SIGNIFICANCE OF LIPIDS IN FERMENTED MEAT TECHNOLOGY

by

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This thesis is dedicated to my parents
Esther Baloi
and the late
Motlhasedi Eweditswe Madisa.

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LIST OF ABBREVIATIONS

A_W	Water activity
BHT	Butylated hydroxytoluene
CLA	Conjugated linoliec acid
COP	Cholesterol oxidative products
DBI	Double bond index
DM	Dry matter
ERH	Equilibrium relative humidity
FAA	Free amino acids
FAME	Fatty acids methyl esters
FFA	Free fatty acids
FFDM	Fat.free dry matter
GDL	Glucono Delta Lactone
GRAS	Generally Regarded As Safe
ID	Internal diameter
IV	Iodine value
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
MAP	Modified atmosphere packaging
ND	Not detected/ not determined
NPN	Non-protein nitrogen
PV	Peroxide value
PTN	Phosphotungstic acid soluble nitrogen
PUFA	Polyunsaturated fatty acids
RH	Relative humidity
SC	Saturated carbonyls
SFA	Saturated fatty acids
SSN	Salt soluble nitrogen
TBA	2-Thiobarbituric acid
TBRS	2-Thiobarbituric acid reactive substances
TC	Total carbonyls
UC	Unsaturated carbonyls
VP	Vacuum packaging

WSN	Water soluble nitrogen

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Sausage

You may brag about your breakfast foods you eat at break of day.
Your crisp, delightful shavings and your stack of last year's hay.
Your toasted flakes of rye and corn that fairly swim in cream.
Or rave about a sawdust mash, an epicurean dream.
But none of these appeal to me, though all of them I've tried—
The breakfast that I liked the best was sausage mother fried.

Old country sausage was its name; the kind, of course, you know.
The little links that seemed to be almost as white as snow.
But turned into a ruddy brown, while sizzling in the pan;
Oh, they were made both to appease and charm the Inner man.
All these new-fangled dishes make me blush and turn aside.
When I think about the sausage that for breakfast mother fried.

When they roused me from my slumbers and left me to do the chores.
It wasn't long before I breathed a fragrance out of doors.
That seemed to grip my spirit, and to thrill my body through.
For the spice of hunger tingled, and 'twas then I plainly knew
That the gnawing at my stomach would be quickly satisfied
By a plate of country sausage that my dear old mother fried.

There upon the kitchen table, with its cloth of turkey red,
Was a platter heaped with sausage and a plate of home-made bread.
And a cup of coffee waiting—not a puny demi-tasse
That can scarcely hold a mouthful, but a cup of greater class;
And I fell to eating largely, for I could not be denied—
Oh, I'm sure a king would relish the sausage mother fried.

Times have changed and so have breakfasts; now each morning when I see
A dish of shredded something or of flakes passed to me,
All my thoughts go back to my boyhood, to the days of long ago,
When morning meat meant something more than vain and idle show.
And I hunger, oh, I hunger, in a way I can not hide
For a plate of steaming sausage like the kind my mother fried.

Edgar A. Guest ("Just Folks". 1917. Reilly & Lee Co.).

CHAPTER 1

1: INTRODUCTION

Sausage is ground seasoned meat in a tubular case of thin skin (Hawker and Cowley, 1995), while "sausage" is said to be an ancient word in many languages (Lissner, 1939). Wurst is an Indo-Germanic word probably derived from Latin, meaning "to turn" or "to twist". Sausage is also known as Kolbasa in Salvic, derived from Hebrew, meaning "all kinds of meat". Leistner (1986a) goes on to say, that the origin of the Danish or Scandanavian word polse is not known, but its likely to have been derived from the Latin word pulvinus, meaning a "cylindrical pillow". Likewise, the origin of the word salami seems uncertain and there are various explanations offered on its origin: Salami is believed to be named after the city of Salamis on Cyprus, destroyed more than 2000 years ago (Bacus, 1984; Anon, 1990). It is also said that it is derived from Latin, simply meaning, "salt".

There are various references about the origin of fermentation as a method to preserve and enhance the sensory properties of foods. The production of fermented sausages is thought to have originated in the countries surrounding the Mediterranean Sea about 1500 BC (Adams, 1986; Anon, 1990). According to Roca and Incze (1990), the natural climate of these countries (including some adjourning areas of Europe) is suitable for the production of dry sausage, e.g. northern Italy, some areas of Hungary and Tessin (Switzerland) have had a reputation for producing high-quality dry sausages for centuries. Chinese sausages are said to have been known in the Chinese North and South Dynasties around 589-420 BC, although these sausages were not fermented or non-fermented and were intended to be consumed hot (Leistner, 1986a). Soy sauce has been documented to have been an ingredient in Chinese fermentations about 3000 years ago (Whitaker, 1978), while sausage is known to have been prepared and consumed by Babylonians about 1500 B.C. (Kinsman, 1980). However, the art of sausage production can in most cases be traced back to southern Europe, from where it first spread to other European countries (Zeuthen, 1995). According to Leistner (1986b), the most well known cured fermented German sausages were probably first produced by Italians about 250 years ago, while Hungarian salami is not more than 150 years old. European emigrants are said to have established fermented sausage production in the USA, South America, and Australia, and the knowledge relating to fermented sausages in the Seychelles, the Philippines and Papua New Guinea is said to be largely a result of European influences (Adams, 1986).

Various regions have developed their own types of sausage according to local climate, temperature, types of spices and meat used, and storage conditions (Anon, 1990). Intensive research in sausage fermentations was, however, not initiated until the traditional empirical methods of manufacture no longer met the requirements of large-scale, low-cost industrial production with short ripening times and highly standardised products. Apparently this happened in the 1930's and the 1950's in USA and Europe respectively, when the first systematic studies on the microbiology and production of fermented sausage were first published (Lücke, 1985). Currently, starter cultures contribute a great deal to the safety of the processing of dry sausage as well to the improvement of the flavour and colour of the product (Roca and Incze, 1990). Fermented sausages and fermented whole meat products are an important part of many diets throughout the world, providing valuable protein, fat nutrients and desirable flavours, over and above the considerable extended shelf life of perishable meat from days to weeks and months, even at warmer temperatures (Campbell-Platt, 1995).

The aim of this study is to establish the lipid stability of salami under three different storage temperatures (4, 12 and 25 ^{0}C) in order to find the most suitable storage temperature for salami in South Africa. Using the suitable storage temperature and lipid stability, the effect of incorporating polyunsaturated fatty acids (PUFA) into pig feed on lipid deterioration will be established as will the viability of replacing pork backfat with other unconventional fat sources (ostrich and sheeptail) in salami manufacture.

CHAPTER 2

2: LITERATURE REVIEW

2.1 INTRODUCTION

According to Campbell-Platt (1987), "a food is fermented if it has been subjected to the action of microorganisms or enzymes, so that desirable biochemical changes cause significant modification of the food". Fermentation is known and has been associated with foods like beer, wine, bread, a variety of milk products, sauerkraut, meat products, soya and rice products of Asia (Lücke and Hechelmann, 1987; Anon, 1990). Fermentation that is normally used in conjunction with smoking and drying in meat products is the oldest preservation method known to man (Bacus, 1984; Campbell-Platt, 1995; Incze, 1998). However, the production of fermented sausages (Anon, 1990; Roca and Incze, 1990), is likely to have originated through chance rather than any scientific knowledge, when people learned that meat would not spoil if it was finely chopped, mixed with salt and spices and dried in rolls. For sausage fermentation to be effective, it has to meet two basic requirements, it should not only improve on the keeping quality of the product, ensuring a long shelf life, but it should also assure good sensory characteristics such as excellent flavour, taste and consistency (Lücke and Hechelmann, 1985; Anon, 1990; Roca and Incze, 1990).

Fermented sausages are ground meat, mixed with salt and curing agents, stuffed into casings and subjected to a fermentation process in which microorganisms play a crucial role (Lücke, 1985; Anon, 1990; Roca and Incze, 1990; Lücke, 1994). A typical fermented dry sausage is basically 60-70 % raw lean meat of a single or a mixture of species plus 30-40 % fat tissue, chopped into small pieces, mixed with about 2-3 % salt, curing agents (nitrate and /or nitrite), some sugar, spices and or seasoning, then stuffed into casings (Lücke, 1994). Fermentation and ripening (drying) are either done naturally under the prevailing climatic conditions or in climatised chambers to ensure preservation without heat treatment (Bischoff, 1982; Lücke, 1994). The choice of meat used depends on eating habits, religious beliefs and meat prices (Lücke, 1985; Anon, 1990).

Traditionally, fermentations were dependent on the chance action of "wild" microorganisms. These are the desirable "natural flora" of a food, which over a period of time build up on the equipment, shelves, tables and air in the processing room (Hierro et al., 1997). These desirable organisms, or "house flora", grow and thrive in the food and produce metabolites, harmful and suppressive to the growth of the competing undesirable spoilage and pathogenic organisms (Anon, 1990; Lawrie, 1995). In order to control and speed up the fermentation process, batches were inoculated with residues from previously matured mixes using a technique called "back slopping" (Bacus, 1984; Lücke, 1985; Campbell-Platt, 1995). However, in large-scale productions where low-cost production with short ripening times, and highly standardised product quality were imperative, this technique became less applicable and the starter culture concept was conceived. The use of starter cultures brought an acceleration of the carbohydrate breakdown process, shortened ripening periods (Fernandez et al., 1995), and led to an increase on finished product uniformity, a reduction in losses due to spoilage and an improvement on flavour and product safety (Lücke, 1985; Anon, 1990).

