} \times \frac{\text{Weight of soya isolate (g)}}{1}$	29.60	29.60	29.60	29.60
Total protein content of 2500 g batch of salami (g)	**Total meat protein content of 2500 g salami (g) + Protein content of backfat in salami (g) + Protein content of soya protein isolate in 2500 g salami (g)**	394.81	391.19	387.57	393.36
Moisture to total protein ratio of salami	$\frac{\text{Total protein content of 2500 batch of salami (g)}}{\text{Total water content of 2500 g batch of salami (g)}}$	1:0.24	1:0.24	1:0.24	1:0.24

Assumptions accepted for purpose of salami formulation

Emulsions were all included at 25% of raw salami weight
All salamis dried to 30% moisture loss
Fat content of backfat = 85%
Moisture content of backfat = 10%
Protein content of backfat = 5%
Moisture content of lean meat = 75%
Protein content of lean meat = 20%
Fat content of lean meat = 0%
Protein content of soya protein isolate = 90%

differences and not to formulation differences. Salami formulation was successful in obtaining chemical equivalency between different formulations. Theoretical fat content of the different salami formulations varied between, 15.97 and 17.22% after manufacturing (Table 4.4). The theoretical moisture content of salamis after manufacturing varied between 63.58 – 64.15% (Table 4.4). The moisture to meat protein ratios and the moisture to total protein ratios for all treatments were 1:0.23 and 1:0.24 respectively (Table 4.4). Theoretical chemical variations between different salami formulas were small enough not to introduce significant variation between the different salami treatment groups.

Tonalin® composition

Tonalin® TG 80 was the food grade source of CLA used in this experiment. The letters TG refer to the fact that the fatty acids are in the triglyceride form. The number 80 refers to the approximate total CLA content of 80%, with the C18:2*c*9,*t*11 isomer occurring at 39.70% and the C18:2*t*10,*c*12 isomer occurring at 39.69% (Figure 4.1). Analysis in our laboratory indicated that the Tonalin® used in this experiment actually contained 77% total CLA. Smaller amounts of oleic acid (14.53%), stearic acid (2.58%), palmitic acid (1.59%), vaccenic acid (0.78%), heneicosanoic acid (0.61%), linoleic acid (0.41%), α-linolenic acid (0.06%) and arachidic acid (0.05%) made up the rest of the FA profile of Tonalin® TG 80 (Figure 4.1). The source of Tonalin® TG 80 is safflower oil which is purified and triple distilled into a highly concentrated product intended for use as a dietary supplement and by the food industry (TONALIN® TG 80 data profile sheet Rev. 1.0, BASF). Throughout this study the term “added CLA” refers to Tonalin® TG 80.

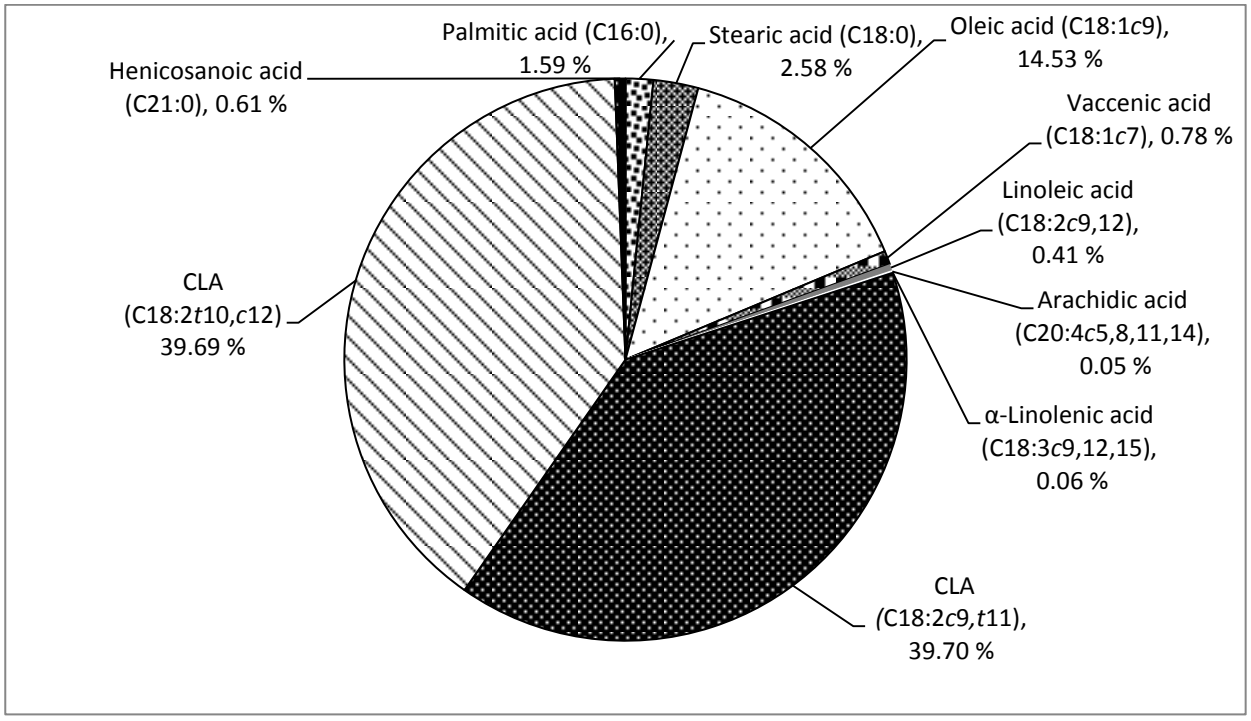


Figure 4.1. The proximate composition of Tonalin® TG 80 as determined with sodium methoxide FAME preparation and GC analysis.

Lean pork and pork BF preparation

Fresh, lean pork and good quality, firm pork BF was bought from a local butchery a few days before model preparation. The meat was cubed and pooled before being weighed into 1000.00 g (for all four treatment groups), ±818.81 g (for CLA100 treatment group) and ±743.81 g (for Control, CLA25 and CLA50 treatment groups). The BF was minced through an Okto mincer (13 mm plate size), weighed off according to the amount needed for each emulsion and also vacuum packaged. All meat ingredients was then stored at -18°C until used.

Emulsion scale up and preparation

Due to the size of the bowl cutter used for the model preparation (see 4.2.1) there is a restriction on the amount of emulsion that can be prepared for each batch. The emulsions needed to be scaled up to a minimum amount of 2 kg per emulsion for efficient and proper functioning of the bowl cutter (Table 4.5). For the Control, CLA25 and CLA50, 625 g of the corresponding emulsions was used for each batch and for the CLA100 batch 550 g of the emulsion was used (Tables 4.1 and 4.2). The emulsions were prepared a day before the actual model preparation and frozen to -18 ^{o}C until use.

Table 4.5. Emulsion components scaled up for 2 kg emulsions.

Emulsion component (g)	Control (100% BF)	CLA25 (25% RDA)	CLA50 (50% RDA)	CLA100 (100% RDA)
BF	1052.64	821.09	589.50	0.00
Tonalin®	0.00	231.55	463.14	1052.64
Water	842.11	842.11	842.11	842.11
Soya protein isolate	105.25	105.25	105.27	105.25
Total	2000.00.	2000.00	2000.00	2000.00

Method for emulsion preparation

1. The water (partly consisting of small ice chips) and soya protein isolate was mixed in the bowl cutter until a smooth texture was obtained.
2. The pork BF and/or Tonalin® was added and the ingredients were emulsified until no individual pockets of BF or Tonalin® were visible.
3. The appropriate amount of each emulsion was weighed off and frozen as flat slabs until needed.

Method of salami preparation

The same spice mixture, curing salt mixture, and starter cultures were used as for Chapter 3 (Tables 3.2 and 3.3). Salami models were manufactured exactly as described in Chapter 3 (p. 38) except that at step 4 one of the following was added: a slab of pre-emulsified pork BF for the Control group and; a slab of pre-emulsified pork BF plus Tonalin® for the CLA25 group and CLA50 groups and a slab of pre-emulsified Tonalin® for the CLA100 group. Three batches of salami were

manufactured for each treatment with intervals of three months between batches. This was to make provision for variation in processing conditions between batches.

Weight loss measurement

Weight loss was determined as described in Chapter 3, p. 42 of this dissertation. The exception was that weight loss was monitored until a 30% loss in weight was reached. This was used to determine the end of the ripening period.

4.2.2 Salami sampling

Salami sampling was conducted as described in Chapter 3, p. 39 of this dissertation.

4.2.3 Microbial analysis

Microbial analysis was conducted as described in Chapter 3, p. 39 of this dissertation.

4.2.4 Chemical analysis

pH

The pH measurement was conducted as described in Chapter 3, p. 41 of this dissertation.

Total Acidity

Total acidity was conducted as described in Chapter 3, p. 41 of this dissertation.

a_w (water activity)

Water activity measurement was conducted as described in Chapter 3, p. 42 of this dissertation.

Total lipid extraction

The total lipid extraction was conducted as described in Chapter 3, pp. 39-40 of this dissertation.

Free Fatty Acid (FFA) analysis

Free fatty acid analysis was performed to determine the levels of lipid hydrolysis. This was carried out using the method by Pearson (1968) by using lipid from the Folch extraction.

Peroxide Value (PV) analysis

Peroxide value analysis was performed to determine the levels of primary lipid oxidation products according to AOAC nr. 965.33 (2000). It was performed using lipid from the Folch extraction.

Fatty Acid Methyl Ester (FAME) preparation for GC analysis

Fatty acid methyl ester preparation was conducted as described in Chapter 3, p. 40 of this dissertation.

GC analysis

GC analysis was conducted as described in Chapter 3, pp. 40-41 of this dissertation.

Determination of CLA as a percentage of the RDA for CLA

The determination of the CLA as a percentage of the RDA for CLA was conducted as described in Chapter 3, p. 42 of this dissertation.

TBARS analysis

TBARS analysis was conducted as described in Chapter 3, p. 41 of this dissertation.

Colour analysis

Colour analysis was conducted as described in Chapter 3, p. 42 of this dissertation.

Texture analysis

Texture analysis was conducted as described in Chapter 3, p. 42 of this dissertation.

Sensory analysis

Sensory analysis was conducted as described in Chapter 3, p. 42-43 of this dissertation.

4.2.5 Statistical analysis

This experiment was a 4 x 4 x 3 x 3 factorial design representing the four Tonalin® inclusion levels, four processing stages, three batches and three replicates per treatment.

An analysis of variance procedure (NCSS, 2007) was used to determine the effect of Tonalin® inclusion level and processing stage, on proximate composition, microbial stability, chemical stability and fatty acid composition of salami. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

4.3. RESULTS AND DISCUSSION

4.3.1 Drying parameters of salami

Results discussed in this section are associated with the ripening process of this type of cured, fermented meat product.

Drying time and final moisture loss

A 30% moisture loss was used to determine the end of the drying process. Although the Control group finished drying more than two days before the other groups (Figure 4.2) at 26.67 ± 2.57 days compared to the more than 29 days of the other treatment groups, it was not statistically significant. These results indicated that the direct addition of CLA had no effect on the drying process even over a wide range of pork BF replacement levels. This is interesting seeing that the CLA (as an oil) did not remain in a stable emulsion and exuded into the meat causing a smearing effect which reportedly would have prevented efficient moisture loss during drying.

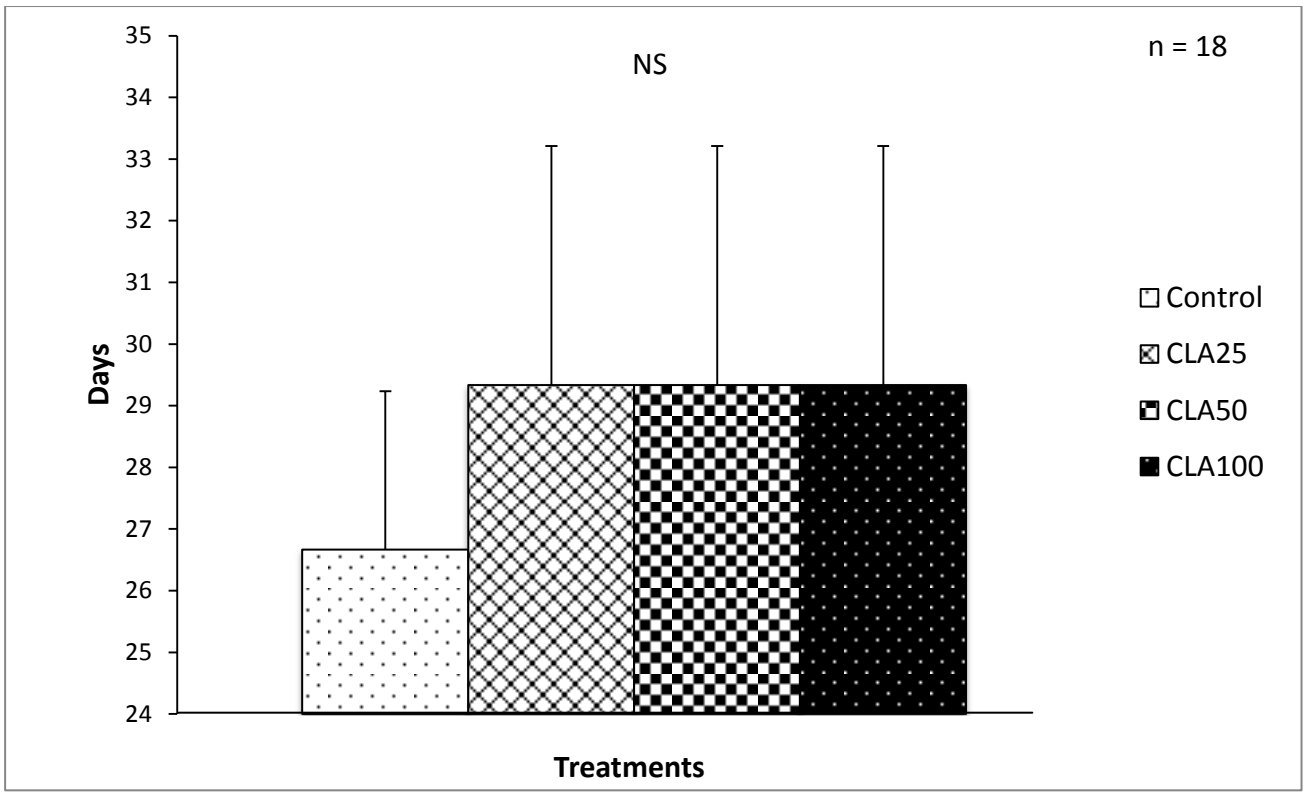


Figure 4.2. The number of days needed for each salami treatment group to reach a 30% loss in moisture. NS = Not significant

The final weight loss (Table 4.6) was also not significantly affected by Tonalin® inclusion levels. This can partly be ascribed to the precise monitoring of weight loss. It should also be mentioned that the end of the drying period was calculated on the basis of a 30% loss in weight from the total salami weight and not as a 30% loss in moisture of the total moisture content. The lack of significant difference in weight loss between salamis from different Tonalin® inclusion levels were surprising since it was previously reported that pre-emulsification of oils had a retarding effect on weight loss of salami (Bloukas *et al*., 1997).