2.2 Classification of sausages

Sausages can be classified in a variety of ways. Kinsman (1980) classified sausages in six categories, namely, fresh sausage, cooked sausage, cooked smoked sausage, uncooked smoked sausage, dry and or semi-dry sausages and meat specialities. Fermented sausages can be classified according to the raw material used (Table 1), i.e. meat type and fat content (Campbell- Platt, 1995; Lücke, 1994), weight loss during processing (degree of drying), final moisture content, or moisture loss variations brought about by meat tissues used. Further classification of fermented sausages is on the basis of a linear relationship between percentage moisture and the product's water: protein ratio (Acton and Dick, 1976; Lücke, 1985; Zeuthen, 1995), which has to be approximately 1 (one) for dry sausages (Garcia de Fernando and Fox, 1991). In Germany, fermented sausages are classified on the basis of the collagen content: and free protein ratio (Lücke, 1994). Anon (1990) categorises fermented sausages as dry and semi-dry fermented sausages. According to him, semi-dry fermented sausages (e.g. summer sausage, cervelat, chorizo, mortadella) have short processing/ageing periods (3 days to 3 weeks), high water activity (a_w), 40-60 % water content, are mildly spiced and smoked, highly perishable, and therefore require refrigeration. Dry fermented sausage e.g. Milano Genoa salami, are aged for several months, are relatively dry, heavily spiced and smoked, have a low a_w and moisture content (< 40 %) and should subsequently be shelf stable.

Roca and Incze's (1990) classification scheme (Table 2) includes fermentation period as a variable and presents fermented dry sausages as spreadable or sliceable sausages. According to them, spreadable sausages are finely ground, have a short fermentation period (3-5 days), contain $NaNO_2$ as a curing agent, the final product has about 35-42 % moisture and a_w of around 0.95-0.96.

Table 1: Parameters influencing the quality of fermented sausages (Lücke, 1994)

Parameter	Variable	Guidelines[a]
Raw material	Animal species (beef/pork/poultry)	pH ≤ 5.8: good microbial quality: no antibiotics
	Age at slaughter	
	Fats/oils in pig feed;	
	Types of fatty tissue (back/belly	No soft or rancid fat
	Formulation (fat content)	
Additives	Sodium chloride	Initial a_w 0.955-0.965
	Curing agent (nitrite/nitrate)	Addition of 100 mg $NaNO_2$/kg[b]
	Sugar amount	0.2-0.7 %
	Sugar type (glucose/sucrose /lactose/dextrins)	0.2-0.5 % of rapidly fermentable sugar
	Lactic acid bacteria	pH reduction to ≤ 5.3 during fermentation[c]
	Acidulants	
	Micrococcaceae	
	Ascorbate	
	Spices	
Comminution	Method (grinder/cutter)	Low temperature (to avoid melting of fat)
	Degree (coarse/fine)	
Filling	Filling equipment	No air inclusions
	Casing material (natural/ Collagen based/ cellulose-based)	Permeability high for vapour and smoke, low for oxygen: shrinkable, peelable
	Casing diameter	
Ripening	Fermentation climate	No air inclusions
	— temperature	≤ 25 °C
	— time	Until pH ≤ 5.3
	— humidity (% ERH)	No vapour condensation; ERH in chamber 5-10 units below ERH of product
	Ageing/drying climate	
	— temperature	≤ 15 °C until a_w < 0.90
	— humidity (% ERH)	ERH in chamber 10-15 units below ERH of product
	— air movement	Uniform drying
	— time	
Surface treatment	Smoke	No growth of undesired moulds
	Mould starter	

[a] Certain deviations are possible if proper precautions are taken (e.g. low ripening temperature).

[b] Lower amounts require lower fermentation temperatures or faster acid formation: use of nitrate instead of nitrite requires lower fermentation temperature. [c] Necessary rate depends on fermentation temperature.

They emphasised that raw material of high quality, containing correct amounts of added carbohydrates, having been subjected to low smoking and storage temperatures, with a high relative humidity and an inclusion of some additives is necessary for minimising consistency and colour retention problems. Acidulants or organic acids may be added for the desirable rapid pH decline.

Sliceable sausages have a coarse consistency and a short or longer ripening period. Short ripened sliceable sausages with a final water content of between 30 and 40 %, a_w value around 0.92-0.94, contain $NaNO_2$ or KNO_3 as curing agents, mono or disaccharides and acidulants like Glucono Delta Lactone (GDL). *Micrococcus* strains were apparently necessary as starter cultures when KNO_3 was used as a curing agent. The purpose of these starter cultures was to enhance extension of shelf life, and provide good sensory and microbial qualities. Subsequently the use of lactic acid bacteria as starter has resolved the problem and eliminated the need for using GDL (Roca and Incze, 1990).

Table: 2 Classification of fermented sausages (Roca and Incze, 1990)

Product type	Period of fermentation	Final water content	Final a_w content	Examples
Spreadable (fine mix)	3-5 days	34-42 %	0.95-0.96	German Teewurst frische Metwurst
Sliceable				
Short ripening	1-4 weeks	30-40 %	0.92-0.94	Summer sausage
Long ripening	12-14 weeks	20-30 %	0.85-0.86	Hungarian salami, Italian salami, French saucisson

Sliceable dry fermented sausages like some types of salami can be ripened for as long as 14 weeks (Table 2). Ripening time is a function of casing diameter, while $NaNO_2$ and or KNO_3 are incorporated to maintain the consistent levels of nitrite necessary for keeping microbial populations at a low level. Fermentation and ripening are done at low temperatures (6 and 15 ^{0}C), resulting in a final product that has a_w of 0.85-0.90 and 20 to 30 % water content. At these moisture and a_w levels the product is relatively shelf- stable ((Leistner and Roedel, 1975).

According to Roca and Incze (1990), the manufacture of fermented dry sausages occurs in three main phases, namely selection of raw materials (including slaughtering and de-boning of meat), sausage "emulsion" preparation and stuffing, smoking and ripening and/or drying. The manufacturing steps are explained as, formulation (mixing and cutting of ingredients), fermentation and ripening (Diaz et al., 1993; Fernandez et. al., 1995),

Since the quality of the final product is highly dependent on the initial quality of the raw materials (Table 1), good manufacturing practices should be used throughout sausage preparation. Wholesome meat free from clots and fat of high bacterial quality, plus good quality dry ingredients are a necessity (Bacus, 1984; Lücke, 1994). A pH of between 5.6-6.0 (not exceeding 6.3) is critical for pork, and the fat should be fresh and not oxidised (Roca and Incze, 1990). To keep the temperature of the mix low during chopping (Table 1), the fat and meat should be frozen to $- 8\ ^{0}C$ and $-4\ ^{0}C$ respectively (Roca and Incze, 1990), or "preconditioned" to yield meat temperature of between -4.4 to $-2.2\ ^{0}C$ prior to breaking, grinding and chopping (Bacus, 1984). This is also essential for proper drying, because excessive friction of the raw materials causes smearing of the fat which then forms a film over the lean parts, creating a barrier that impedes moisture loss from the meat surface (Bacus, 1984; Roca and Incze, 1990). According to Campbell-Plat, (1995), salt used was originally not pure sodium chloride but contained sodium or potassium nitrate. This was so because fermented sausages produced with sodium chloride alone developed a grey centre and quickly became rancid (Leistner, 1995). Currently NaCl, $NaNO_2$ or KNO_3 are used in combination in dry sausage formulations. The nitrate helps to preserve the pink curing colour and contributes to direct curing by reduction to nitrite. At least 100 mg $NaNO_2$ /kg should be added to achieve this purpose (Table 1). Seasoning and spices provide flavour and aid safe fermentation by inhibiting undesirable or pathogenic microorganisms (Campbell-Platt, 1995). This is especially the case during the early stages of the process when the other inhibitory "hurdles" like pH are not fully developed (Leistner, 1995). Spices also have an antioxidant effect (Campbell-Platt, 1995).

The type and amount of carbohydrate determines the rate and extent of lactic acid formation and the sausage microflora. Monosaccharides are preferred over disaccharides and polysaccharides (Table 1). This is because monosaccharides are readily available for hydrolysis and lactic acid production leading to a decline in pH. Di- and polysaccharides have to be converted by the microorganisms into monosaccharides before they can be available for fermentation, and some disaccharides are also not readily converted (Anon, 1990; Roca and Incze, 1990; Lücke, 1994). The use of sugars is critical to accelerate the pH drop necessary for colour, aroma and safety of the product in short-period ripened sausages (Roca and Incze, 1990). Either over-filling or under-filling of the casing should be avoided since they are both detrimental to product quality. Very little oxygen should be incorporated, and vacuum stuffers are highly recommended (Table 1). Smearing should be avoided and product stuffing temperature of between -2.2 and $+1.1\ ^{0}C$ (Bacus, 1984) and/ or between 0 and

1 ^{0}C (Roca and Incze, 1990) is recommended, as higher temperatures can also cause smearing (Bacus, 1984; Roca and Incze, 1990; Lücke, 1994), which may later cause drying and rancidity problems (Lücke, 1985).

Fermented dry sausage preservation is accomplished not by a single factor, it is rather achieved through the "hurdle effect", i.e. an interaction or synergistic effect of a series of factors or hurdles (Leistner, 1995). Factors such as the competitive flora (natural from meat substrate and/ or starter culture), added salt, sugar, nitrite and/ or nitrate, the decrease in redox potential, pH (acidity) and a_w, combined fulfil the task of dry sausage preservation (Roca and Incze, 1990; Leistner, 1995). According to Leistner (1995), the addition of nitrite to the sausage mix is very important at the start of fermentation for the microbial stability of the product (especially for the inhibition of *Salmonella*), since the other preservation hurdles are not fully developed at this stage of processing.

Oxygen from the air is incorporated into the sausage batter during chopping and mixing, resulting in a high redox potential. Nevertheless, growth of bacteria (aerobic fermentation), which sets in at the start of fermentation causes a reduction in redox potential. This low redox potential, enhances the bactericidal effectiveness of nitrite, inhibits aerophilic bacteria (present in the raw meat which could otherwise initiate putrefaction) and offers a selective advantage to lactic acid bacteria.