Table 4.6. The final weight loss at the end of ripening for each salami treatment group.

Treatment	Control	CLA25	CLA50	CLA100	Sign. Level
Final moisture loss (%)	30.65 ± 0.57	30.34 ± 0.84	29.98 ± 0.99	30.11 ± 0.93	Ns.

Means with different superscripts within the same column differ significantly. NS = Not significant

pH and total acidity

Although CLA inclusion level had no effect, processing stage had a significant ($p < 0.001$) effect on salami pH. The effect of processing stage was most probably due to the formation of lactic acid from the LAB as ripening progressed (Figure 4.3). The average pH of the four treatment groups declined from ±5.74 after manufacturing to ±5.34 after fermentation and to ±5.08 after drying (Figure 4.3).

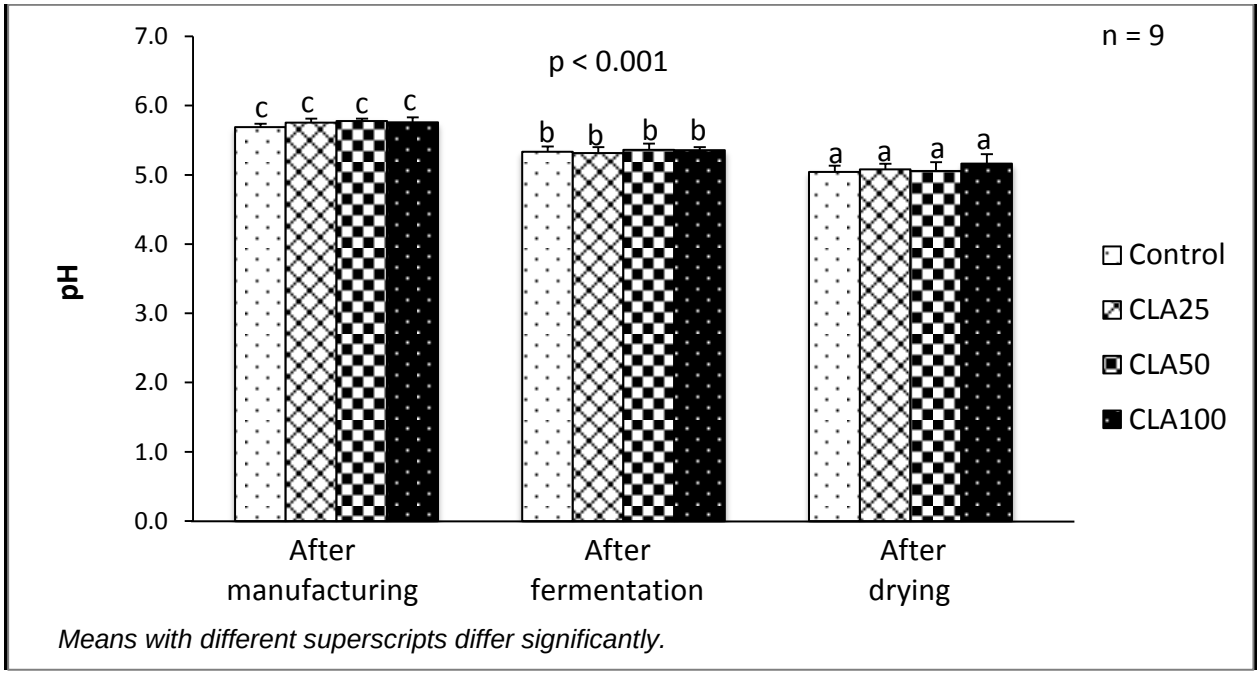


Figure 4.3. The pH of the four salami treatments during processing.

As in the case of pH, CLA inclusion level did not affect the total acidity of salamis (Figure 4.4). As expected, processing stage had a statistically significant ($p < 0.001$) effect on the total acidity of salami, confirming the formation of organic acids in the salami as a result of LAB metabolism (Figure 4.4). From Figure 4.4 it was clear that total acidity increased as processing progressed. Directly after manufacturing, total acidity was ±0.90 mL of 0.1 N NaOH and after the drying stage had increased to over 1.70 mL of 0.1 N NaOH (Figure 4.4).

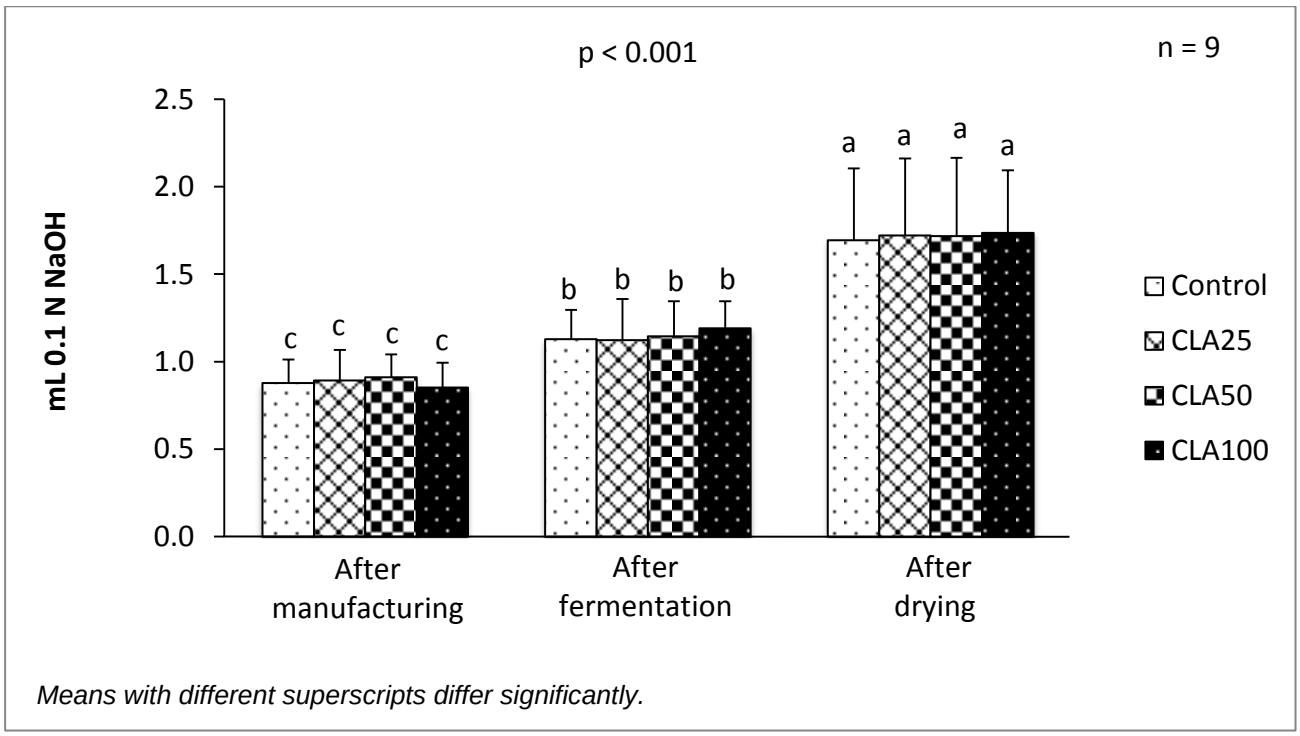


Figure 4.4. Total acidity of the four salami treatments during processing.

4.3.2 Proximate composition of salami

Moisture content

The actual moisture content of the salami after manufacturing (Table 4.7) were in close agreement with the theoretical moisture content of ±64% calculated for salami from the different treatments during formulation (Table 4.4). No significant differences in moisture content were observed between Tonalin® inclusion levels within each of the processing stages (Table 4.7). This implies that Tonalin® inclusion level had no effect on the release of moisture from salami within a specific processing stage. This also indicates that the pre-emulsification of the BF or CLA oil did not create any significant difference in moisture content or moisture release. This is in contrast to the findings of other researchers which indicated that the pre-emulsification of oils had a negative effect on moisture content (Josquin *et al*., 2011) and weight loss within the set time (Bloukas *et al*., 1997). The different processing stages significantly($p < 0.001$) influenced the moisture content of the salamis. Overall the moisture content decreased from around ±64.48% directly after manufacturing to around ±51.01% moisture after drying (Table 4.7). This can be ascribed to an increase in LAB count during fermentation of salami, resulting in an increase in total acidity and a decrease in salami pH. As the pH of salami decreases meat proteins move towards its isoelectric point where it release water.

Table 4.7. The proximate composition of salami as influenced by Tonalin® inclusion level and processing stage.

Processing stage	Tonalin® inclusion level	Fat (%)	FFDM (%)	Moisture (%)
After manufacture	**Control**	11.71 ± 0.76[a]	23.28 ±1.79[a]	64.20 ± 0.93[b]
	CLA25	11.99 ± 1.31[a]	23.06 ± 2.25[a]	64.25 ± 1.11[b]
	CLA50	11.61 ± 0.72[a]	22.24 ± 1.26[a]	64.88 ± 1.29[b]
	CLA100	11.59 ± 0.77[a]	22.42 ± 1.40[a]	64.59 ± 1.32[b]
After fermentation	**Control**	12.29 ± 0.47[a]	23.93 ± 1.50[a]	63.04 ± 0.99[b]
	CLA25	12.61 ± 0.80[a]	23.46 ± 1.23[a]	63.01 ± 1.06[b]
	CLA50	12.62 ± 0.63[a]	23.12 ± 1.45[a]	63.16 ± 1.85[b]
	CLA100	11.94 ± 1.40[a]	24.43 ± 1.95[a]	63.81 ± 2.34[b]
After drying	**Control**	18.40 ± 1.63[c]	32.27 ± 1.82[b]	49.35 ± 1.84[a]
	CLA25	16.93 ± 1.23[bc]	31.65 ± 0.99[b]	50.56 ± 2.24[a]
	CLA50	16.31 ± 1.11[b]	31.80 ± 1.04[b]	51.99 ± 1.21[a]
	CLA100	15.65 ± 2.09[b]	31.51 ± 1.19[b]	52.15 ± 3.90[a]
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts within the same column differ significantly.

Fat content and fat free dry matter content

The actual fat content of the salami after manufacturing (Table 4.7) were in close agreement with the theoretical fat content of ±11.5% calculated for salami from the different treatments during formulation (Table 4.4). Tonalin® inclusion levels had no significant effect on fat content of salami directly after manufacturing or after fermentation. The actual fat content of the salami after drying (Table 4.7) were in close agreement with the theoretical fat content of ±16.5% calculated for salami from the different treatments after 30% moisture loss (Table 4.4). The exception was the Control group of which the fat content after drying were nearly 2% higher than that of salami from the other Tonalin® inclusion levels. The fat content of the Control salami (18.40 ± 1.63%) were in fact significantly ($p < 0.001$) higher than the fat content of salami from the CLA50 (16.31 ± 1.11%) and CLA100 (15.65 ± 2.09%) groups. The only possible explanation for this could be the very high oil (Tonalin®) content of the CLA50 and CLA100 salamis (Table 4.2). The high oil content might have resulted in destabilization of the emulsion and the resultant loss of measureable amounts of oil through drip loss during the relative high temperature of fermentation and drying. This was confirmed visually in the ripening room where drops of oil formed on the floor below the hanging salamis.

The significant ($p < 0.001$) effect on fat content introduced by processing stage (Table 4.7) could be attributed to the change in the moisture content of the salamis. As the salamis dried, the fat to total mass ratio of each salami increased. The fat content of salamis after manufacture was ± 11.5%. There was a slight but not significant increase in fat content to ±12.5% after fermentation. A statistically significant ($p < 0.001$) increase to ±17% fat was observed after drying. Tonalin® inclusion level had no significant effect on fat free dry matter content within any of the processing stages (Table 4.7). The only significant ($p < 0.001$) difference was found between the processing stages where the FFDM content increased significantly ($p < 0.001$) from ±22% after manufacturing

to ±32% after drying. This can also be attributed to the loss in moisture during manufacturing which concentrate the remaining ingredients.

4.3.3 Microbial stability parameters

The partial replacement of pork BF with CLA at three increasing inclusion levels did not had a significant effect on the a_w of salami within any of the processing stages (Figure 4.5). Processing stage had a statistically significant ($p < 0.001$) effect on a_w (Figure 4.5). From after manufacturing until after drying, the a_w was found to decrease from an average a_w of ±0.94 to ±0.90. This effect was attributed to moisture loss and the resulting concentration of solutes such as sodium chloride, sugars and lactic acid as examples, which drove down the amount of free water available to partake in chemical reactions. Microbiological tests were selected for quantification and evaluation of the mixed starter culture and to monitor common pathogenic and spoilage microorganisms associated with this type of fermented meat product.

Lactic acid bacteria

Tonalin® inclusion level had no significant effect on LAB within any of the processing stages (Table 4.8). In literature it was reported that CLA was found to be capable of inhibiting the growth of some strains of lactic acid bacteria at a very low concentration of 25 µg per mL of MRS media (Sieber, Collomb, Aeschlimann, Jelen & Eyer, 2004). This experiment failed to demonstrate this effect. Even at very high CLA inclusion levels no significant difference were observed in LAB count between the Control and Tonalin® treatment groups within all processing stages.