Lactic acid bacteria suppress undesirable pathogenic and spoilage microorganisms, e.g *Listeria*, *Salmonella* and pathogenic staphylococci by producing lactic acid responsible for the reduction in pH (Leistner, 1995). The initial pH has an influence on the growth of pathogens, especially if the raw material pH was optimal for lactic acid bacteria such as *Lactobacillus plantarum, Pediococcus pentosaceus* etc. The ultimate pH of dry fermented sausage which is a factor of added sugars and ripening temperature (Leistner, 1995), is very important for the keeping quality of sausage. The low pH of fermented sausages is not only important bacteriologically, but it also enhances the drying speed, because water evaporation is easier around the isoelectric point of meat proteins (pH 5.4-5.5), corresponding to the lowest water binding capacity of the product (Wismer-Pedersen, 1971; Roca and Incze, 1990).

The growth and metabolic activity of microorganisms and their survival are highly dependent on the a_w of the substrate. Microbial spoilage, food poisoning and fermentation takes place if the a_w of the substrate is favourable for the growth and metabolic activity of the microorganisms present (Roca and Incze, 1990). The a_w of fermented sausages continues to fall throughout the ripening period (Baumgartner et al., 1980; Fernandez et al., 1995; Leistner, 1995; Papadima and Bloukas, 1999).

The extent and rate of a_w decline depends on the porosity of the sausage mix, sausage diameter, amount of fat in the sausage, ripening temperature and the relative humidity (RH) of the ripening chamber relative to time (Baumgartner et al., 1980; Bacus, 1984; Stiebing and Roedel, 1988; Leistner, 1995; Papadima and Bloukas, 1999).

Table 3 lists microbial species commonly used as starter cultures for fermented sausages. The role these organisms play in sausage fermentation is also outlined.

Table 3: Microorganisms as starter cultures for sausage fermentation (Lücke et al., 1990)

Microbial group	Species used as starter[a]	Useful metabolic activity	Benefits to sausage fermentation
LAB	*L. plantarum*, *L. pentosous*, *L. sake*, *L. curvatus*, *P. pentosaceus*, *P. acidilactici*.	Formation of lactic acid	Inhibition of pathogenic and spoilage bacteria. Acceleration of colour formation and drying.
Catalase-positive cocci	*S. carnosus*, *S. xylosus*, *M. varians*	Nitrate reduction and oxygen consumption Peroxide destruction Lipolysis?	Colour formation and stabilisation. Delay in rancidity. Aroma formation. Nitrate reduction. Removal of excess nitrate.
Yeasts	*D. hansenii*	Oxygen consumption Lipolysis	Delay rancidity. Aroma formation.
Moulds	*P. nalgiovense* biotypes 2,3,6	Oxygen consumption Peroxide destruction Lactate oxidation Proteolysis Lipolysis?	Colour stability. Delay rancidity. Aroma formation. Aroma formation. Aroma formation.

[a] Abbreviations: *D.*, *Debaryomyces*; *L.*, *Lactobacillus*; LAB., Lactic Acid Bacteria; *M.*, *Micrococcus*; *P.*, *Pediococcus*; *S.*, *Staphylococcus*; *P.*, *Penicillium*.

Microorganisms are added to meat products (Table 3) as starter cultures to ensure product safety, shorten fermentation time, extend shelf life and for a unique product quality and consistency (Roca and Incze, 1990). Microorganisms employed as starter cultures should therefore comply with certain requirements. They must be salt tolerant, grow in the presence of at least 6 % sodium chloride (based on water content) and at least 100 mg/kg sodium nitrite. They should also have a growth temperature range of between 15 and 40 ^{0}C, with an optimum at 30 ^{0}C, homofermentative (no CO_2 production), non-proteolytic and not harmful to humans (Roca and Incze, 1990). According to Jessen (1995), starter cultures should be GRAS (Generally Regarded As Safe) approved, being free from any chemical or microbial impurities that may cause health risks. They should in addition possess activities that control fermentation (acidification) and contribute positively to product colour and flavour development as well as increased product stability. The strain should be phage resistant in order to perform optimally during fermentation, not give rise to metabolites that inhibit or prevent manufacture and should consist of strains that are phenotypically and genetically stable.

2.3 Changes occurring during dry fermented sausage production

The fermented sausage meat undergoes a variety of changes during fermentation, ripening and or drying. These physical changes include changes in consistency (firmness), pH, colour development, fat content and decreases in weight, a_w and moisture. All these changes as well as proteolysis, glycolysis, lipolysis and lipid oxidation, are directly responsible for the final quality of the product (Roca and Incze, 1990).

2.3.1 Physico-chemical changes

Fermented sausages lose weight during fermentation and ripening due to dehydration. As a result the dry matter (DM), fat content, protein content and salt content increases while the moisture content decreases (Papadima and Bloukas, 1999). Demeyer et al. (1974) reported a 15 to 20 % increase in DM during the ripening of a dry fermented sausage. They observed a crude fat content higher than the total fatty acid content and attributed this partly to the presence of glycerol and non-fatty acid containing lipids such as cholesterol in the crude fat. Samelis et al. (1993) observed a final weight loss of 23.7 and 32.3 % in the coarse and finely ground sausages. The coarsely ground sausage had a higher drying rate, indicating that under the same drying conditions, water migrated more easily from the core to the surface, with very little interference from the large fat particles (Samelis et al., 1993). The DM increased from 48.4 to 80.7 % and 50.2 to 73.9 %, in coarse and finely ground products respectively, while the fat increased from 32.3 to 53.5 % in coarsely ground, and 36.7 to 52.7 % in the finely ground sausage. The fat content was not significantly different between the two batches.

When lipase is added a decrease in the water content of dry fermented sausages takes place during ripening (Fernandez et al., 1995). Moisture content of the sausages decreased from about 60 % on the fresh product to final values of between 40 and 45 % for all batches. Products with a shorter ripening period (14 days) experienced the least moisture loss, indicating that the rate of dehydration was higher during the last stages of ripening. Due to this water loss, the sausages lost 15 to 20 % of their weight, indicating maturity, and the total fat content increased relative to water loss throughout ripening. From an initial fat content of 23 % (w/w per 100 g raw sausage), the final product contained 35 to 39 % fat, an equivalent of 59 to 63 % fat in the DM (w/w per 100 g dry sausage). However, the batches with 250 and 500 lipase units had lower final fat levels (30 %: or 59 % DM). This phenomenon could possibly be attributed to the loss of polar glycerides (probably monoglycerides resulting from the high lipolytic activity observed in these batches), in the aqueous phase during lipid extraction (Fernandez et al., 1995). Zalacain et al. (1995) also observed non-significant differences in the total fat content (% DM) of ripened dry fermented sausages with starter or lipase from *Candida cylindracea*. Molly et al. (1996) reported a 17 to 25 % water loss, irrespective of the differences in addition of glucose, antibiotics and micrococcus inoculum in Belgian sausages. They reported slight non-significant increases in DM and crude fat (ethyl ether extract) between the treatments, with a crude fat content ranging between 32 and 36 % across the different experiments, after 21 days of ripening. Traditional Greek sausages lost weight on drying, and the fat level, storage conditions and ripening period had a significant effect on weight loss (Papadima and Bloukas, 1999). Weight loss was found to be dependent on temperature, RH of the ripening room, air speed, ripening time, degree of comminution of meat, sausage diameter and the amount of fat in the sausage (Roedel and Klettner, 1981; Roedel, 1986; Stiebing and Roedel, 1987). Papadima and Bloukas (1999) reported that weight loss was significantly affected by the interactions between fat level and storage conditions, and fat level and storage time. The lower the fat level, the higher ($p < 0.05$) the weight loss and weight losses increased in a linear pattern relative to storage time. Sausages with 10 and 20 % fat levels were affected by storage conditions. At 20 % fat level, a 20 % weight loss was realised after 3 days storage in the ripening room (13-15 ^{0}C, 85-95 % RH, 0.1ms^{-1} air speed), while the same weight loss was realised after 12 days for cold stored sausages (3-7 ^{0}C, 65 to 75 % RH). The ripening room with controlled climatic conditions, viz. higher temperature and regulated air speed diminished the time necessary to attain a predetermined weight loss. The higher weight loss of the ripening room sausages has also been attributed to their lower pH values (5.0 to 5.3 after 7 days of storage), which is around the isoelectric point of meat products (pH 5.4-5.5) coinciding with the lowest water holding capacity of the product (Wismer-Pedersen, 1971). Weight loss was negatively correlated with pH ($r = -0.639$, $p < 0.001$).

A_w, defined as the ratio of the equilibrium of water vapour over a system and the vapour pressure of water at the same temperature (Roca and Incze, 1990), or free water available for microbial or chemical reactions in a substrate, influences the growth and metabolic activity of microorganisms. The a_w of meat and meat products is in the range of 0.99 to 0.70, and meat products have a lower a_w than fresh meat and therefore a better shelf life (Leistner and Roedel, 1975). The low a_w of processed meat products is affected by many variables. These include, the degree of comminution, ripening period, withdrawal or addition of water, addition of sodium chloride or other salts, addition of fat or fat content, casing permeability, temperature and the RH of the air (Roca and Incze, 1990; Cook, 1995). According to Wirth (1988, 1989), the granulated fat in non-cooked sausages helps to loosen the sausage mixture, aiding the continuous migration of moisture from the inner layers of the product; a process necessary for undisturbed fermentation and aromatisation. The a_w of lipase treated dry fermented sausages decreased gradually throughout ripening, from 0.97 on the raw sausage mix to between 0.90 and 0.92 and 0.86 and 0.87 for the 14 and 28 days ripened products respectively (Fernandez et al., 1995). There was no significant observed effect on the a_w brought about by the addition of lipase. Dry fermented sausages inoculated with single strains or a mixed culture of lactobacilli, staphylococci and micrococci and a sterile control batch caused a reduction in a_w from 0.97 on day zero of ripening to final values of 0.85-0.86 after 50 days of ripening (Hierro et al., 1997). They reported a gradual and constant loss in water content, from about 63 % in the fresh sausage to a final value of 25-30 % moisture content on the sausage after drying. The fat content was similar for all treatments, culminating in a final value of about 53 % DM. No adverse variations in moisture content, fat content and a_w as a result of the presence or absence of a starter inoculum were observed. Due to weight loss and the resultant increase in salt content during storage, the a_w of traditional Greek sausages (refrigerator or ripening room), decreased during storage (Papadima and Bloukas, 1999). The decrease in a_w was significantly affected by storage condition ($p < 0.05$), storage period ($p < 0.001$), and the interaction of fat level and storage period ($p < 0.01$). A_w was inversely proportional to fat level. Nevertheless the sausage with 30 % fat still had a high a_w ($a_w > 0.95$) after 14 days, which made it highly perishable, while the 10 % fat ripening room stored sausage had an a_w value of below 0.9 and a pH value of around 5.0, rendering it stable at ambient temperature.