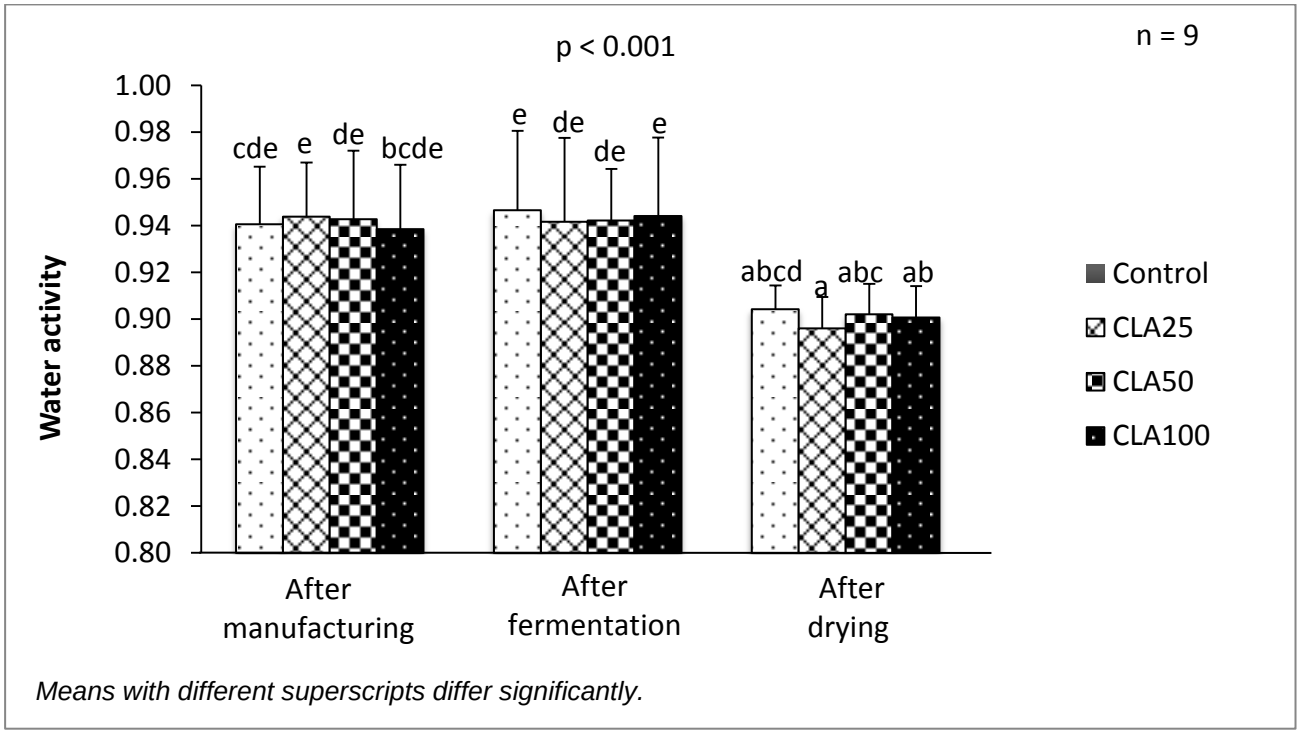


Figure 4.5. Water activity of the four salami treatments during processing.

Table 4.8. Microbial stability of salami as affected by CLA inclusion level and processing stage.

Processing Stage	CLA-Level	Lactic acid bacteria (log cfu/g)	Coliforms (log cfu/g)	Yeasts (log cfu/g)	Moulds (log cfu/g)
After manufacture	**Control**	5.09 ± 0.10^{a}	3.45 ± 0.16^{b}	3.14 ± 0.42^{a}	1.44 ± 0.80^{a}
	CLA25	5.13 ± 0.09^{a}	3.48 ± 0.25^{b}	3.20 ± 0.55^{a}	1.54 ± 0.92^{a}
	CLA50	5.07 ± 0.14^{a}	3.36 ± 0.31^{b}	3.20 ± 0.55^{a}	1.56 ± 0.94^{a}
	CLA100	5.12 ± 0.08^{a}	3.29 ± 0.35^{b}	2.92 ± 0.73^{a}	1.30 ± 0.88^{a}
After Fermentation	**Control**	7.94 ± 0.11^{b}	3.25 ± 0.50^{b}	2.31 ± 1.81^{a}	2.92 ± 2.00^{ab}
	CLA25	7.97 ± 0.12^{b}	3.73 ± 0.35^{b}	2.40 ± 1.85^{a}	3.68 ± 0.89^{bc}
	CLA50	7.83 ± 0.32^{b}	3.89 ± 0.17^{b}	2.36 ± 1.86^{a}	3.12 ± 1.73^{ab}
	CLA100	7.94 ± 0.15^{b}	3.40 ± 0.40^{b}	2.37 ± 1.81^{a}	3.81 ± 0.46^{bc}
After drying	**Control**	7.91 ± 0.17^{b}	0.56 ± 0.84^{a}	6.69 ± 0.77^{b}	4.35 ± 2.25^{bc}
	CLA25	7.99 ± 0.19^{b}	0.68 ± 1.02^{a}	6.90 ± 0.50^{b}	5.38 ± 0.80^{c}
	CLA50	7.98 ± 0.25^{b}	0.96 ± 1.01^{a}	6.94 ± 0.35^{b}	4.80 ± 1.46^{bc}
	CLA100	8.01 ± 0.24^{b}	0.76 ± 1.07^{a}	7.01 ± 0.66^{b}	4.95 ± 1.17^{bc}
Significance level		p< 0.001	p< 0.001	p< 0.001	p< 0.001

Means with different superscripts in the same column differ significantly.

Processing stage had a statistically significant ($p < 0.001$) effect on LAB count. After manufacturing the LAB counts for all four treatment groups were already in excess of log 5 cfu/g (Table 4.8). This was due to the high inclusion level (estimated at log 6 cfu/g) of the mixed lactic starter culture added to the batter during manufacturing. A significantly ($p < 0.001$) higher concentration of LAB was found after fermentation (log ± 7.90 cfu/g) compared to after manufacturing. This can be explained by the growth of the LAB during the fermentation step. As no significant difference was found between the LAB counts after fermentation or after drying it can be deduced that the LAB population stabilized after fermentation and remained stable even after drying. The increase in acidity, decrease in pH, decrease in a_w as well as numerous other physical and chemical processes did not affect the LAB in any way confirming the suitability of the Bactoferm™ T-D-66 mixed starter culture for use in this type of product. It is also clear that Tonalin® inclusion had no significant effect on starter efficiency.

Coliforms

As in the case for LAB, Tonalin® inclusion level had no significant effect on coliforms within any of the processing stages (Table 4.8). Coliform counts were however significantly ($p < 0.001$) affected by processing stage (Table 4.8). The coliform counts (Table 4.8) directly after manufacturing (contaminated raw product) and those after ripening did not differ significantly even when the time difference of 48 h is considered. After drying a significant decrease in coliform count compared to the end of fermentation was observed. The temperature of the salamis during ripening, fast decrease in pH and inclusion of sodium chloride, nitrate and nitrite probably explain this inhibition of growth. At the end of drying these values were below the National limit of 100 cfu/g (SANS 885). No direct effect by Tonalin® inclusion level was found. A small difference ($p < 0.05$) between the three different batches of salami was found. The interaction between Tonalin® inclusion level with

processing stage was also found not to contribute any significant differences between the treatment groups.

Yeasts and moulds

Yeast and mould counts were quantified separately to get a better idea of how each group of these microorganisms were affected by Tonalin® inclusion level and processing stage. Both yeasts and moulds were totally unaffected by Tonalin® inclusion within processing stage. Processing stage had however a statistically significant ($p < 0.001$) effect on yeast and mould counts (Table 4.8). When the actual counts from these two groups of microorganisms were compared, the following was apparent: the yeasts had counts in excess of log 3 cfu/g after manufacturing which then decreased to counts of log ±2.40 cfu/g after fermentation and then increased to roughly double the initial counts at log ±6.70 - 7.01 cfu/g after drying. The moulds increased steadily from log ±1.50 cfu/g after manufacturing to log ±5 cfu/g after drying. The reason for these increases during these processing stages is due to the favourable growth conditions for this group of organisms. As stated on page 57, the environment was not regarded as an observable parameter with regards to this study.

4.3.4 Lipid stability parameters

Free fatty acids, peroxides and TBARS were measured to observe the stability of the salami lipids throughout processing. Nitrate, reduced to nitrite by *Lactobacillus plantarum* and nitrite, with known antioxidant effects on meat lipids (Gray & Pearson, 1984), were added to al the salami batters in equal amounts. Therefore any contributing antioxidant effect from nitrate and nitrite on lipid stability should have a constant effect on salami from all treatment groups.

Free Fatty Acids

In the degradation of lipids the first products to form is FFA through lipid hydrolysis (Fritsch, 1981). An increase in FFA normally implies a decrease in lipid quality (Atinafu & Bedemo, 2011). No significant effect for Tonalin® inclusion level was found within the treatment groups after the manufacturing and the fermentation processing stages (Figure 4.6). After drying the control group had a significantly ($p < 0.001$) lower FFA content compared to the CLA25 and CLA50 treatment groups indicating a higher degree of lipid hydrolysis for the two CLA supplemented groups. This may mean that CLA in Tonalin® may be more susceptible to hydrolysis. A statistically significant ($p < 0.001$) effect was observed for the effect of processing stage on free fatty acid content. After drying the FFA content of salami from all treatments were significantly ($p < 0.001$) higher than after manufacturing and fermentation. This lipid degradation may be ascribed to starter culture activity since it is known that some starter culture organisms have lipolytic activity (Molly *et al*., 1996;

Lizaso *et al.,* 1999), the products of which contribute to the formation of salami odour and flavour (Ordóñez *et al*., 1999).

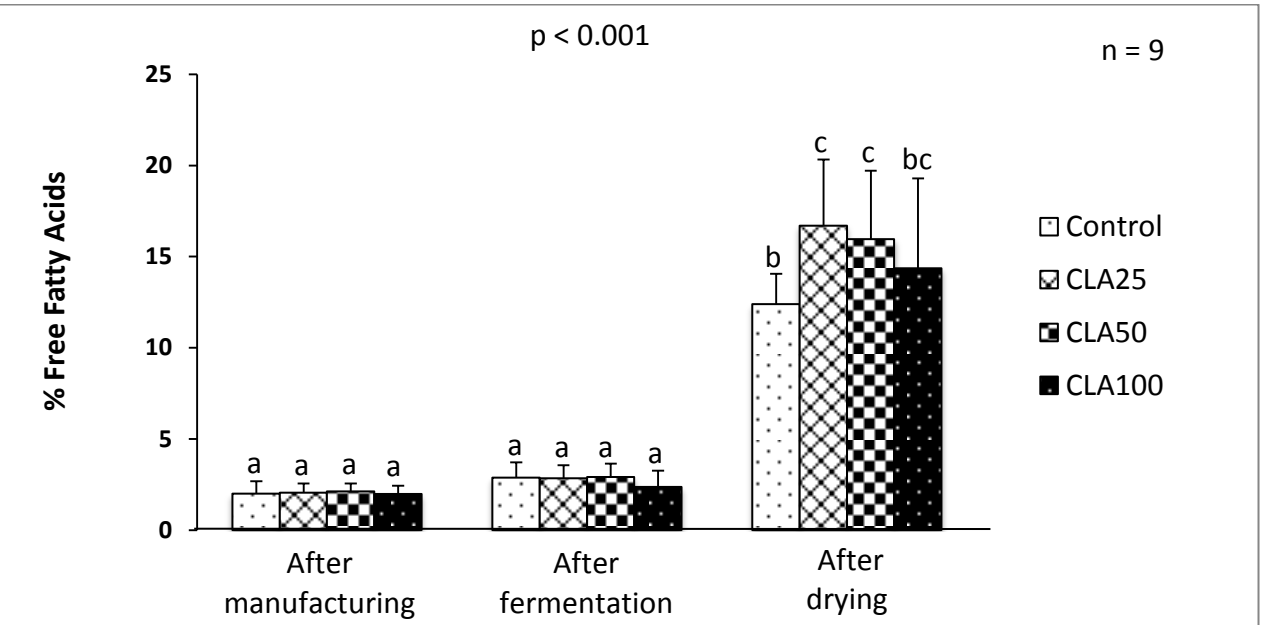


Figure 4.6. Free fatty acid development of the four salami treatment groups during salami processing.

Peroxide Value (PV)

In one of many possible degradation reactions that can affect FA, FFA are oxidised to some form of primary oxidation products known as peroxides (Fritsch, 1981). Peroxides are formed in the early stages of oxidation and are in themselves unstable and highly susceptible to further degradation to secondary products of oxidation (Farhoosh & Moosavi, 2009). Although the CLA25 and CLA50 groups had the highest levels of lipid hydrolysis after drying (Figure 4.6), it was the Control group that formed the highest level of peroxides after drying at the end of salami processing (Figure 4.7). This level was still below the acceptable limit of 25 mEq peroxide/kg of fat (Narasimham *et al*., 1989). Although no significant differences were observed in peroxide values between Tonalin® inclusion levels after manufacturing and after fermentation, all the CLA inclusion groups had consistently and significantly ($p < 0.001$) lower levels of peroxide formation compared to the control salami after drying. This may be attributed to the antioxidant effect of CLA reported in literature (Ip *et al*., 1991; Du *et al*., 2000; Hur *et al*., 2004).

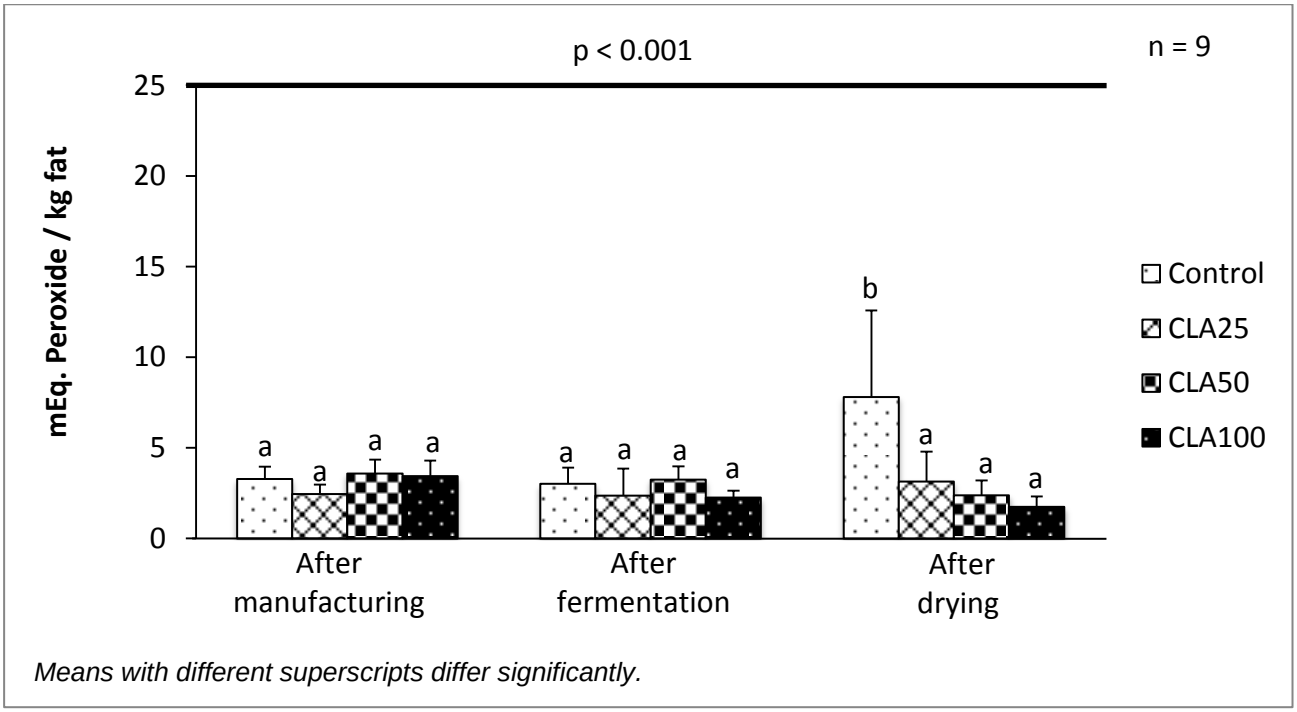


Figure 4.7. Peroxide formation of the four salami treatment groups during salami processing within the 25 mEq peroxide/kg of fat limit.

TBARS

Secondary oxidation products were observed for all treatment groups throughout the salami manufacturing process (Figure 4.8), indicating the further oxidation of primary oxidation products. These products known as aldehydes were quantified as TBARS. The only significant ($p < 0.001$) difference in TBARS was found between the Control and CLA100 after the fermentation step. This difference disappeared after the drying step indicating the formation of further degradation products. The TBARS value of the Control group after fermentation was also the highest TBARS value reported for any group at any processing step and at 0.53 ± 0.25 mg malonaldehyde/kg, salami may have had developed a rancid odour and or taste (Wood *et al*., 2008). Although not statistically significant, the TBARS values of the CLA treatment groups decreased somewhat after the drying step compared to the TBARS of salami from the Control group. The large standard deviations may be explained by the fact that TBARS analysis may not be as accurate in complex samples due to interference from amino acids, sugars and nitrite (Janero, 1990).

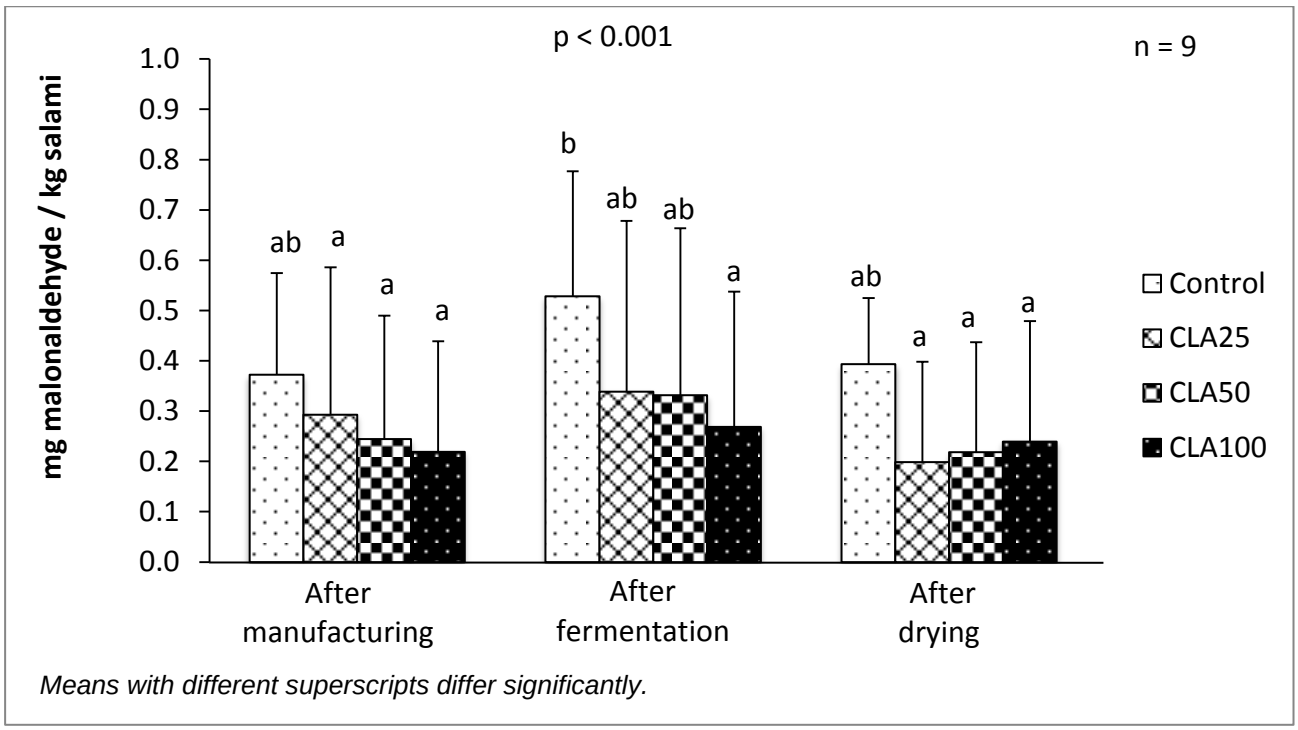


Figure 4.8. Thiobarbituric acid reactive substances of the four salami treatment groups during salami processing.

4.3.5 Fatty acid content, composition and ratios

Fatty acid composition was monitored after drying (at the end of the salami manufacturing process) since analysis of variance revealed that processing stage had no effect on fatty acid composition. These results relate to important fatty acids, fatty acid composition as well as ratios of technological and nutritional importance.

Fatty acid composition

Every single fatty acid quantified was significantly ($p < 0.001$) affected by Tonalin® inclusion level (Table 4.9). All the SFAs such as C14:0, C16:0, C17:0, C18:0 and C20:0 decreased as the CLA inclusion level increased (Table 4.9). This is due to the fact that the SFAs occurring at around 43% was systematically replaced with CLA (Warnants *et al*., 1998). The only SFA found to increase was C21:0 which was attributed to the Tonalin® product being a source of C21:0 (Figure 4.1). A similar replacement of MUFAs and PUFAs with CLA (Warnants *et al*., 1998) resulted in a lowering effect for MUFAs such as C16:1*c*9, C18:1*c*9 and C18:1*c*7 for PUFAs such as C18:2*c*9,12, C18:3*c*9,12,15, C20:2*c*11,14, C20:3*c*11,14,17 and C20:4*c*5,8,11,14 and trans fatty acids (TFA) such as C18:1*t*9. In contrast, CLA level increased significantly ($p < 0.001$) with each inclusion level. The level of C18:2*c*9,*t*11 was very low in the Control and increased exponentially with each higher CLA inclusion and C18:2*t*10,*c*12, which was not detected in the Control, followed the same pattern.

Table 4.9. The fatty acid composition of salami as affected by CLA inclusion at the end of processing.

CLA-Level (%)	Control (n=9)	CLA25 (n=9)	CLA50 (n=9)	CLA100 (n=9)	Sign. level
C14:0	1.52 ± 0.04^{d}	1.21 ± 0.05^{c}	0.89 ± 0.04^{b}	0.22 ± 0.05^{a}	p < 0.001
C16:0	25.58 ± 1.13^{d}	21.26 ± 1.06^{c}	16.47 ± 0.97^{b}	6.35 ± 0.99^{a}	p < 0.001
C16:1*c*9	2.45 ± 0.10^{d}	2.00 ± 0.09^{c}	1.56 ± 0.12^{b}	0.56 ± 0.14^{a}	p < 0.001
C17:0	0.24 ± 0.12^{c}	0.19 ± 0.11^{bc}	0.13 ± 0.09^{ab}	0.06 ± 0.05^{a}	p < 0.001
C18:0	10.73 ± 0.78^{d}	9.46 ± 0.76^{c}	7.87 ± 0.77^{b}	4.45 ± 0.33^{a}	p < 0.001
C18:1*t*9	0.11 ± 0.04^{c}	0.06 ± 0.03^{b}	0.03 ± 0.02^{a}	0.13 ± 0.06^{c}	p < 0.001
C18:1*c*9	43.44 ± 1.75^{d}	38.59 ± 1.01^{c}	32.62 ± 0.83^{b}	20.06 ± 1.21^{a}	p < 0.001
C18:1*c*7	2.96 ± 0.59^{d}	2.69 ± 0.14^{c}	2.28 ± 0.13^{b}	1.34 ± 0.11^{a}	p < 0.001
C18:2*c*9,12(*n*-6)	10.39 ± 0.62^{d}	8.79 ± 0.22^{c}	6.72 ± 0.19^{b}	2.68 ± 0.47^{a}	p < 0.001
C18:2*c*9,t11(*n*-6)(CLA)	0.11 ± 0.04^{a}	6.85 ± 0.50^{b}	14.79 ± 0.96^{c}	31.38 ± 1.67^{d}	p < 0.001
C18:2*t*10,*c*12(*n*-6) (CLA)	ND	6.72 ± 0.49^{a}	14.79 ± 0.83^{b}	31.63 ± 1.53^{c}	p < 0.001
C18:3*c*9,12,15(*n*-3)	1.49 ± 0.12^{d}	1.21 ± 0.10^{c}	0.90 ± 0.06^{b}	0.26 ± 0.06^{a}	p < 0.001
C20:0	0.18 ± 0.03^{d}	0.16 ± 0.02^{c}	0.13 ± 0.02^{b}	0.06 ± 0.01^{a}	p < 0.001
C20:2*c*11,14(*n*-6)	0.47 ± 0.02^{d}	0.38 ± 0.02^{c}	0.28 ± 0.02^{b}	0.05 ± 0.03^{a}	p < 0.001
C20:3*c*11,14,17(*n*-3)	0.08 ± 0.01^{b}	0.06 ± 0.01^{b}	0.03 ± 0.03^{a}	0.03 ± 0.07^{a}	p < 0.001
C20:4*c*5,8,11,14(*n*-6)	0.25 ± 0.02^{b}	0.25 ± 0.03^{b}	0.24 ± 0.03^{b}	0.17 ± 0.09^{a}	p < 0.001
C21:0	0.01 ± 0.01^{a}	0.10 ± 0.02^{b}	0.26 ± 0.03^{c}	0.54 ± 0.04^{d}	p < 0.001

Means with different superscripts in the same row differ significantly.

Fatty acid ratios

As in the case of individual fatty acids, all the fatty acid ratios were also significantly influenced by Tonalin® inclusion level (Table 4.10). Total SFAs and MUFAs (Table 4.10) were found to decrease significantly with each increase in Tonalin® inclusion level as explained in the previous paragraph. Although most of the individual PUFAs decreased, the total PUFAs increased significantly ($p < 0.001$) with each increase in Tonalin® inclusion level (Table 4.10). This is explained by the contribution made by the two CLA isomers to the total PUFA level. The MUFA/SFA ratio increased significantly ($p < 0.001$) with increased CLA level due to the fact SFAs decreased more severely than MUFAs with increased CLA content. The PUFA/SFA ratio increased significantly ($p < 0.001$) due to the decrease in SFA content and increase in PUFA content (Table 4.10). Increased MUFA and PUFA levels deteriorate meat product quality (Wenk *et al.,* 1990), however higher levels of MUFAs and PUFAs are better in terms of human nutrition and health (Warnants *et al*., 1998; Cordain *et al*., 2005; Muchenje *et al.,* 2009).

As the CLA content of salami increased with each Tonalin® inclusion level an increase in double bonds was found and this was reflected in a significant ($p < 0.001$) increase in the Iodine value (IV) of salami (Table 4.10). An IV of 60-70 is recommended, higher values may indicate problems with soft fat (Barton-Gade, 1983; Fischer, 1989b). Only the Control group had an IV within this range. A Double Bond Index (DBI) of less than 80 is suggested for good quality fat (Barton-Gade, 1983; Davenel *et al*., 1999). A statistically significant ($p < 0.001$) increase in DBI was observed with increased CLA content of salami. Only the Control group had a DBI of less than 80. Peroxidizability index (PI) gives an indication of the susceptibility of lipids to oxidation (Pamplona *et*

al., 1998). A significant ($p < 0.001$) increase in PI of salami lipids was observed with increased CLA content of salami. The atherogenicity index decreased significantly ($p < 0.001$) with increase in Tonalin® inclusion level. This is as a result of the decrease in SFA content with increased CLA content. It can be seen as positive from a human health point of view as a decrease in the atherogenicity index results in a decrease in atherogenic (blood lipid raising) agents which is associated with mutagenic, carcinogenic and cytotoxic properties (Kanner, 1994; Chizzolini *et al*., 1998). Omega-6 FA increased dramatically while omega-3 FA decreased dramatically with increased Tonalin® inclusion levels (Table 4.10). These changes led to a situation where the *n*-6/*n*-3 ratio was increasing warped, especially for CLA100. The nutritional guideline for *n*-6/*n*-3 PUFAs is a ratio of < 4:1, but this was far exceeded due to the high levels of CLA in CLA25, CLA50 and CLA100 compared to the Control. Lipids in the CLA100 treatment had *n*-6/*n*-3 ratio as high as 237:1. The Control's *n*-6/*n*-3 ratio already exceeded this limit and is a well documented FA problem in pork (WHO, 2003; Scollan *et al*., 2006; Wood *et al*., 2006). Although many of the above mentioned FA ratios fell outside the limits or levels desirable for meat technology and human health, it should be kept in mind that the aim of this study was the replacement of pork backfat with an oil (Tonalin®) with its own nutritional and health benefits such as cholesterol lowering, therapeutic and anti-oxidant effects (Pariza *et al.,* 1985; Lee *et al*., 1994; Belury *et al*., 1996; Park *et al*., 1999; Du *et al.*, 2000; Miller *et al*., 2001; Smedman & Vessby, 2001; Hur *et al*., 2004).

Table 4.10. Fatty acid ratios of the four salami treatment groups of nutritional and technological importance.