According to Demeyer et al. (1974), the production of organic acids is primarily responsible for the pH decrease in fermented products observed during fermentation and the early stages of ripening, however this trend is counterbalanced by the production of ammonia during the late stages of ripening. It is said that this latter increase in dry sausage pH could also be a result of the increased concentration of buffering substances and the decreased dissociation of the electrolytes already present in the sausage meat (Demeyer et al., 1979). Similar results were reported by Lois et al. (1987) in "chorizo" manufactured with and without addition of sugars, by Astiasaran et al. (1990) in "chorizo", "saucisson" and "salami", and by Samelis et al. (1993) in coarsely and finely ground Greek dry sausage without starter culture. A relatively high initial pH (6.1) was observed on both batches of the Greek sausage. In the finely ground batch pH decreased to 5.5 by the 4th day and to a final value of 5.4. The pH of the coarsely ground batch however increased on further ripening, a factor they attributed to ammonia and amine production during the late stages of this phase. Lois et al. (1987) reported a drop in pH from 5.9 to 4.8 and 5.3 for the batch with and without added sugars respectively, followed by a gradual increase in pH in both batches during ripening. Fernandez et al. (1995) reported a drop in pH during fermentation and the early stages of ripening followed by stabilisation or marginal changes during the last stages of the process. However, there were no significant differences between the control batches and the pancreatic lipase treated sausage containing less than 200 lipase units. The batches with 200 and 500 lipase units resulted in significantly higher final pH values. A significant difference in final pH of fully ripened dry fermented sausage was observed between lipase treated sausage (final pH value of 5.02), and the other sausage containing a starter (final pH of 4.71) (Zalacain et al., 1995). According to Montel et al. (1993) bacterial starter culture combinations had a significant influence on the drop of pH during dry fermented sausage ripening. The pH of the non-inoculated control batch was significantly lower than that of inoculated batches at the end of fermentation, and the pH of the non-inoculated control sausages remained relatively constant throughout ripening. Sausages inoculated with *Pediococcus* species showed very little change in pH, while sausages containing *Lactobacillus sake* exhibited the highest pH drop, more significantly in the later stages of ripening.

Consistency or texture is a function of moisture content or a_w and pH. A pH value below 5.40 (necessary for protein solubilisation) or a_w below 0.9, are said to be critical for a positive change in consistency (Roca and Incze, 1990).

The true cured colour of dry fermented sausages is attributed to nitrosyl-haemoglobin and nitrosyl-myoglobin (Roca and Incze, 1990). According to Papadima and Bloukas (1999), fat level and storage time significantly affected the lightness (L*), redness (a*), and yellowness (b*) of fermented Greek dry sausages. The interaction of fat level and storage time had a significant effect on redness (a*) and yellowness (b*) of fermented sausages ripened at 13 to 15 ^{0}C (85-95 % RH, 0.1ms^{-1} air speed) and at 3 to 7 ^{0}C (65–75 % RH). Storage conditions had a significant effect ($p < 0.001$) only on yellowness (b*), indicating that ripening room stored sausages were more yellow, and this effect was more pronounced in sausages with 10 and 20 % fat levels. The higher the fat level, the higher the L* value, which implied that the product with more fat was lighter. Papadima and Bloukas (1999) nevertheless observed a significant decrease ($p < 0.05$) in L* values around the 3rd day of storage, a factor they explained as sausage darkening due to weight loss. High correlations were observed between lightness and weight loss (r = (0.859, $p < 0.001$) and yellowness and weight loss (r = (0.863, $p < 0.001$). Increases ($p < 0.05$) in Hunter a* values were observed at all storage conditions during the first 3 days, a factor attributed to the formation of nitrosyl-myoglobin. Nitrosyl-myoglobin is a product of the reaction between myoglobin and nitrites under mildly acidic conditions, conditions that prevail in nitrate and or nitrite added fermented sausages during fermentation and the early stages of ripening. However, a decrease in Hunter a* values was observed on the 10 % fat level sausages, especially those stored in the ripening room. This was explained to be a possible result of oxidation of nitrosyl-myoglobin, accelerated by the increased salt content of the product and/ or the pro-oxidant effect of the salt (Savic and Savic, 1996).

2.3.2 Biochemical reactions responsible for the flavour of a fermented sausage

The development of flavour in meat products is a very complex process, due to the high number of reactions involved (Table 4). According to Toldra (1998), flavour compounds are generally a resultant of either enzymatic or chemical reactions such as lipid oxidation, Maillard reactions and others. The fermentation process of dry sausages is based on the interaction between meat, fat, bacterial growth, physico-chemical phenomena and biochemical processes. Taste and flavour of dry sausages are due to products originating from fermentation of carbohydrates (glycolysis), proteolysis, lipolysis, lipid oxidation, spices and curing salts (Demeyer et al., 1986; Verplaetse, 1994; Dainty and Blom, 1995; Campbell-Platt, 1995; Navarro et al., 1997). According to Dainty and Blom (1995), fermented sausage flavour is to a greater or lesser extent brand specific. It is further complicated by the influences from interactions between the smoke, salt, nitrate and nitrite and their decomposition products, as well as bacterial fermentations and the enzymes inherent in the meat (Table 4). The inherent dry sausage flavour is the result of a complex equilibrium between volatile compounds originating from the meat, smoke and seasoning (Johansson et al., 1994). Proteolysis and lipolysis constitute the main biochemical reactions in the generation of flavour or flavour precursors, the reactions being due to proteases and lipases, respectively. The degree of contribution of either endogenous enzymes or those of microbial origin naturally in the product or added as starter culture will however mainly depend on the type of process employed (Berdaque et al., 1993; Molly et al., 1996; Toldra, 1998).

Table 4: Components of aroma and flavour of fermented sausages (Lücke, 1994)

Compounds added as such:
Salt
Spices
Smoke constituents
Products of microbial degradation of carbohydrates:
Lactic acid
Acetic acid
Products of protein degradation by microbial or meat enzymes:
Amino acids
Peptides
Volatile fatty acids
Carbonyl compounds
Products of lipid degradation:
Medium- and long-chain fatty acids (formed by microbial or meat lipases)
Carbonyl compounds (from hydroperoxides)
Volatile fatty acids
Hydrocarbons
Transformation products from additives (e.g. smoke or spice constituents)

2.3.2.1 Glycolysis.

The endogenous glucose and phosphorylated intermediates, plus any carbohydrates added as an ingredient are used by endogenous flora of the meat, or starter culture if added, as a source of energy for growth (Roca and Incze, 1990). This carbohydrate fermentation results in an accumulation of organic acids, especially lactic acid (the principal product of fermentation), but to some extent acetic acid, ethanol, acetoin and carbondioxide are also produced (Anon, 1990). Papadima and Bloukas (1999) observed a decrease in pH, which they attributed to bacterial fermentation of leek carbohydrates in their product (since they did not add any sugars), and explained this to explain the production of organic acids, mainly lactic from carbohydrate fermentation. Johansson et al. (1994) observed a rapid increase in lactic acid during the first 3 days of ripening followed by a considerable increase between the 3rd and the 7th day. D-lactic and L-lactic acid increases were comparable, with resultant concentrations of 13.0 mg/g and 15.3 mg/g DM respectively, after 63 days of storage. However, minimal amounts of acetic acid were observed throughout ripening (1.3 mg/g DM). They interpreted the accumulation of acetic and lactic acid and the drop in pH to be a result of *Pediococcus* and *Staphylococcus* species metabolic activity. De Ketelaere et al. (1974) observed a reduction in carbohydrate content, a significant accumulation of lactic acid and small amounts of acetic acid. Smaller but significant amounts of propionic and butyric acids also occurred while the total carbonyl value never exceeded 0.5 mmole/100 g DM. Although sugar fermentation is linked with the production of lactic acid, Molly et al. (1996) demonstrated that microbial fermentation of sugars is also capable of producing some volatile molecules believed to play a role in flavour development. In the absence of glucose, total carbonyl products were lowered and an inhibitory effect of antibiotics on total carbonyls was not observed. According to them, about 65 % of the aldehydes formed originated from the carbohydrate fraction. Bacteria played a role in the formation of aldehydes originating from the carbohydrate fraction, but not from the lipid fraction. Lois et al. (1987) observed a decrease in carbohydrate concentration in "chorizo" formulated with an addition of fermentable sugars and one without sugars. The fermentable carbohydrates in the batch without added sugars are likely to have originated from the spices, because the carbohydrate content of the added garlic and paprika were 30.9 and 18 3 % respectively and these spices contributed 0.68 % carbohydrates to the initial meat mixture. The pH of the dry sausages was slightly higher in the absence of antibiotics, and they observed a highly negative correlation between bacterial counts and pH (r = 0.85) after 21 days of ripening. This was probably a reflection of the effectiveness of the antibiotics on the inhibition of fermentation.