CLA-Level	Control (n=9)	CLA25 (n=9)	CLA50 (n=9)	CLA100 (n=9)	Sign. level
MUFA (%)	48.96 ± 2.06^{d}	43.34 ± 1.23^{c}	36.49 ± 1.06^{b}	22.09 ± 1.40^{a}	$p < 0.001$
PUFA (%)	12.79 ± 0.64^{a}	24.26 ± 1.12^{b}	37.76 ± 1.77^{c}	66.20 ± 2.71^{d}	$p < 0.001$
SFA (%)	38.26 ± 2.04^{d}	32.38 ± 1.94^{c}	25.75 ± 1.73^{b}	11.70 ± 1.35^{a}	$p < 0.001$
MUFA/SFA	1.29 ± 0.13^{a}	1.35 ± 0.12^{ab}	1.42 ± 0.11^{b}	1.90 ± 0.11^{c}	$p < 0.001$
PUFA/SFA	0.34 ± 0.03^{a}	0.75 ± 0.08^{b}	1.48 ± 0.15^{c}	5.76 ± 0.88^{d}	$p < 0.001$
IV (Calculated)	66.12 ± 2.01^{a}	80.89 ± 2.56^{b}	98.04 ± 2.86^{c}	134.18 ± 3.41^{d}	$p < 0.001$
DBI	76.59 ± 2.32^{a}	93.65 ± 2.95^{b}	113.44 ± 3.29^{c}	155.13 ± 3.94^{d}	$p < 0.001$
PI	16.32 ± 0.68^{a}	27.38 ± 1.16^{b}	40.33 ± 1.70^{c}	67.56 ± 2.55^{d}	$p < 0.001$
Atherogenicity Index	0.51 ± 0.04^{d}	0.39 ± 0.03^{c}	0.27 ± 0.02^{b}	0.08 ± 0.01^{a}	$p < 0.001$
***n*-6 (%)**	11.22 ± 0.64^{a}	22.99 ± 1.08^{b}	36.83 ± 1.80^{c}	65.91 ± 2.73^{d}	$p < 0.001$
***n*-3 (%)**	1.57 ± 0.13^{d}	1.28 ± 0.11^{c}	0.93 ± 0.08^{b}	0.29 ± 0.06^{a}	$p < 0.001$
***n*-6 / *n*-3**	7.19 ± 0.73^{a}	18.12 ± 1.50^{a}	39.74 ± 4.51^{b}	236.66 ± 54.58^{c}	$p < 0.001$

Means with different superscripts in the same row differ significantly.

4.3.6 Physical parameters

The physical condition of the salami was measured at the end of drying on the finished product. Colour and texture (shear and compression) were selected as criteria to monitor physical quality of salami from the different treatments. The results in Figures 4.9 and 4.10 revealed a number of significant effects of CLA inclusion level on the physical parameters of the salami.

Colour

The results for colour analysis of different salamis are depicted in Figure 4.9. In terms of lightness, the Control and CLA25 groups were significantly ($p < 0.001$) lighter than the CLA100 group. No variation between treatment groups in terms of redness was found. The Control and CLA25 groups were found to be significantly ($p < 0.001$) yellower than the CLA100 group. For chroma, defined as the vividness of colour, the only significant ($p < 0.001$) difference was found between the Control and CLA100 groups with the Control group being more vivid. For hue angle, defined as the development of colour from red to yellow, the Control and CLA25 group had a significantly higher value than the CLA50 group. From these results it became clear that CLA at different inclusion levels affected the colour of various treatment groups. At the highest inclusion level the product was darker, yellower and less vivid. Meat colour is important in the consumer purchasing decision and may thus be affected (Wiegand *et al*., 2002; Mancini & Hunt, 2005; Pérez-Alvarez *et al*., 2011).

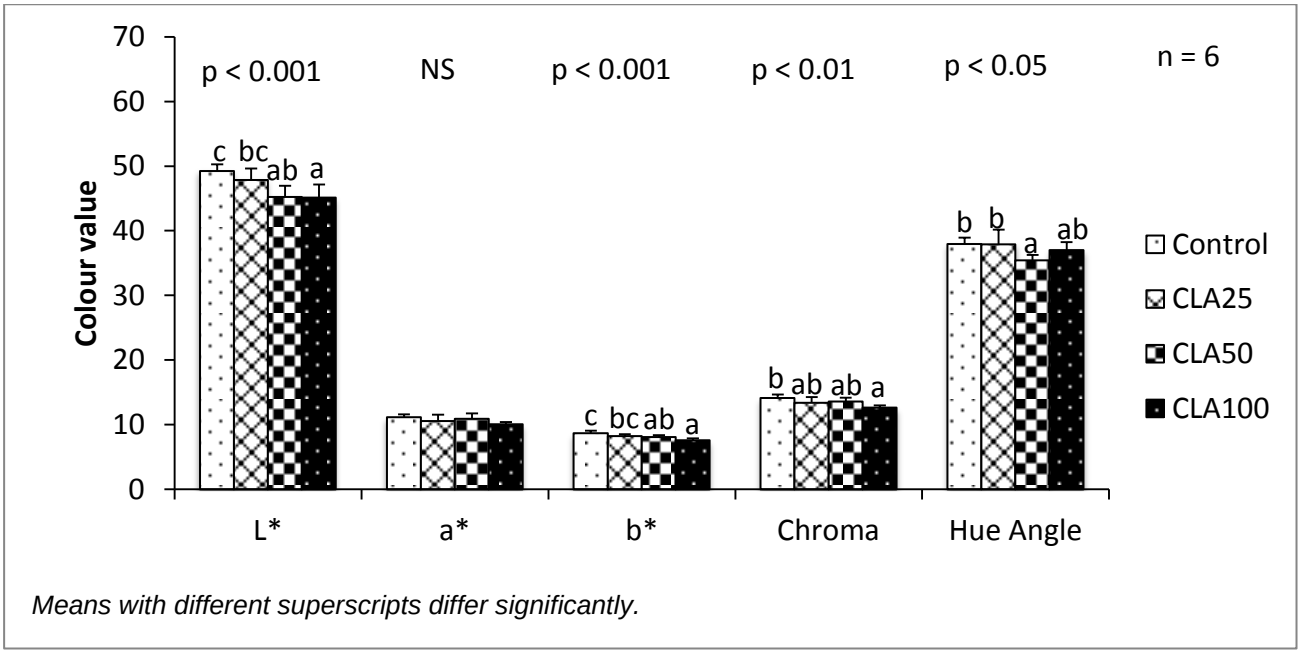


Figure 4.9. Comparison of the four salami treatment groups for the main colour parameters at the end of processing.

Texture measurements

For texture measurement of salami compression and shear force were used as parameters (Figure 4.10). A stepwise increase in compression force needed to compress the sample to 10% of its original height was found with the CLA50 and CLA100 groups differing significantly ($p < 0.001$) from the Control. For shear force, the force needed to cut through the sample, the CLA100 group needed significantly more force to cut through the sample than the other treatment groups. Thus, an increase in CLA inclusion level led to a proportional increase in hardness. This may be explained by the fact that pork BF loosens up the meat in a normal sausage batter and decreasing

the amount of BF used will lead to clumping and hardening of the sausage structure (Bloukas *et al*., 1997; Muguerza *et al.*, 2001; Beriain *et al*., 2011).

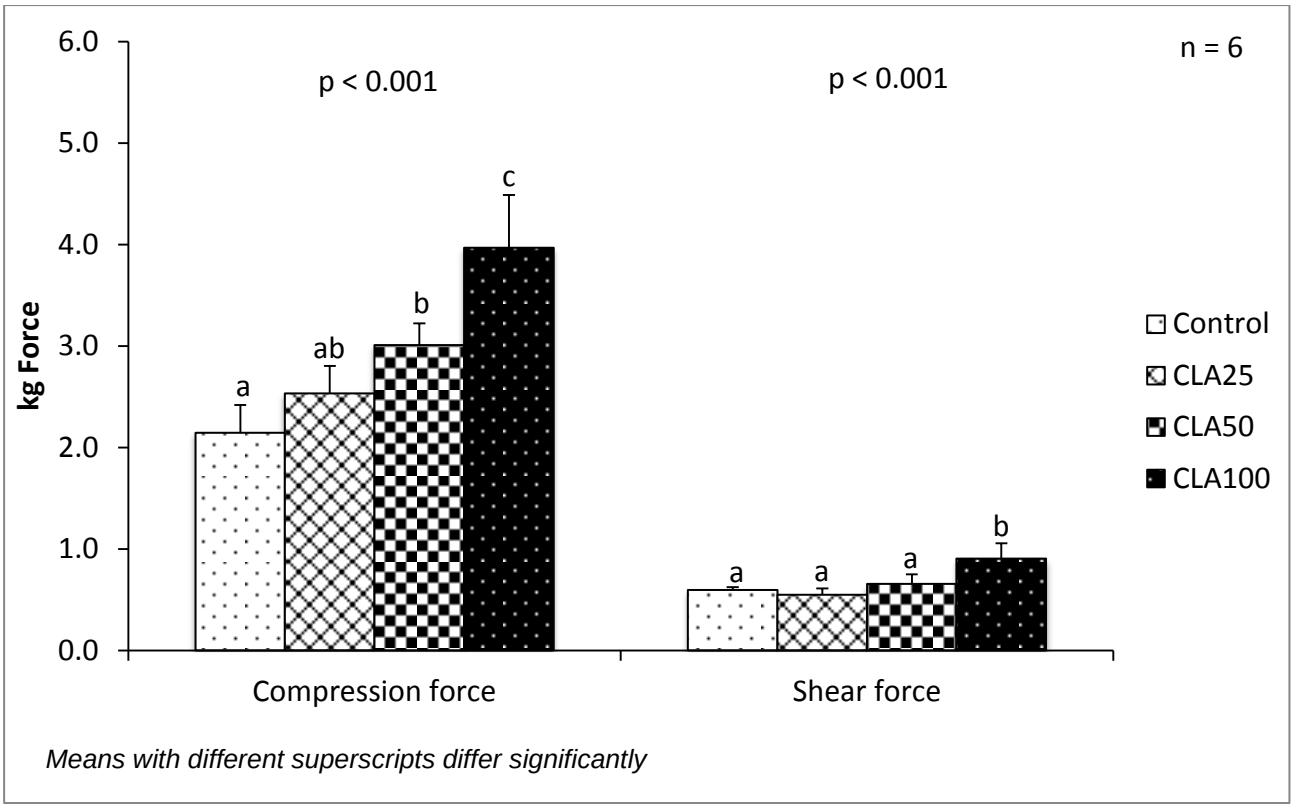


Figure 4.10. Comparison of the four salami treatment groups for compression force and shear force at the end of processing.

4.3.7 Sensory analysis

The sensory analysis was performed on the salamis after drying at the end of processing using a 75 member consumer panel. The panel could not identify any significant differences between any of the four treatment groups for aroma, taste, firmness or overall acceptability (Figure 4.11). This indicates that CLA inclusion at any level did not affect the perceived liking of the sensory properties of the various salami groups. The scores for all four attributes fell between “neither like nor dislike” and just under “like moderately”. This could be a result of the low level of smoking and very low levels of spices used to minimise the introduction of any extra variables.

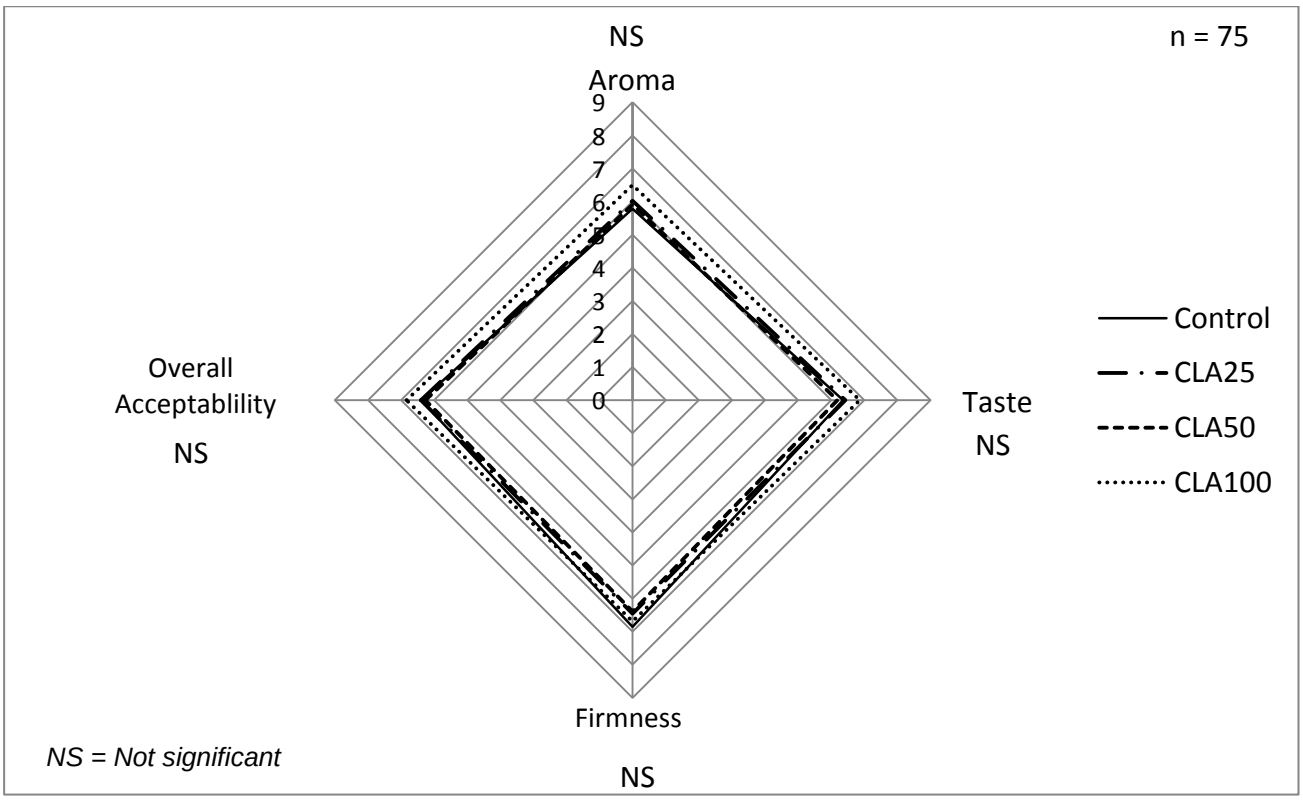


Figure 4.11. A spider plot of the sensory scores of four attributes for each of the four salami treatment groups.

4.3.8 Actual CLA content of the salamis

It was important to determine if the actual CLA content as well as the quantity of each particular isomer was present at quantities close to those formulated for, for the 28 g portion size.