2.3.2.2 Proteolysis

Myofibrillar proteins, especially myosin and actin are important proteins in the formation of the structure of dry sausages (Katsaras and Peetz, 1988). They act through a three phased binding process, entailing activation, diffusion and stabilisation. The salt added during bowl cutting solubilises the proteins (activation), and the solubilised proteins diffuse from the myofibrils to form a matrix of protein and water outside the muscle cells, binding together meat, fat and connective tissue particles (diffusion). Finally the system is stabilised by a formation of a gel caused by the drop in pH (5.6 and below) that occurs during fermentation (stabilisation). Mihalyi and Kormendy (1967) reported a loss in solubility of myofibrillar and sarcoplasmic proteins due to denaturation in a dry Hungarian sausage, while decreases of 40 to 68 % and 54 to 63 % in myofibrillar and sarcoplasmic proteins solubility respectively were observed by Dominguez (1988) in "chorizo" ripened for 45 days.

During post-mortem proteolysis, muscle proteins are hydrolysed by cathepsins and calpains into polypeptides. Polypeptides are then degraded into shorter chain peptides, which can be metabolised into free amino acids by aminopeptidases. Peptides can undergo further reactions to form non-volatile taste compounds or volatile aroma compounds (Diaz et al., 1993; Toldra, 1998). These compounds include non-protein compounds which affect the pH and flavour of the product (Hierro et al., 1997). Volatile compounds important in the flavour of fermented sausages are aldehydes, alcohols, and acids resulting from the degradation of branched-chain amino acids like leucine, isoleucine, valine, and those with low threshold like phenylalanine or methionine (Montel et al., 1998). In dry sausages, proteolysis is either microbial (natural meat flora or from added starter) and /or endogenous (from meat enzymes like calpains, cathepsins and trypsin-like proteinases) (Verplaetse, 1994; Johansson et al., 1996).

De Keteleare et al. (1974) observed a decrease in total soluble crude protein (myofibrillar protein, and "sarcoplasmic proteins") by the end of ripening. The presence or absence of starter culture had no significant effect on these compounds. The low pH brought about by lactic acid accumulated from carbohydrate metabolism by microorganisms (indigenous to the meat or from added starter culture) caused denaturation of the salt soluble proteins during ripening (Ten Cate, 1960). Water-soluble nitrogen compounds (WSN) increased during dry fermented sausage ripening (Roca and Incze, 1990). Mihalyi and Kormendy (1967) observed an increase in non-protein nitrogen (NPN) compounds in dry fermented Hungarian sausages during ripening (100 days). Proteolysis in "chorizo" manufactured with and without added fermentable sugars was confirmed by an increase in NPN and ammonia ($NH_4^{(}$) production (Lois et al., 1987). The rate of hydrolysis was higher in the sample with fermentable sugars, possibly because of the low pH, which stimulated hydrolysis of myofibrillar proteins.

Diaz et al. (1993) demonstrated the effect of incorporation of meat enzymes on proteolysis in fermented sausages. A rapid increase in WSN, NPN, and phosphotungstic acid soluble nitrogen (PTN) during fermentation, followed by stabilisation during ripening was observed in the pronase E (a mixture of proteinases, amino- and carboxypeptidases) treated batches, while all fractions increased moderately throughout processing in the control batch. Total volatile nitrogen increased consistently throughout ripening for all the batches. In general, the higher the added quantity of pronase E, the higher the increase in the nitrogen fractions. The levels and changes in NPN during ripening of the control compares favourably with reported studies on Spanish (Lois et al., 1987) and European (Dierick et al., 1974) dry sausages.

Johansson et al. (1994) observed an increase in NPN throughout processing. A rapid increase in NPN occurred during the first 7 days of processing, while an electrophoretogram of water soluble and salt soluble proteins showed a major reduction in these fractions during the first 3 days of processing. Water soluble proteins and salt soluble proteins with molecular weights in the ranges 20 to 30 kDa and 50 kDa (for water soluble and salt soluble proteins respectively), had almost disappeared by the 7th day. These protein fractions denatured because of the rapid drop in pH that occurred during this period.

Garcia de Fernando and Fox (1991) demonstrated the occurrence of proteolysis during fermented sausage processing by monitoring changes in nitrogen fractions. WSN increased from about 20 % to approximately 30 % at the end of ripening, with the greatest increase observed during fermentation. WSN permeates (peptides less than 10 kDa) increased significantly, while slight increases were observed on the PTN, and the free amino acids fractions. The salt-soluble nitrogen (SSN) fraction decreased rapidly during fermentation and moderately during ripening. Dominguez (1988) observed increases of NPN in both traditionally made “chorizo” and industrially processed “chorizo”.

A linear relationship between processing temperature and overall increase in free amino acids (FAA) and NPN was observed during the curing of Iberian ham (Martin et al., 1996). There was a marked difference in the final FAA content of the two types of ham matured under different climatic conditions, viz. 20 to 30 ^{0}C. A possible explanation was that the higher temperature stimulated the responsible endo and exopeptidases. However, the delayed observed reduction in the liberation of FAA was attributed to several factors, namely the reduction in exopeptidase activity towards the end of processing (Toldra et al., 1993), inhibitory effect of the salt and dessication, or the degradation of FAA to volatile compounds such as amines and Maillard reaction products (Antequera et al., 1992).

2.3.2.3 Lipolysis, oxidation and flavour.

According to Gandemer (1998), the animal species is the main factor responsible for variations in the fatty acid composition of triacylglycerols, especially in polyunsaturated fatty acids (PUFA), which accounts for 2-3 % in cattle, 7-15 % in pork, 20-25 % in chicken and more than 30 % in rabbit. PUFA are principally composed of C18 PUFA, especially linoleic and a small proportion of linolenic acid (Gandemer, 1998). The fatty acid composition of the polar lipid consists of between 45 to 55 % PUFA, made up of linoleic acid (14-30 %), long-chain PUFA such as arachidonic acid (8-14 %), and C22 fatty acids such as 22:4 n-6, 22:5 n-3 and 22:6 n-3 (Gandemer, 1997). According to Buscailhon et al. (1994), the polyunsaturated fraction of French ham contained 4.6 % linoleic acid and 0.4 % linolenic acid, while the fatty acid composition of the polar lipids in this product consisted of 49 % PUFA. The other long-chain PUFA accounted for at least 17 %, and consisted of arachidonic acid (11 %), and traces of 20: 2 n-6, 20: 3 n-6, 22: 4 n-6, 20: 5 n-3, 22: 5 n-3 and 22: 6 n-3 (Bascailhon et al., 1994)

The fats in the meat can be classified as depot (intermuscular) fat and tissue or intramuscular fats. Intramuscular fats exist in close association with protein and contain a high proportion of the total phospholipid content of meat. This fraction is highly susceptible to oxidation and although it exists in small quantities it has a strong influence on meat quality (Buckley and Connoly, 1980). The high sensitivity of phospholipids to oxidation can be explained in two reasons. Firstly, phospholipids contain long chain PUFA that are very sensitive to oxidation. Secondly, phospholipids are membrane components in close contact with catalysts of lipid oxidation located in the aqueous phase of the muscular cell (Gandemer, 1998). Phospholipids are the lipid class most sensitive to oxidation while unsaturated fatty acids are liberated by lipolysis (Chizzolini et al., 1998). According to Allen and Foegeding (1981), intramuscular lipids are involved in many quality traits of meat products, such as nutritional value (energy, fatty acid and cholesterol supply), sensory attributes (tenderness, juiciness, colour and flavour) and technological properties (shelf life). Among the sensory attributes, the one mainly related to intramuscular lipids is flavour. Lipids contribute positively to the development and deterioration of flavour (Gandemer, 1998). The distinctive flavour of dry fermented sausages is according to Roca and Incze (1990) related at least in part, to the hydrolytic and oxidative changes occurring in the lipid fraction during ripening. Lipolysis and oxidation are the two main biochemical processes that take place in the lipid fraction during ripening of fermented sausages. The oxidation of lipid moieties in fermented meat products has a direct effect on the sensory quality of the final product. They are influenced by a large number of factors such as: processing (heating, mincing, mixing); storage conditions; pH; a_w; lipid composition; metals; haem compounds; nitrite and salt and additives (Demeyer et al., 1974; Quintanilla et al., 1996; Chizzolini et al., 1998; Novelli et al. 1998.). According to Chizzolini et al. (1998) and Novelli et al. (1998), heat treatment has a negative effect on cellular structure, inactivates enzymes, and releases oxygen from oxymyoglobin, creating conditions conducive to formation of hydrogen peroxide. Shredding, mincing and mixing disrupts the muscle structure, and in this way increases the surface area exposed to oxygen and other oxidation catalysts. In the meat industry, raw materials storage could be very important because lipid oxidation is an autocatalyzed reaction in which some intermediate and final oxidation products have pro-oxidant effects. According to Novelli et al. (1998), an initial stage of oxidation due to free radical chain reactions, which can take place during storage, could be aggravated by processing and in turn accelerate oxidation directly or indirectly via the autocatalysis mechanism. Verplaetse, (1994), said lipolysis and oxidation have to be seen as two different processes in which microorganisms have a substantial impact only on the lipolysis products.