Concentration of each CLA isomer per 28 g portion of salami

The concentration of C18:2*c*9,*t*11 and C18:2*t*10,*c*12 per 28 g portion of salami was significantly ($p < 0.001$) affected by Tonalin® inclusion level (Table 4.11). Both isomers were found to be present in almost equal amounts which were in line with the formulation of the Tonalin® product for each isomer (Table 4.11). The C18:2*c*9,*t*11 isomer was found at a very low concentration in the Control group and the C18:2*t*10,*c*12 isomer was not detected in the Control group. This confirmed the marginal ability of pigs as monogastric animals to synthesize meaningful amounts of CLA as reported in literature (Chin *et al*., 1992; Schmid *et al.,* 2006). The concentration of CLA for the three CLA treatment groups was found to increase stepwise in accordance with the amount of Tonalin® included in the salami batter with significant ($p < 0.001$) differences between each treatment group.

Table 4.11. CLA content of salami as affected by Tonalin® inclusion at the end of processing.

CLA-Level	C18:2c9,t11 (mg/28 g salami)	C18:2t10,c12 (mg/28 g salami)	mg Total CLA /28 g salami
Control (n=9)	7.60 ± 2.54[a]	ND	7.60 ± 2.54[a]
CLA25 (n=9)	429.12 ± 32.24[b]	423.45 ± 31.70[a]	852.57 ± 63.86[b]
CLA50 (n=9)	869.41 ± 134.81[c]	884.19 ± 126.79[b]	1753.60 ± 261.37[c]
CLA100 (n=9)	1689.77 ± 212.44[d]	1745.32 ± 210.94[c]	3435.09 ± 422.44[d]
Sign. level	p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly ND = Not detected

Percentage of the RDA of each of the four treatment groups

The percentage of the RDA of CLA per 28 g portion size was significantly (p < 0.001) affected by Tonalin® inclusion level. The Control group was found to supply a negligible percentage of 0.22% of the RDA (which represents the 100% mark on the y-axis) (Figure 4.12). Through the inclusion of Tonalin® directly into the salami batter the following RDA results were obtained for the three CLA treatment groups: 24.36% of RDA for CLA25, 50.10% of RDA for CLA50 and 98.15% of RDA for CLA100. All of these were found to be very close to the values calculated during formulation (Table 4.4). It also indicates how easy it is to supply very precise levels of CLA through direct addition of CLA to the salami product. It would never be possible to obtain these high levels of CLA in meat products through dietary supplementation of CLA in pigs.

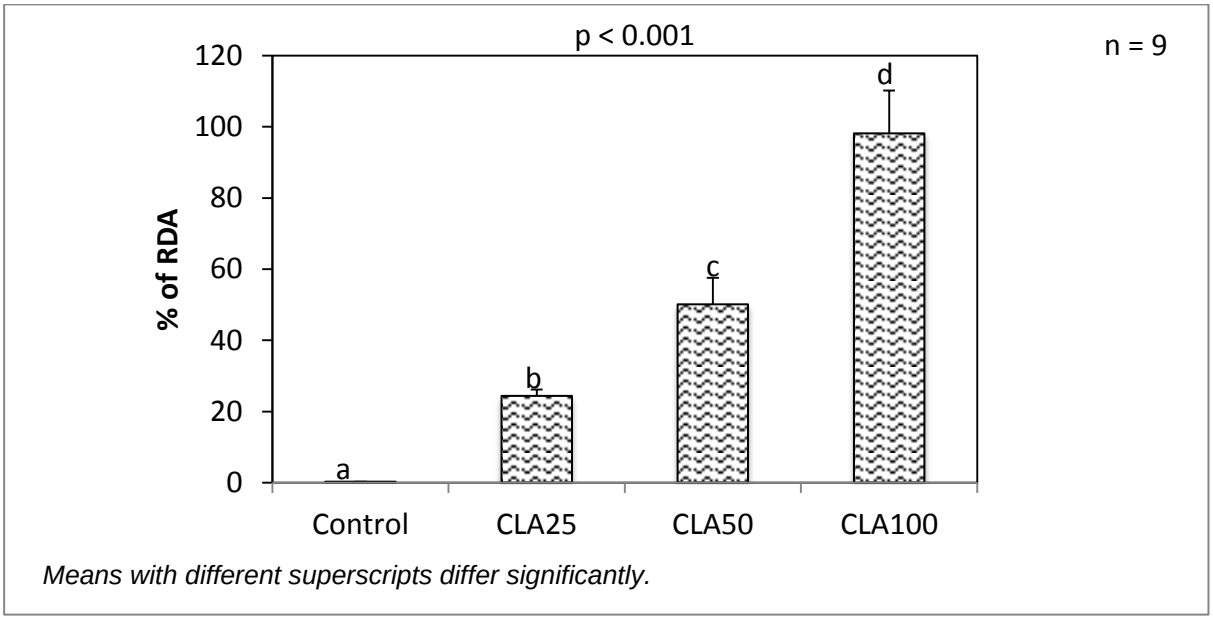


Figure 4.12. The concentration of CLA for each of the salami treatment groups expressed as percentages of the RDA.

4.3.9 Effects of Tonalin® inclusion on extended cold storage of salami

The salamis of all four treatments groups were also subjected to a 30 day vacuum packaged storage at 4°C to observe any possible effects these conditions may have on the stability of salami.

Parameters not statistically affected by Tonalin® inclusion level during refrigerated storage

The following chemical and microbial parameters seemed to be unaffected by Tonalin® inclusion level after vacuum packaging and refrigerated storage for one month (Table 4.12): pH; a_w; total acidity; peroxide value; LAB counts; coliform counts; yeast and mould counts. Although the pH of all four treatment groups were unaffected by CLA inclusion at different levels after the 30 day storage period, pH values were generally lower than at the end of drying (Table 4.12; Figure 4.3). This indicates that lactic acid formation still occurred during refrigerated storage as a result of the still active mixed starter culture. Even though various microbial and chemical processes continued during storage, water activity levels after 30 days storage was also comparable to a_w levels after drying (Table 4.12; Figure 4.3). CLA inclusion had no significant effect on the total acidity after storage. As the pH continued to decrease slightly during storage the total acidity increased further indicating the formation of more lactic acid by the mixed starter culture. Total acidity increased from approximately 1.70 mL of 0.1 N NaOH after drying (Figure 4.4) to ±2 mL of 0.1 N NaOH after 30 days of refrigerated storage for 30 days (Table 4.12).

Table 4.12. Chemical and microbial stability of salami after 30 days of vacuum packaged storage at 4°C.

CLA-Level	Control (n=9)	CLA25 (n=9)	CLA50 (n=9)	CLA100 (n=9)	Sign. level
pH	4.98 ± 0.06	4.99 ± 0.11	4.99 ± 0.10	5.02 ± 0.09	NS
a_w	0.9022 ± 0.0110	0.9030 ± 0.0106	0.9036 ± 0.0120	0.9069 ± 0.0111	NS
Total acidity	1.96 ± 0.36	1.96 ± 0.28	2.02 ± 0.32	2.03 ± 0.37	NS
% Free fatty acids	10.84 ± 2.44^{ab}	10.40 ± 2.40^{ab}	13.84 ± 3.98^{b}	9.66 ± 1.84^{a}	p < 0.05
Peroxide value	2.28 ± 1.38	2.36 ± 1.16	2.34 ± 0.66	1.69 ± 0.67	NS
TBARS value	0.48 ± 0.17^{b}	0.18 ± 0.06^{a}	0.21 ± 0.09^{a}	0.19 ± 0.10^{a}	p < 0.001
Lactic acid bacteria (log cfu/g)	7.88 ± 0.19	7.89 ± 0.25	7.81 ± 0.46	7.94 ± 0.26	NS
Coliforms (log cfu/g)	0.09 ± 0.28	0.14 ± 0.43	0.00 ± 0.00	0.27 ± 0.53	NS
Yeasts (log cfu/g)	4.09 ± 2.44	4.96 ± 1.98	5.32 ± 1.35	5.33 ± 0.71	NS
Moulds (log cfu/g)	4.69 ± 1.48	5.23 ± 0.57	5.11 ± 0.60	4.87 ± 1.04	NS
C18:2*c*9,*t*11 (mg/28 g salami)	7.47 ± 2.64^{a}	463.76 ± 28.27^{b}	820.60 ± 155.85^{c}	1749.46 ± 76.10^{d}	p < 0.001
C18:2*t*10,*c*12 (mg/28 g salami)	ND	447.59 ± 27.24^{a}	815.15 ± 152.45^{b}	1753.25 ± 80.70^{c}	p < 0.001
mg Total CLA /28 g salami	7.47 ± 2.64^{a}	911.36 ± 55.49^{b}	1635.74 ± 308.27^{c}	3502.71 ± 156.61^{d}	p < 0.001
% of RDA	0.21 ± 0.08^{a}	26.04 ± 1.59^{b}	46.74 ± 8.81^{c}	100.08 ± 4.47^{d}	p < 0.001

Different superscripts in the same row differ significantly.

Although no statistical difference were observed in LAB count after refrigerated storage for 30 days (Table 4.12), the LAB counts were slightly lower than after drying (Table 4.8). This implies the transition from the stationary phase to the early death phase in terms of growth. CLA inclusion at different levels had no effect on the coliform counts after the 30 days refrigerated storage (Table 4.12). It was expected that no coliforms would be present after 30 days of storage due to the low pH (lactic acid formation) and low storage temperature. However, colonies were still reported for three of the treatment groups (Control, CLA25 and CLA100) albeit at much lower concentrations than after the drying stage (Table 4.8). Yeasts and moulds were also unaffected by increasing CLA inclusion levels after 30 days of refrigerated storage (Table 4.12). The mould counts for the Control, CLA25 and CLA50 groups were slightly higher after 30 days of storage (Table 4.12) compared to after processing (Table 4.8). A decrease was expected due to the lack of oxygen thought to be present and the fact that most moulds are obligate aerobes (Madigan & Martinko, 2006). The vacuum packaging may have had a sufficient oxygen transfer rate or microbial competition for oxygen was not as fierce as expected. Yeast counts were found to decrease from after the drying stage to after 30 days of storage. Yeasts reportedly flourish in high sugar environments (Madigan & Martinko, 2006). Their decreased concentration after refrigerated storage (Table 4.12) compared to after drying (Table 4.8) can be ascribed to most of the added sugars (dextrose and sucrose) already used during LAB fermentations as well as a lack of oxygen.

The significantly ($p < 0.001$) higher peroxide value for the Control group versus the CLA treatment groups obsered after drying (Figure 4.9) disappeared after the refrigerated storage period (Table 4.12) with no significant differences between any of the treatment groups. The other treatment groups only displayed a slight decrease in PV at the end of the refrigerated storage period (Table 4.12) compared to after drying (Figure 4.9). The large decrease in peroxide value for the Control group from after drying to after storage as well as the slight decrease in peroxide values for the other three groups can probably be ascribed to the further secondary oxidation of the peroxides (Fritsch, 1981).

Parameters affected by Tonalin® inclusion level during refrigerated storage

Free fatty acids, TBARS and actual CLA content seemed to have been affected by Tonalin® inclusion level after vacuum packaging and refrigerated storage (Table 4.12). After 30 days of storage the FFA value for CLA50 was significantly ($p < 0.05$) higher than that of CLA100 (Table 4.12). The FFA content of CLA50 did not differ significantly from the Control or CLA25. After drying the CLA25 group had a significantly ($p < 0.05$) higher percentage of FFA compared to the Control (Figure 4.8), while after 30 days of storage this was no longer the case. These results may indicate that for a certain CLA inclusion level at a certain time during the salami products' lifetime, increased lipid hydrolysis will occur. Compared to the FFA values after drying (Figure 4.8), the FFA values for all four groups were lower after 30 days of refrigerated storage indicating a progression

to the formation of primary lipid oxidation products such as for example, peroxides. *Staphylococcus carnosus* (from the mixed starter culture) is also reportedly capable of converting FFA to other products known to contribute to aroma (Stahnke, Holck Jensen, Nilsen & Zanardi, 2002; Tjener, Stahnke, Andersen & Martinussen, 2004a,b) and this may also have contributed to the lower FFA levels after storage of the salamis.

After 30 days of refrigerated storage the Control had a significantly ($p < 0.001$) higher TBARS value than any of the three CLA inclusion groups. This may be the consequence of the fact that CLA can act as an antioxidant in meat products (Ip *et al*., 1991; Du *et al*., 2001; Hur *et al.,* 2004). The TBARS values after 30 days of refrigerated storage (Table 4.12) were at the same level as the TBARS values after drying (Figure 4.10).

After 30 days of storage significant ($p < 0.001$) effects on the CLA isomers and thus on the total mg CLA per 28 g portion of salami and percentage of RDA were identified (Table 4.12). The concentration of C18:2*c*9,*t*11 decreased in the Control and CLA50 groups and increased in the CLA25 and CLA100 groups in comparison with the concentration of C18:2*c*9,*t*11 after drying (Table 4.11). The C18:2*t*10,*c*12 isomer was undetected in the Control group after 30 days of storage, similar to the result found after drying. A decrease in the CLA50 group and increase in the CLA25 and CLA100 groups were observed from after drying to after 30 days of refrigerated storage. These results were reflective for total mg CLA per 28 g portion of salami and percentage of the RDA. It remains unclear as to why the CLA experienced these changes in concentration. Literature showed that neither storage of meat and dairy products (Shantha *et al.*, 1994; Shantha *et al*., 1995; Hur *et al*., 2004; Martín *et al*., 2008b) nor a dynamic ripening period in cheeses (El-Salam & El-Shibiny, 2012) affected CLA concentration.

4.4 Conclusions

No problems were experienced with the formulation and manufacturing of the different groups of salami. After processing of the salamis the proximate composition of salamis manufactured with different Tonalin® inclusion levels was unaffected when compared to 100% pork BF Control salami. Drying time was somewhat longer although this proved to be statistically insignificant and in line with literature (Josquin *et al*., 2011). The other ripening parameters consisting of pH, total acidity and a_w were also unaffected by CLA inclusion and the level of inclusion. This is seen as a positive result as any deviations in these parameters would have indicated slowdowns in the development of the salami from a freshly manufactured product to the desired finished product.