Lipolysis involves the liberation of long chain free fatty acids (FFA) from mainly the triglycerides, and to some extent from the polar lipid fraction (Quintinilla et al., 1996). The triglycerides are hydrolysed by lipases from the endogenous meat enzymes or exogenous enzymes from microbial origin (natural meat flora and/ or added starter culture), while the phospholipid fraction is hydrolysed by phospholipases (Toldra, 1998). The liberated FFA can be oxidised by radiation, heat, ions and/ or oxidative enzymes to form peroxides, the primary products of oxidation (Buckley and Connolly, 1980; Toldra, 1998). Peroxides can undergo degradation through secondary oxidation to release short chain fatty acids, odour and flavour-producing compounds (Buckley and Connolly, 1980), or further interact with peptides and amino acids forming volatile aroma compounds. Lipid peroxidation is a causative agent of oxidative rancidity which itself results in a deterioration of the organoleptic and nutritive quality of the product (Roca and Incze, 1990). According to Alford et al. (1971) rancidity is the oxidative deterioration of food lipids and it involves the reaction of unsaturated fatty acids with oxygen to yield hydroperoxides, which in turn decompose to products with undesirable taste and odour. Volatile aroma compounds identified in dry sausages include alkanes, alkenes, aldehydes, alcohols carboxylic acids, esters, ketones, sulphur derivatives, terpenes, as well as phenols, chlorides and pyrazines (Chizzolini, 1998; Toldra, 1998). Terpenes are mainly derived from spices (Johansson et al., 1994). Observations have been reported where lipid oxidation compounds made 50 % and more of the total volatile compounds in fermented products that were neither spiced nor smoked (Berdague et al., 1993; Chizzolini et al., 1998). Addition of spices influences the groups of volatiles isolated from fermented products (Berger et al., 1990; Dominguez Fernandez and Zumalacarregui Rodriguez, 1991; Johansson, et al., 1994).

According to Lubieniecki and Schelhorn (1972), hydrolytic changes in dry sausages are mainly due to bacterial lipase activity, and control of bacterial lipase activity has been found to have a positive effect on shelf life, even though tissue lipases were still active. Bacterial metabolism is also said to be an important cause of oxidative changes in unsaturated fatty acids, leading to a build-up of lipid peroxides and carbonyl compounds (Alford et al., 1971).

The release of long-chain fatty acids from neutral lipids, phospholipids and cholesterol during dry sausage ripening has been documented (Demeyer et al., 1974; Dominguez Fernandez and Zumalacarregui Rodriguez, 1991; Johansson et al., 1994; Fernandez et al., 1995). Demeyer et al. (1974) observed a significant decrease in triglyceride bound fatty acids, with a corresponding pronounced increase in FFA, and moderate increases in diglycerides and monoglyceride components of the lipid fraction, during ripening of dry fermented sausages. Polar lipid acids increased late during ripening. They explained the accumulation of diglycerides and FFA as an indication of lipase specificity for position three of the triglycerides. This was deduced from the fact that pork fat represents a particular fatty acid distribution pattern, with stearic acid located at position one (ca. 60 %), palmitic acid (ca. 60-80 %) at positon two and the octadecenoic acids (50-60 %) at position three. The rate of hydrolysis of the individual FFA decreased in the order, linoleic, oleic, stearic and palmitic, indicating specificity of lipolysis for position three of the triglycerides. They observed an erratic behaviour in carbonyl values throughout processing. Carbonyls increased during the first week of ripening, decreased after smoking and increased on subsequent ripening. The initial increase in carbonyl values was attributed to carbohydrate fermentation (De Ketelaere et al., 1974), while the decrease during the later stages of processing was due to further metabolism of lipid peroxides (Cerise et al., 1973). According to Alford et al. (1971) the acceleration of peroxide formation and the increase in the monocarbonyl fraction suggests that the microorganisms present can carry out the reactions involved in the production of rancidity i.e. the resultant peroxides with their subsequent decomposition to monocarbonyls. However, this ability to produce peroxides and monocarbonyls appears to be relatively rare among microorganisms. An increase in peroxides without a concomitant increase in monocarbonyls could either imply that, the microorganisms lack the mechanisms necessary to convert peroxides to monocarbonyls, and that they decompose the monocarbonyls as rapidly as they are formed or they convert the peroxides to other compounds.

Johansson et al. (1994) reported triglyceride hydrolysis into 1,2 diglycerides and FFA and the formation of 1,3 diglycerides and monoglycerides. The formation of 1,3 diglycerides was interpreted as possible acyl migration, where the more thermodynamically stable 1,3 diglyceride form is spontaneously formed from the unstable 1,2 diglycerides (Bloomer, 1990). Similar findings were reported by Demeyer et al. (1974), suggesting a hydrolysis preference for the outer position (mostly bearing an unsaturated fatty acid). They also reported a consistent decrease in triglycerides during ripening, with corresponding increases in FFA, diglycerides and to a lesser extend monoglycerides. Peroxide values (PV) increased on fermentation and then decreased to very low values during ripening. This phenomenon has been attributed to either no fat oxidation occurring due the rapid development of the anaerobic environment in the sausage (Roedel et al., 1992), and the antioxidant effect of smoking (Potthast and Lowe, 1988), or to the fact that the peroxides were decomposed. The phospholipid fraction decreases because this fraction is more unsaturated than the triglyceride fraction (Leseigneur-Meynier and Gandemer, 1991) and is therefore more susceptible to lipid oxidation. Johansson et al. (1994) detected volatiles that originated from sources such as carbohydrates, lipolysis and lipid oxidation, proteolysis, smoking and seasoning. Other volatiles may include compounds from meat which are either of bacterial origin or due to muscle enzymes. They identified aliphatic hydrocarbons, aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, carboxylic acids, esters, nitrogen compounds, sulphur compounds, chloride compounds, terpenes and furans. Terpenes (52 %) and sulphur compounds (20 %) were the most prevalent in the headspace, altogether constituting 23 % of the total chromatogram

Johansson (1996) found that bacterial contribution to FFA was constant, irrespective of endogenous lipolysis, type of meat or bacterial counts. Endogenous and bacterial formation of FFA significantly differed ($p < 0.01$) in beef and pork, endogenous lipolysis being more pronounced in pork than beef. Bacterial production of FFA significantly exceeded that resulting from endogenous enzymes in both types of meat. Linoleic acid (C18:2c, 9, 11) was the most fatty acid commonly liberated in pork, while pälmitic-(C16:0), stearic-(C18:0) and oleic acids (C18:1c, 9) were liberated in both beef and pork.

Molly et al. (1996) in their study of Belgian sausage found that, antibiotics prevented normal development of bacteria but did not affect the degree or type of lipolysis. They reported that the addition of micrococci in the presence or absence of antibiotics did not increase the overall lipolytic activity. They observed that, the major part of the liberated FFA was from the triglycerides, and that a preferential release of unsaturated fatty acids from the total lipid fraction took place compared to saturated (SFA) and monounsaturated fatty acid (MUFA). That PUFA consisting of n-6 e.g. linoleic acid were released more readily than the monounsaturated form, mainly oleic. PUFA increased 3.5 fold, compared to 2.7 and 1.5 fold increases for MUFA and SFA respectively. Molly et al., (196) also observed a high specificity for fatty acid release from the polar fraction compared with the triglyceride fraction, confirming the higher degree of unsaturation found in the polar lipid (phospholipid) fraction of the total fat fraction. Addition of antibiotics significantly decreased carbonyl compound production in the presence of glucose. When glucose was omitted this inhibition was not observed. This specificity for liberation of PUFA from the polar lipid fraction has been reported by other researchers in fermented sausages (Demeyer et al., 1974) and cured ham (Buscailhon et al., 1994), where the degradation of polar lipids by muscle lipases was observed. Significant increases in FFA with a corresponding decrease in phospholipids, and a moderate increase in diglycerides have been observed during dry cured ham ripening, e.g. French ham (Buscailhon et al., 1994), Serrano ham (Gandemer, 1998). Marginal changes in monoglycerides were observed in Serrano ham. Radovanovic et al. (1996) observed a decrease in the triglyceride and an increase in the FFA fractions of the total lipids of "Uzice beef Prshuta", a traditionally matured meat product of Yugoslavian origin consisting of two different parts of meat from beef round and loin. The triglyceride decreased from 88.70 to 85.46 % in the "sol" and 87.10 to 85.43 % in the "rozbratna", while the FFA increased from 1.32 to 3.31 % and 1.55 and 3.15 % for the former and latter respectively. These changes in the lipid fractions confirmed that the products underwent some lipolytic changes during maturing.

An increase in the degree of unsaturation during fermentation and a decline during ripening-drying was observed in salami, chorizo and saucisson (Chasco et al., 1993). The increases in the C_{16} and C_{18} fatty acids quotients were higher in chorizo and the lowest were observed in salami. The changes in the degree of unsaturation was also reflected by the iodine value (IV), a measure of the degree of unsaturation of a fat or oil), which increased or changed marginally during fermentation and decreased during ripening for chorizo and salami. However, IV decreased during fermentation and increased during ripening in one of the commercial brands of saucisson. These variations can be attributed to the liberation of the long-chain fatty acids during fermentation with specificity for PUFA, and the partial destruction of these acids by oxidative processes during ripening and subsequent drying. Free oleic (C18:1) and linoleic (C18:2) acids increased consistently in all types of sausages. The thiobabituric acid value (TBA) increased in all sausage brands, confirming the oxidative changes in the lipid fraction of the dry sausages. A higher increase in TBA was observed in the chorizo than the salami and saucisson. However, the TBA values of the ripened product for all the brands were either less than or in the region of one (1), the critical value for oxidative rancidity (Buckley and Connoly, 1980). This indicated that there was no increase in the oxidative rancidity processes. Peroxide degradation was confirmed by a build-up of volatile compounds (total carbonyls), which increased considerably throughout fermentation and drying. Volatile aldehydes (butanal, hexanal and pentanal) increased significantly in all sausage types. Hexanal had the highest concentrations, decreasing in the order, chorizo, saucisson and salami. There were no traces of propanal however, which is the predecessor of linolenic acid in all products.