Microbial parameters were also completely unaffected by CLA inclusion level. This resulted in unhindered and unaltered functioning of the starter culture, which is of vital importance for product

development, stability and safety (Bacus & Brown, 1986; Buckenhuskes, 1993; Çon & Gökalp, 2000; Erkkilä *et al.*, 2001; Fadda *et al*., 2002). CLA inclusion level also did not affect coliforms or yeasts and moulds as pathogenic or spoilage microorganisms. The inhibition of hyphal growth by CLA has recently been reported in literature (Shareck, Nantel & Belhumeur, 2011) although no similar inhibitory effect which could have led to a decrease in fungal growth was found, even at these high CLA inclusion levels. When the actual lipid stability parameters were studied no effect by CLA was found on initial lipid hydrolysis. Peroxides and TBARS formation was affected positively by CLA inclusion level during processing of the salami. The actual CLA content of salami was greatly improved when CLA groups were compared to the Control group. The level of CLA for a particular formulation was more easily increased through this direct addition method than through dietary supplementation. The actual CLA levels in the finished product were quite close to the levels calculated for in the formulation process.

An incremental replacement of pork BF with a commercial, food grade source of CLA Tonalin® resulted in the successful manipulation of the FA content and FA composition of salami. All MUFAs, PUFAs and SFAs decreased significantly with increased replacement of BF with CLA. This is due to the fact that the BF was the major source of most of these fatty acids. The decrease in pork BF and thus SFAs was acceptable in terms of human nutritional guidelines, but not acceptable in terms of the importance of SFAs in meat technology (Severini *et al*., 2003). CLA isomer levels increased dramatically with each inclusion level. Nutritionally important fatty acid ratios such as MUFA/SFA and PUFA/SFA ratios improved dramatically, but complicate the meat technology aspects of this type of meat product (Wenk *et al.,* 1990; Verbeke *et al.,* 1999, Teye *et al.,* 2006). A dramatic decrease in the atherogenicity index was also identified which further proves the improvement in nutritional properties of the salami. Fatty acid ratios indicative of lipid oxidation stability appeared to have been negatively influenced, especially due to the massive contribution CLA made to PUFA content.

Various colour attributes of the finished salamis were significantly affected by CLA inclusion level in a negative way. Tonalin® inclusion at the highest level led to a product which was darker, yellower and less vivid in colour. Meat colour is important in the consumer purchasing decision and may thus be affected (Mancini & Hunt, 2005). The texture of the salamis became increasingly harder with increasing CLA inclusion level. A consumer panel could not detect this hardening effect or any other changes in the sensory attributes of salami.

After storage of the finished salamis at 4°C for 30 days it was found that CLA inclusion level did not adversely affect the microbial stability of the salamis. Lipid stability was improved for TBARS, not affected for peroxide values and negatively affected for percentage free fatty acids for one CLA inclusion group (CLA50). In contrast to literature (Shantha *et al.,* 1994; 1995; Hur *et al*., 2004;

Martín *et al*., 2008b) changes in the concentrations of both CLA isomers were found after 30 days of refrigerated storage with no clear indication of what could be responsible for these changes.

Making use of partial replacement of pork BF with CLA, the FA composition of salami was greatly improved in terms of human nutrition and consumers did not seem to mind or notice the negative effect on meat technology. Through the use of direct addition of CLA, the actual CLA levels of the finished products were quite close to the RDA levels calculated for in the formulation process. The 100% RDA for CLA was easily formulated for a 28 g portion of salami. The ripening and microbial parameters of the salami was unaffected by CLA inclusion and this product may even have an improved shelf life due to the positive effects of CLA on lipid stability.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Various chronic diseases are increasingly linked to meat consumption (Weiss *et al.,* 2010). This results in consumers eating less meat (Verbeke *et al*., 1999). Red meat consumption is associated with being high in fat and contributing to cancers (Valsta *et al.,* 2005). Processed meat is also associated with high levels of sodium chloride which has been linked to hypertension and various other lifestyle diseases (Ruusunen & Puolanne, 2005). In the case of pork this is especially important as pork has been singled out as a major source of SFAs (Verbeke *et al*., 1999; Aida *et al.*, 2005), the consumption of which has been linked to cardiovascular disease, cancer and inflammatory and autoimmune diseases (Simopoulos, 2002). Consumers demand healthier meat and meat products and they are expecting the incorporation of health enhancing ingredients to their meat product (Zhang *et al*., 2010).

These consumer pressures forced the meat industry to react (Weiss *et al*., 2010). Various strategies have been implemented in an attempt to improve the nutritional value of pork. The use of leaner pigs which are slaughtered at lower live weights have been suggested although this strategy introduces new problems such as the deteriorating effect of PUFAs on fat quality (Warnants *et al.,* 1998). Increasing the slaughter weight again leads to a situation where SFAs become a problem due to increased lipid saturation (Babatunde *et al*., 1966; Allen *et al*., 1967; Staun, 1972; Martin *et al*., 1972). Saturated fatty acids are not viewed in a completely negative light. Due to their oxidative and processing stability they are highly valued by the meat processor (Bruwer *et al*., 1991). The inverse relationship between the nutritional and technological qualities of FAs (Hugo & Roodt, 2007) seems to be open for manipulation depending on the type of meat product under review.

The incorporation of non-meat lipids into existing meat and meat products in an attempt to create healthier versions of existing meat products (Juárez *et al.*, 2009) is another strategy. This may improve the “image” of meat products as well as address the changing needs of consumers. These products may be regarded as meat-based functional foods. Lipids are the bioactive compounds in food that have received the most quantitative and qualitative attention in terms of development of healthier meat products (Jiménez-Colmenero, 2007). Quality of the fat tissue in pork is highly influenced by the amount of dietary fatty acids and the composition thereof (St. John *et al.*, 1987; Cardenia *et al.,* 2011). In an attempt to improve the image of pork a lot of research has been done on dietary supplementation with CLA. Conjugated linoleic acid was discovered in 1979 by Michael Pariza and co-workers. It is a collective name for a group of positional and geometric isomers of

linoleic acid (Thiel-Cooper *et al*., 2001). The most biologically active forms are the C18:2*c*9,*t*11 and C18:2*t*10,*c*12 isomers on which this study was focused.

It was found that monogastric animals e.g. pigs do not actively synthesize significant amounts of CLA by themselves, but that supplementation of CLA in their diets led to measurable increases in CLA content (Chin *et al.*, 1992; Schmid *et al.,* 2006). Conjugated linoleic acid introduces various positive effects on human health as well as technological improvements to pork (Corino *et al.*, 2003; Lauridsen *et al.*, 2005; Hah *et al.*, 2006; Schmid *et al*., 2006; Martin *et al.,* 2008; Martín *et al.*, 2008b; Marco *et al.*, 2009; Toomik *et al*., 2012). It has positive effects on cancer, cardiovascular disease, diabetes, body composition, the immune system and bone health in humans (Schmid *et al*., 2006; Khanal *et al*., according to Zhang *et al*., 2010). In pigs it was found to increase fat firmness and muscle marbling, both factors which increases the economic value of pork with no negative effects on derived products such as dry-cured loin (Martín *et al*., 2008c).

In the first experiment of this study 40 Duroc X Landrace gilts weighing on average 35 kg were divided into two groups. Of these, 20 gilts were fed on a diet containing 0.5% SFO and the other 20 gilts were fed on a diet containing 0.5% CLA (Luta-CLA® 60, BASF). Each of these groups was further divided into two slaughter weight groups. Ten gilts from each group were slaughtered at ±70 kg and the other 10 gilts were slaughtered at ±90 kg. The lean pork and backfat of the loins were pooled within each of the four treatment groups and utilized to creat novel, cured and fermented pork sausages (salami). An attempt was made to determine if quality is influenced by slaughter weight and dietary supplementation of CLA. In terms of this study, quality was defined in terms of microbial, physical and lipid stability parameters as well as fatty acid composition and fatty acid ratios.

Major effects on the fatty acid composition and fatty acid ratios of the BF used in the manufacturing of the salami were observed with both dietary supplementation and increased slaughter weight having significant effects. Backfat thickness increased significantly ($p < 0.01$) as a result of increased slaughter weight (Nürnberg *et al*., 1998) and BF hardness increased significantly ($p < 0.001$) due to the effects of increased slaughter weight and CLA dietary supplementation which increased the quality of the pork (Martín *et al*., 2008c). As reported in literature CLA dietary supplementation led to higher degrees of lipid saturation, even at lower slaughter weight (Smith *et al*., 2002; Wiegand *et al*., 2002; Lauridsen *et al*., 2005).

With regard to the MUFAs and PUFAs, various effects were observed due to slaughter weight, dietary supplementation of CLA or as in some cases, due to a combined effect from both of these parameters. The total UFA content of salami were significantly ($p < 0.001$) influenced by both slaughter weight and dietary treatment. The lower content of PUFAs of salami in especially the

90CLA group may be attributed to the more saturated profile of more mature fat tissue in heavier pigs and also to the fact that the longer exposure to dietary CLA in the heavier pigs will also lead to increased lipid saturation (Migdał *et al*., 2004). In terms of nutritionally important FA ratios only the 70SFO group managed to have acceptable MUFA/SFA (Cordain *et al*., 2005; Muchenje *et al*., 2009) and PUFA/SFA ratios (WHO, 2003; Scollan *et al*., 2006; Wood *et al*., 2008). The 70SFO group with a low slaughter weight, lowest SFA and highest UFA content had an IV considered as very poor from a fat quality point of view whereas the 90CLA group with a higher slaughter weight, the highest SFA content and the lowest UFA had the best fat quality in terms of IV (Barton-Gade, 1983; Girard *et al*., 1988; Fischer, 1989b).

The microbial, physical, sensory and lipid stability parameters were either largely unaffected or inconsistently affected by CLA dietary supplementation and slaughter weight. In terms of the ripening parameters, the CLA dietary treatment groups showed a slightly shorter drying time. Consistently shorter drying times for salamis supplemented with CLA through dietary manipulation could prove to be profitable as more salamis could be produced in a given amount of time. The pH and total acidity were unaffected by different diets and slaughter weights, in contrast to what was expected especially due to the effect dietary CLA has on muscle fibre composition and increased buffering capacity of the muscle (Migdał *et al*., 2004). No significant effects on a_w could be gleaned from slaughter weight and diet as variables. The microbial parameters revealed that neither slaughter weight nor diet affected the proper function of the LAB critical to the stability and quality of the salami (Fadda *et al*., 2010). No effects on the spoilage bacteria were observed although there was a limited inhibitory effect exhibited by CLA supplementation on the yeast and mould counts of the 70CLA group compared to the 70SFO group ($p < 0.001$). There have been various reports of FAs having antifungal activity in literature (Kabara *et al*., 1972; Bergsson *et al*., 2001; Wood & Lee, 1981) although no such reports relating to CLA having antifungal properties in food could be found. The lipid stability parameters as free fatty acids, peroxide values and TBARS were completely unaffected by diet and slaughter weight.

Dietary treatment and slaughter weight both affected the DBI significantly ($p < 0.001$) with higher slaughter weights and dietary CLA supplementation resulting in lower DBI values. Again the 70SFO group had the lowest fat quality due to its high DBI being above the cut-off point for good quality fat (Prabucki, 1991). Peroxidizability index was only significantly ($p < 0.001$) influenced by slaughter weight. Backfat from the two higher slaughter weight groups of 90SFO and 90CLA had lower PI values and thus better fat quality than the 70SFO and 70CLA groups (Pamplona *et al.,* 1998). Furthermore, it was determined if an increase in CLA levels could be made, it would improve the health aspects of salami and possibly grant it the status of being a significant source of CLA in the human diet. In practice this proved to be mainly ineffective and largely unachievable. Even for the highest slaughter weight group supplemented with CLA (90CLA) the effective CLA

level was only just over 1% of the RDA of 3.5 g CLA/day (Ha *et al.*, 1989) and cannot be considered a very successful approach to increase human consumption of CLA. Other, more direct methods of supplementation was thought to prove to be more successful.

In terms of colour CLA dietary supplementation was found to have no effect on *L**, *a** and *b** colour values as reported in literature (Joo *et al*., 2002; Tischendorf *et al*., 2002; Intarapichet *et al*., 2008) although in terms of Hue angle the 90CLA group was less red. Texture measurements revealed conflicting results where the 70SFO group had significantly ($p < 0.05$) higher shear force and the 90CLA group had significantly ($p < 0.01$) higher compression force. The higher shear force of the 709SFO group could not be explained, but the higher compression force of the 90CLA group was attributed to dietary CLA supplementation (Joo *et al*., 2002; Wiegand *et al*., 2002; Lo Fiego *et al*., 2005; Marco *et al*., 2009). Dietary CLA supplementation and slaughter weight did not affect the sensory properties of the salami according to the results of the 75 member consumer panel. Similar results were reported in literature where dietary fat supplementation (Alonso *et al*., 2012) and dietary CLA supplementation (Migdał *et al*., 2004) did not affect the sensory properties of pork.

None of the chemical parameters of any of the four treatment groups were affected in any significant way by slaughter weight or diet after one month cold storage at 4°C. Of all the microbial parameters monitored after one month cold storage only the yeast and mould counts showed a significant ($p < 0.001$) difference with the 70SFO group having a higher count than the other three treatment groups which could not be explained.

In the second experiment of the study, direct addition was evaluated as a means to increase the CLA content of salami. Lean pork and pork BF was utilized in the manufacturing of four distinct groups of novel cured and fermented pork sausages (salami). The aim of this part of the study was to increase the CLA content of a cured, fermented and dried sausage pork (salami) to three increasing percentages (25%, 50% and 100%) of the Recommended Dietary Allowance for conjugated linoleic acid per 28 g portion of salami. This was accomplished through the direct addition of CLA (Tonalin® TG 80) in a pre-emulsified oil form with proportional decreases in the normally used pork BF content of the salamis. The Tonalin® TG 80 consisted of FAs of which 80% was in the triglyceride form and 77% of this consisted of almost equal parts of the two biologically active isomers of CLA namely the C18:2*c*9,*t*11 and C18:2*t*10,*c*12 isomers. The three CLA inclusion groups were compared to a Control group with a 100% pork BF in emulsion for any possible effects on the microbial, physical and lipid stability parameters as well as fatty acid composition and fatty acid ratios. This work was repeated in four separate phases, each spanning over a three month timeline.

In terms of the drying parameters the Control group dried slightly faster than the three Tonalin® inclusion groups, although not statistically significant. Thus, direct addition of CLA did not affect the drying process even over a wide range of pork BF replacement levels. This was an unexpected result seeing as the Tonalin® did not remain in a stable emulsion and caused a smearing effect which was expected to hamper the release of moisture from the salamis (Bloukas *et al*., 1997). In contrast to the research of Josquin *et al*. (2011) there was also no negative effect on the moisture content when using pre-emulsified oil. The pH and total acidity of the salamis were completely unaffected by Tonalin® inclusion level, further enforcing the conclusion that the salami drying process as a whole was unaffected by CLA inclusion level. Actual fat content of the four groups of salamis after manufacturing was in close proximity to each other and to the values estimated during the formulation of each treatment group. After drying there was a significant ($p < 0.001$) difference in fat content with that of the Control group being the highest. This was attributed to drip loss of the destabilized Tonalin® emulsions which was then visually confirmed in the ripening room. Microbial parameters were largely unaffected by CLA inclusion level. Nowhere was this more important than for the activity of the LAB which may reportedly (Sieber *et al*., 2004) have been inhibited by CLA at very low concentrations, although this was not the case.

Lipid stability was largely unaffected by the inclusion of CLA in salami at different levels. After drying the CLA25 and CLA50 groups had significantly ($p < 0.001$) higher FFA levels compared to the Control, indicating a possible increased susceptibility of Tonalin® to lipid hydrolysis. This trend in increased lipid degradation for the CLA25 and CLA50 groups was not reflected in the peroxide values of these two groups. The Control group had significantly ($p < 0.001$) higher PV after drying although this was still well within the limit for peroxides of 25 mEq peroxide/kg of fat (Narasimham *et al*., 1989). For the TBARS no effect of CLA on the formation of secondary oxidation products was observed.

All the FAs were significantly affected by the inclusion of Tonalin® at different levels mainly due to the fact that the pork BF originally used as a source of lipids were replaced at various levels. Significant ($p < 0.001$) decreases in SFAs and significant ($p < 0.001$) decreases in individual PUFAs were found as Tonalin® inclusion increased, although the total amount of PUFAs increased due to the increase in CLA. The amount of MUFAs also increased significantly ($p < 0.001$) with higher inclusion level. Increased MUFA and PUFA levels deteriorated fat quality (Wenk *et al*., 1990). These MUFAs and PUFAs added as Tonalin® were already in a liquid form, thus the salami texture would definitely be affected. This was reflected in the high IV and DBI for all three CLA treatment groups associated with fat quality (Barton-Gade, 1983; Fischer, 1989b; Davenel *et al*., 1999). Higher levels of MUFAs and PUFAs are better in terms of human health (Warnants *et al*., 1998; Cordain *et al.,* 2005; Muchenje *et al*., 2009). The PI results of the three CLA treatment groups were also significantly increased ($p < 0.001$) although the actual PV were actually lower

than that of the Control. The atherogenicity index decreased significantly ($p < 0.001$) with increase in Tonalin® inclusion level. From a human health point of view a decrease in the atherogenicity index results in a decrease in atherogenic (blood lipid raising) agents which is associated with mutagenic, carcinogenic and cytotoxic properties (Kanner, 1994; Chizzolini *et al*., 1998).

In terms of colour parameters, Tonalin® at different inclusion levels affected the colour of various treatment groups in different ways. At the highest inclusion level the product was darker, yellower and less vivid. Meat colour is important in the consumer purchasing decision and may thus be affected (Wiegand *et al*., 2002; Mancini & Hunt, 2005; Pérez-Alvarez *et al*., 2011). An increase in Tonalin® inclusion level led to a proportional increase in hardness. This was explained by the fact that pork BF loosens up the meat in a normal sausage batter and decreasing the amount of BF used will lead to clumping and hardening of the sausage structure (Bloukas *et al*., 1997; Muguerza *et al.*, 2001; Beriain *et al*., 2011). In contrast to the objective measurements on texture, the 75 member consumer panel could not identify any significant differences between any of the four treatment groups for firmness or for any of the other sensory parameters (aroma, taste, or overall acceptability). Thus the negative effect on texture that pre-emulsified oil might have on salami quality could in reality be effectively less than thought.

The concentration of C18:2*c*9,*t*11 and C18:2*t*10,*c*12 per 28 g portion of salami was significantly ($p < 0.001$) affected by Tonalin® inclusion level with both isomers found to be present in almost equal amounts which were in line with the formulation of the Tonalin® product for each isomer. The percentage of the RDA of CLA per 28 g portion size was significantly ($p < 0.001$) affected by Tonalin® inclusion level. From a negligible percentage of 0.22% of the RDA for the Control treatment up to 98.15% of the RDA for the CLA100 group was found. The partial replacement of pork BF and direct addition of CLA to salami proved to be an effective method of increasing CLA levels in salami in an attempt to improve the health aspects of salami to the point where it could be regarded as a functional food.

The vast majority of parameters were completely unaffected by Tonalin® inclusion after 30 days of refrigerated storage. The TBARS value of the Control was found to be significantly ($p < 0.001$) higher than any of the three CLA inclusion groups. This was thought to be due to the antioxidant effect of CLA on meat products (Ip *et al*., 1991; Du *et al*., 2001; Hur *et al.,* 2004). After 30 days of storage, significant effects ($p < 0.001$) on the CLA isomers and thus on the total mg CLA per 28 g portion of salami and percentage of RDA were identified with the concentrations of the two CLA isomers increasing for some treatment groups and decreasing for others. No explanation could be formulated as to why the CLA experienced these changes in concentration. According to available literature, neither storage of meat and dairy products (Shantha *et al.*, 1994; 1995; Hur *et al*., 2004;

Martín *et al*., 2008b) nor a dynamic ripening period found in products like cheese (El-Salam & El-Shibiny, 2012) affected CLA concentration.

From this research it was concluded that the nutritional and health properties of a high fat meat product can be manipulated with CLA through both dietary and direct means. There was however major differences in the results from these two methods. When dietary supplementation was used, fat quality was improved although this negatively affected the nutritional profile of the fat. The chemical, microbial and sensory parameters remained largely unaffected and ripening parameters were somewhat improved. The actual CLA content of this product was far below the level that would give the product functional food status in terms of CLA content. When direct supplementation with a reduction in pork BF was used, the fat quality was negatively affected in that the final product had a higher content of UFAs which degraded the rheological properties of the product. This did however not seem to be noticeable to consumers and the high UFA level did not decrease lipid stability. Other chemical and microbial parameters were also not negatively affected. Direct addition of CLA in the form of Tonalin® showed that various levels of the RDA for CLA and a standard portion of salami with close to a 100% of the RDA could be formulated with relative ease and no negative effects on the production process (eg. ripening parameters).

FUTURE RESEARCH

In terms of the greater success achieved in using a direct method of CLA supplementation over dietary supplementation, research could continue on this method. Other more stable pre-emulsification methods such as the use of sodium alginate in the place of soya protein isolate could be investigated. A similar study to Chapter 4 involving the use of the other food grade form of Tonalin®, namely Tonalin® 60 WDP in a salami model system should be considered. This product differs from Tonalin® TG 80 in that it is a white to off-white powder prepared by spray drying Tonalin® TG 80 onto milk powder. This product may be more stable in a salami batter compared to the pre-emulsified oil form, however it only contains around 57% CLA with C18:2*c*9,*t*11 and C18:2*t*10,*c*12 in a 50:50 ratio. The product in powder form will most probably affect the salami in various chemical, microbial and rheological ways and there will be a need to establish if a 100% of the RDA for CLA could be reached using this product with a much lower concentration of CLA.

CHAPTER 6

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CHAPTER 7

SUMMARY/OPSOMMING

Summary

The consumption of meat is increasingly linked to various diseases and this has already affected the growth of this sector of the food industry in some countries. Pork is seen as one of the major contributors to this problem. The meat industry reacted by using strategies such as dietary supplementation and direct addition of healthier lipids to manipulate the nutritional value of meat. The positive effects of CLA on human health are well documented and various strategies have been successfully employed in increasing the levels of CLA in different animal models such as pigs and eventually pork products. The effects CLA may have on a fermented meat product like salami has not been studied yet. No research have been reported where it was attempted to increase the nutritional value of salami, maintain acceptable product quality and include a therapeutically high level of CLA with the belief that it will benefit human health.

In the first experiment of this study, 40 Duroc X Landrace gilts weighing on average 35 kg were randomly divided into two groups fed either a diet containing 0.5% sunflower oil (SFO) or a diet containing 0.5% conjugated linoleic acid (Luta-CLA® 60, BASF). These groups were further divided into two slaughter weight groups of ±70 kg and ±90 kg. After slaughter the lean meat and backfat from the loins of these animals were pooled by treatment group and utilized to manufacture salami. The aim was to determine if salami quality is influenced by slaughter weight and dietary supplementation of CLA. Both variables had major effects on the fatty acid composition and fatty acid ratios of the muscle and fat raw material as well as salami. The fatty acids and fatty acid ratios of technological importance were mostly positively influenced while the fatty acids and fatty acid ratios of nutritional and health concern were mostly negatively influenced by increased slaughter weight and dietary CLA supplementation. The microbial, physical, sensory and lipid stability parameters of salami were unaffected or inconsistently affected by both variables. Although dietary CLA was deposited successfully in muscle and fat, the deposition level was low. Consumption of a 28 g portion of salami manufactured from CLA enriched pork could only supply in 1% of the RDA for CLA. It could be concluded that although dietary supplementation of pork with CLA improved the technological properties of fat tissue it could not be considered a very successful approach to increase human consumption of CLA.

In the second experiment of this study the aim was to increase the CLA content of salami to three different percentages (25%, 50% and 100%) of the RDA for CLA per 28 g portion of salami. This was accomplished through the direct addition of CLA (Tonalin® TG 80) in a pre-emulsified form

with proportional decreases in the normally used pork BF content of the salamis. The salamis from these three treatment groups were then compared to a 100% pork BF control group for any possible effects on the microbial, physical and lipid stability parameters as well as fatty acid composition and fatty acid ratios. Microbial and sensory parameters were largely unaffected with varying effects on the physical and lipid stability parameters. Major effects on the fatty acid composition and fatty acid ratios of the salamis were observed. The partial replacement of pork BF and direct addition of CLA to salami proved to be an effective method of increasing CLA levels in salami in an attempt to improve the health aspects of salami to the point where it could be regarded as a functional food.

Keywords: dietary supplementation; direct addition; pork; conjugated linoleic acid; slaughter weight; backfat; sunflower oil; salami; stability; nutrition

Opsomming

Die inname van vleis word toenemend verbind met verskeie siektetoestande en dit het reeds gelei tot ‘n negatiewe uitwerking op die groei van hierdie sektor van die voedselindustrie in verskeie lande. Varkvleis word gesien as een van die hoof bydraers tot hierdie probleem. Die vleisindustrie het hierop reageer deur strategieë te ontwikkel, soos dieëtaanvulling en direkte toediening van meer heilsame lipiede om die voedingswaarde van vleis te verbeter. Die positiewe effekte van gekonjugeerde linoleïensuur (GL) op menslike gesondheid is goed gedokumenteer en verskeie strategieë is alreeds suksesvol aangewend om die vlakke van GL in verskeie diermodelle soos varke en varkprodukte, te verhoog. Die effekte wat GL mag hê op gefermenteerde produkte soos salamie is nog nie bestudeer nie. Nog geen navorsing is al gerapporteer waar daar gepoog is om die voedingswaarde van salamie te verhoog, aanvaarbare produk kwaliteit te handhaaf en ‘n terapeutiese hoë vlak van GL in te sluit met die doelwit dat dit menslike gesondheid sal bevoordeel nie.

In die eerste deel van hierdie betrokke studie is 40 jong sôe van ‘n Durok x Landras kruising in twee groepe verdeel. Een groep is ‘n dieët gevoer wat 0.5% GL ingesluit het en die ander groep is ’n dieët gevoer wat 0.5% sonneblomolie ingesluit het. Elkeen van hierdie twee groepe sôe is verder verdeel in een van twee slagmassas van ±70 kg of ±90 kg. Na slagting is die maervleis en rugvet van elk van die vier groepe gebruik in die vervaardiging van salamie. Die doel was om vas te stel of salamie kwaliteit deur slagmassa en dieëtaanvulling van GL beïnvloed word. Beide veranderlikes het ‘n groot effek gehad op die vetsuursamestelling van die spier en vet roumateriaal sowel as die salamie. Die vetsure en vetsuurverhoudings van tegnologiese belang is meestal positief beïnvloed terwyl die vetsure en vetsuurverhoudings van voedings- en gesondheidsbelang

meestal negatief beïnvloed is deur slagmassa en dieetaanvulling van GL. Die mikrobiese, fisiese, sensoriese en lipiedstabiliteit van die salamie is baie min deur beide veranderlikes beïnvloed. Hoewel dieëtaanvulling gelei het tot die suksesvolle deponering van GL in spier en vet, was die neerleggingsvlak baie laag. Die inname van ‘n 28 g porsie van salami van GL verrykte salami kon net in 1% van die aanbevole daaglikse inname (RDA) van GL voldoen. Daar is tot die gevolgtrekking gekom dat hoewel die dieëtaanvulling van varke met GL die tegnologiese eienskappe van die vetweefsel verbeter, dit nie beskou kan word as ‘n baie suksesvolle benadering om die menslike inname van GL te verhoog nie.

In die tweede deel van hierdie studie was die doel om die GL inhoud van salamie te verhoog tot drie verskillende persentasies (25%, 50% en 100%) van die RDA per 28 g porsie salamie. Dit is teweeg gebring deur die direkte byvoeging van GL (Tonalin® TG 80) in ‘n vooraf ge-emulsifiseerde vorm met ‘n proporsionele verlaging van die rugvet inhoud van die salamie. Die salamie van die drie GL verrykte groepe is dan vergelyk met ‘n 100% rugvet kontrole vir enige moontlike effekte op die mikrobiese, fisiese, lipiedstabiliteit en vetsuursamestelling. Mikrobiese en sensoriese eienskappe was grootliks nie geaffekteer nie met variërende effek op die fisiese en lipiedstabiliteit parameters. Verhoogde GL inhoud het ‘n baie groot effek gehad op vetsuursamestelling en vetsuurverhoudings. Die gedeeltelike vervanging van rugvet en direkte toevoeging van GL by salamie is ‘n doeltreffende metode om GL vlakke in salamie te verhoog tot ‘n vlak waar salamie beskou kan word as ‘n funksionele voedsel.

Sleutelwoorde: dieëtaanvulling; direkte insluiting; varkvleis; gekonjugeerde linoleïensuur; slagmassa; rugvet; sonneblomolie; salami; stabiliteit; voedingswaarde