Dominguez Fernandez and Zumalacarregui Rodriguez (1991) reported a marked increase in FFA in "chorizo", manufactured traditionally (without starter culture, sugars, curing agents or additives) and industrially, confirming the occurrence of lipid hydrolysis in both types of sausage. The results agree with previous work done on dry fermented sausages, (Cerise et al., 1973; Demeyer et al., 1974) including Hungarian salami which is characterised by a high FFA value, > 5 % (Nagy et al., 1989). Oleic and palmitic acid increased slightly during ripening. Carbonyl values were low, except in an isolated case of 370 µmoles/100 g DM observed on the 45th day in one of the traditionally produced batches. The highly erratic carbonyl values behaviour reported compared favourably with values cited by Demeyer et al. (1974). The static behavior of carbonyl compounds during ripening has been explained to be a result of either the antioxidant action of the smoke (Demeyer et al., 1974), the destruction of these compounds by some microorganisms or to the unavailabilty of microorganisms capable of changing peroxides to carbonyls (Dominguez Fernandez and Zumalacarregui Rodriguez, 1991). TBA values reported were lower than the critical value for rancidity i.e. TBA > 1 (Buckley and Connoly, 1980). Except for a TBA of 2.21 on one of the traditionally produced batches, the rest of the results indicated that oxidative rancidity had not occurred. This inhibition in the development of rancidity on chorizo was possibly due to the antioxidant effect of nitrite, smoke residues and spices, though the antioxidant effect of garlic at 1 % is said to be negligible (Dominguez Fernandez and Zumalacarregui Rodriguez, 1991). Lois et al. (1987) observed an intense build-up of acidity in chorizo manufactured with and without inclusion of sugars (traditionally). The final % FFA of about 3.0 indicated a high rate of lipolysis.

A Greek dry sausage with no added starter culture and varying degrees of coarseness (Samelis et al., 1993), showed a build-up of FFA to the value of 2.99 % on the 14th day, in the finely ground sausage. However, FFA values decreased during further ripening in all types of sausages. This has been attributed to either the metabolic activities of the micro flora or to the chemical conversion of the FFA into carbonyl compounds (Alford et al., 1971). The main fatty acids in the residual fat (less FFA) were, oleic (C18 1), palmitic (C16:0), linoleic (C18:2), stearic (C18:0) and palmitoleic (C16:1) and the same profile was observed in the FFA fraction throughout production. The hydrolysed fat was rich in linoleic and low in the other acids. There was a general increase in palmitoleic and linoleic fatty acids. Palmitic and stearic fatty acids decreased, while oleic acid decreased and increased in concentration in coarsely and finely ground batches respectively.

Using the hydrolysis coefficient *k*(FA) i.e. a measure of a change in concentration of a fatty acid under examination in relation to the available substrate, microbial lipase specificity was established. According to Samelis et al. (1993), the hydrolysis coefficient can be a positive, a zero or a negative value, where a positive value implies that the fatty acid in question is preferentially released from the lipids. Its concentration therefore increases in the FFA fraction. A negative value implies that the fatty acid is not released at all or its released from the lipids at a lower rate than the rate of lipolysis and consequently its concentration in the FFA fraction decreases. A value of zero means that the fatty acid is released at about the same rate as the hydrolysis rate. Quantitative and qualitative differences were found between the batches. The k(C18:1) was a positive value in the fine batch and negative in the coarse batch, (qualitative) while quantitatively some k(FFA) doubled in the coarse batch. The C16:n fatty acids sequence of release was C16:1 > C16:0, and the shorter chain fatty acids (C16:0 and C16:1) were released more readily from the lipids than their iso-unsaturated acids with two more carbons (C18:0 and C18:1). The SFA coefficients, k(C16:0) and k(C18:0) were negative throughout the process, implying that the rate of release of this group from the lipid was less than the average rate of hydrolysis. Except for day 7, the k(C16:1) values were higher than kC18:1), and a strong linear dependence of the hydrolysis coefficient k(C18:n) on the number of double bonds, was observed for the 18 carbon chain fatty acids. The sequence of release for the homologous fatty acids from the lipid fraction was C18:2 > C18:1 > C18:0 and C16:1 > C16:0, while the iso-unsaturated fatty acids were released in the order C16: 0 > C18:0 and C16:1 > C18:1 (Samelis et al., 1993).

This microbial lipase selectiveness towards certain fatty acids in Greek dry sausage was observed for at least a month of processing (fermentation and ripening), and it appeared not to be dependent on the differences in lipolysis rates observed in the two batches (coarse and fine ground, without starter). Dominguez Fernandez and Zumalacarregui Rodriguez (1991), also reported preferential release of unsaturated fatty acids from sausage lipids, which is said to be responsible for the high content of linoleic acid in the FFA fraction. This has been attributed to the specificity of lipolysis for position 3 of the pork fat triglycerides (Demeyer et al., 1974), where most of the octadecanoic acids (50-60 %) are bound. Cantoni et al. (1967), reported that selected lipolytic strains were capable of liberating the unsaturated fatty acids (oleic, linoleic, palmitoleic) more readily than the saturated ones (stearic, palmitic) and Samelis et al. (1993) interpreted this action to be due to micrococcal lipases.

The acid value (g oleic acid/100 g lipid) of sausages containing *Candida cylindracea* lipase were significantly higher than those of a sausage containing *Lactobacillus plantarum* and *Staphylococcus carnosus* as starter culture. This indicated a higher rate of hydrolysis in the sausages with fungal lipase as compared with sausages containing the bacterial starters (Zalacain et al., 1995). Insignificant changes, ($p < 0.05$), were observed in PV and TBA values in both types of sausages, indicating that the intense increase in FFA produced by the fungal lipase action did not increase rancidity. FFA were detected in the order oleic > linoleic > palmitic > stearic in the sausage with starter, while myristic, palmitic, palmitoleic, stearic and oleic were significantly higher ($p < 0\ 01$) in sausage with the lipase than in the sausage with starter. Linoleic acid was significantly higher, at a higher level of significance ($p > 0.05$) in the sausage containing lipase, whereas linolenic acid levels were not significantly different in the two types of sausages. There was no difference in MUFA between the two types of sausages, the sausage with lipase showed a greater percentage of SFA, and lower levels of PUFA than the sausage with starter culture.

Increases in principal fatty acids in sausages incorporating pancreatic lipase in their formulation have been reported. The ratio of SFA to PUFA was however higher in sausages with lipase than the ones with starter (Fernandez et al., 1991). Zalacain et al. (1995) concluded that, this specificity of lipase for the liberation of SFA could have a positive effect on reducing the possibility of rancidity development in dry fermented sausages, because of the low final levels of the highly oxidisable PUFA. Short chain fatty acids were generated either by the degradation of sugars or by lipid oxidation (Girard and Bucharles, 1991; Lücke, 1994). Acetic acid was significantly higher ($p < 0.05$) in the sausage with lipase, while propionic ($p < 0.001$) and butyric acids ($p < 0.01$) were significantly higher in the sausage with starter. The changes in valeric and isovaleric acids were not significantly different in the two types of sausages. Zalacain et al. (1995) interprets this to mean that there was no undesirable growth of heterofermentative organisms in either types of sausage.

Triglycerides decreased continuously throughout ripening in both the control and the pancreatic lipase treated batches, resulting in an increase in FFA, diglycerides and monoglycerides (Fernandez et al., 1995). The rate of lipolysis was rapid during fermentation and moderate on ripening. The triglyceride degradation in lipase treated batches was directly proportional to the concentration of the added enzyme, to such a degree that batch 500 (containing 500 units pancreatic lipase) had lost up to two thirds of it's initial triglycerides content by the end of ripening. Monoglyceride content increased in all batches, although the increase was significantly higher in pancreatic lipase treated sausages containing more than 60 units of enzyme. Although trace amounts of diglycerides were initially detected, the batches with lipase units of above 180 had final diglyceride contents which were 2-4 times higher than the control batches. FFA content increased significantly in lipase treated batches, especially in lipase treated batches containing between 90 and 500 units. Values were similar to FFA contents of dried sausage with a long ripening period (Lois et al., 1987). Fernandez et al. (1995), attributes the high rate of hydrolysis observed during fermentation, to the pH (6.0) of the sausage and the processing temperature (22 ^{0}C) during this phase, both of which were near the optimum for pancreatic lipase activity (pH 7.0 and 37 ^{0}C). They suggested that, the slow rate of hydrolysis observed during ripening could be used to control and avoid excessive lipid breakdown, and over-maturation of the product in commercial dry fermented sausage production.

Zalacain et al. (1996) reported a significantly higher acidity value ($p < 0.01$) in a sausage that contained both starter culture (*Candida cylindracea*) and lipase, over one that contained only the starter culture. The acidity (g oleic acid/100 g lipid) increased in both types of sausages during fermentation and ripening, even though the sausage without lipase had a final acidity value similar to that of the sausage containing lipase after only one week of ripening. This indicates that lipolysis occurred in both types of sausages during ripening. However the sausage with lipase had a higher lipolysis rate, progressive throughout processing, while lipolysis was less intense in the sausage without lipase. The acidity of both types of sausages at the end of ripening compares well with reported values, (Dominguez Fernandez and Zumalacarregui Rodriguez, 1991; Fernandez et al., 1991). Short-chain fatty acids, which could be from either hydrolysis of carbohydrate or lipid oxidation (Girard and Bucharles, 1991; Lücke, 1994) were detected. Acetic acid increased significantly in both types of sausages during all stages of ripening. Larger amounts of the acid were observed in the sausage with lipase than in those containing the starter throughout fermentation and ripening. Propionic, butyric, valeric and isovaleric acids decreased during ripening only in the sausage without lipase, while in the finished product, the sausage with lipase had higher concentrations of all the above mentioned short-chain fatty acids. Significant increases in myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids were observed in the sausage with lipase at all stages of ripening, while linolenic acid was significantly lower in the sausage with starter and lipase than in the one with starter alone at all stages of processing. This has been interpreted to either mean that linolenic acid was liberated to a lesser extent or that more of this acid was utilised in the catabolic processes to produce volatile compounds (Zalacain et al., 1996). Throughout all phases of ripening, free SFA were higher in the sausage with starter and lipase. MUFA were higher in the sausage with starter only, while PUFA were higher in the sausage with starter plus lipase but only after 72 hours of ripening., indicating that in spite of the intense lipolytic activity, there was no significant increase in oxidative rancidity. Changes in TBA values were marginal in both types of sausages. TBA values decreased during the first week of ripening and increased during the following week in both types of sausages. The decline in TBA value was significant only with the batch containing the starter alone although the differences between sausage type were not significant. These TBA values demonstrated that in spite of the high lipolytic activity, there was no significant increase in the oxidative rancidity processes and that relative to changes in the lipid fraction, the incorporation of lipase in sausage formulation shortens the ripening time of dried fermented sausages. MacDonald et al. (1980) observed a significant reduction in TBA values of pork cured with nitrite, even at levels as low as 50 mg/kg compared with pork cured with salt, citric acid or butylated hydroxytoluene (BHT). This indicated that nitrite reduces or inhibits lipid oxidation better than BHT and citric acid.

Hierro et al. (1997) demonstrated the effect of endogenous meat enzymes and starter culture on the lipolytic changes occurring in dried fermented sausages by using sterile ingredients and processing under sterile conditions. The FFA content increased in all batches throughout ripening and in the order: control; *Lactobacillus plantarum; Lactobacillus plantarum* plus *Staphylococcus* species; and *Lactobacillus plantarum* plus *Micrococcus* species. The *Staphylococcus* species inoculated batch had a final FFA content that was three times higher than the amounts in the control batch. Hierro et al. (1997) reported that 70 % of the lipolytic activity was due to meat tissue enzymes when mixtures of cultures were used. Therefore, because endogenous enzymes provide the primary lipolysis, the role of the micrococci is to reduce nitrates and nitrites. Individual fatty acid concentrations decreased in the order: oleic > palmitic > stearic > linoleic, with traces of palmitoleic and myristic acids occurring during fermentation and ripening. A comparison of the concentration of the individual acids at the beginning of fermentation and at the end of ripening show the following order of release: C18:2 > C18:1 > C16:1 > C14:0 > C16:0 > C18:0. This trend was similar in both the control and inoculated batches, although C18:2 and C18:1 were higher in the control batch. The latter findings indicate that both microbial and meat endogenous enzymes preferentially hydrolysed the outer fatty acid of the triglyceride molecule or have a preference for the polar lipid fraction (mainly phospholipid), since it contains higher levels of PUFA than the apolar one. The higher rate of release observed for C18:2 and C18:1 in the control batch probably indicates that meat enzymes have a greater specificity for the sn-3 position or polar lipids than do the microbial enzymes (Hierro et al., 1997). Acetic acid was the main short-chain fatty acid detected, especially in the lactobacilli inoculated batches. This has been explained to imply that this acid is formed mainly by carbohydrate fermentation (Demeyer et al., 1986).

2.4 GENERAL CONCLUSIONS

Fermented sausage flavour is produced by a complex process effected by fermentation of carbohydrates, proteolysis, lipolysis and lipid oxidation, seasoning and curing agents (Demeyer et al. 1986; Roca and Incze, 1990; Verplaetse, 1994; Campbell-Platt, 1995; Dainty and Blom, 1995; Navarro et al, 1997). The role of lipolysis is essential because the FFA produced are the substrates for the oxidative changes responsible for flavour and aroma development. Highly unsaturated fatty acids are preferentially released, and as a result of their oxidation, products like peroxides, aldehydes, alcohols, ketones etc. are formed.

The composition of the lipid fraction is strongly influenced by species and animal feed. The stability of the lipid fraction in turn is highly dependent on the composition and degree of unsaturation of the fatty acid molecules making up the lipid. Long chain PUFA are preferentially released from the lipid fraction and phospholipids are highly susceptible to oxidation since they are richer in unsaturated fatty acids than the triglyceride fraction.

Although lipolysis and lipid oxidation in European fermented sausages has been documented (Lois et al. 1987; Astiarana et al. 1990; Johansson et al. 1994; Navarro et al. 1997), the changes in the lipid fraction of South African fermented sausages have not been studied.

The aim of the current study therefore was to gain knowledge about the effects of storage temperature, dietary PUFA enrichment and the varying of the raw materials on the lipid stability of a South African salami.

CHAPTER 3

3: THE EFFECT OF STORAGE TEMPERATURE ON THE LIPID COMPONENT OF A FERMENTED SAUSAGE

ABSTRACT

The influence of temperature on the lipid stability of a fermented sausage (salami) was studied by subjecting a vacuum-packaged product to three storage temperatures, namely 4, 12 and 25 ^{0}C for thirty days. The following chemical measurements were made during processing and after 15 and 30 days storage: composition of the lipid fraction, free fatty acids (% oleic), fatty acid composition, iodine-, peroxide-, carbonyl- and TBA values. During processing, there was a significant buildup of FFA (% oleic), hydroperoxides, carbonyl- and TBA values indicating lipid hydrolysis with some degree of oxidation. During storage, increases and decreases in the levels of individual free fatty acids and reduction in peroxides confirmed lipid hydrolysis and oxidation. Oxidation was confirmed by significant increases in TBA and carbonyl values during storage. Significantly larger changes were observed at 25 ^{0}C, indicating that temperature has a significant effect on the changes in the lipid fraction of a fermented sausage. Of the temperatures studied, 4 ^{0}C was the best, 12 ^{0}C was satisfactory and the most practical, while 25 ^{0}C was unacceptable in terms of storage of salami in South Africa.

3.1 INTRODUCTION

According to Leistner and Roedel (1975), meat products with pH values above 5.2 and a_w values above 0.95 are highly perishable and should be stored at or below +5 ^{0}C. The expected bacteriological shelf life of these products is about two weeks, and maybe prolonged by lowering storage temperatures. At pH values of between 5.2 and 5.0 (inclusive) or a_w values of between 0.95 and 0.91 (inclusive) meat products are perishable and should be stored at temperatures below +10 ^{0}C. The bacteriological shelf life of these products is about four weeks and can be longer at lower temperatures. Meat products with pH values below 5.2 and a_w values below 0.95 or only pH < 5.0 or only $a_w < 0.91$ are "shelf-stable" and don't require refrigeration. The shelf life of these products is often not limited by bacterial loads but by chemical or physical spoilage, especially rancidity and discolouration.

Storage conditions, including time, had a significant effect on the pH of traditional Greek sausages stored under cold conditions (3 to 7 ^{0}C) and/ or in the ripening room (13 to 15 ^{0}C) (Papadima and Bloukas, 1999). Even though sugars were not added to the mix on formulation, the pH of the sausages decreased on storage. A significant negative correlation ($r = -0.798$, $p < 0.001$) was observed between pH value and lactic acid bacterial level by the 3rd day. The pH of the sausages in the ripening room was lower ($p < 0.05$) than that of the cold room stored sausages. After 14 days production, the pH value of the ripening room stored sausages was below 5.0, rendering them relatively shelf-stable, while the sausages in the cold store had a pH of above 5.2 and were therefore still highly perishable. According to Papadima and Bloukas (1999), storage of traditional sausages in the ripening room leads to a faster and more extensive decrease in pH, compared with cold stored ones and this ensured a better keeping quality.

Ripening temperature has been shown to have a direct effect on moisture content, pH, a_w and firmness of a typical "cervelat" sausage (Baumgartner et al., 1980). The decline in moisture was directly proportional to the ripening temperature. There were no significant differences in the moisture content of sausages ripened at 15 and 20 ^{0}C or 25 and 30 ^{0}C, whereas there was a significant difference in the moisture content of sausages at 20 and 25 % after 4 days of ripening and throughout the entire processing period. They observed a slight but evident liquefaction of fat on the surface of the sausage fermented at 30 ^{0}C. The a_w of "cervelat" decreased throughout ripening. The decrease was rapid at 30 ^{0}C during the first 3 days, while a slight difference in the rate of decline of a_w was observed between 15 and 20 ^{0}C. At the 25 ^{0}C ripening temperature the rate of decrease of a_w was more rapid than at the two lower temperatures. There was a decline in pH at all ripening temperatures. A slow decrease in pH was observed up to the 21st day after which very little change in pH took place at the 15 ^{0}C ripening temperature. The decrease in pH was rapid at 20 and 25 ^{0}C and even more rapid at 30 ^{0}C during the first 3 days of ripening. A significant increase in firmness was observed at all ripening temperatures when the pH of the sausage decreased below 5.4, and this was after 9, 4, 3 and 2 days at 15, 20, 25 and 30 ^{0}C ripening temperatures respectively. Roca and Incze (1990) also mentioned a strong relationship between pH and water loss on sausage consistency, and a pH of 5.4 or a_w of 0.90 were critical for the proper development of this parameter in dry fermented sausages. Baumgartner et al. (1980) observed an increase in firmness from 12N to 26N in the sausage ripened at 15 ^{0}C when the pH dropped below 5.4. High correlations between the measured parameters were observed at all temperatures over the ripening period (28 days). The correlation coefficients decreased in the order, a_w and water content ($r = +0.9895$), water content and firmness ($r = -0.9135$), a_w and firmness ($r = -0.8963$), pH and firmness ($r = -0.8787$), pH and water content ($r = -0.6963$ and pH and a_w ($r = -0.612$).

Sorensen and Samuelsen (1996) demonstrated the effect of environmental conditions on lipolysis by cell-free extracts from the meat starter cultures of *Staphylococcus xylosus* and *Derbaryomyces hansenii*, using pork fat emulsions as model systems. Maximum lipolysis was observed in the region of 37 ^{0}C for both organisms. However a rapid decline in lipolysis was observed at temperatures above 37 ^{0}C, and the lipases seemed to be inactivated at 40 ^{0}C. Lipases from both cultures retained about 20 % of their ability to liberate fatty acids at low temperature (5 ^{0}C). At a constant temperature of 37 ^{0}C, the optimal pH for lipolysis was 7.0 and 6.5 for the *S. xylosus* and *D. hansenii* lipases respectively, and lipolysis declined rapidly at pH above these optima. At pH values between 5.5 and 6.0, lipolysis declined sharply for the *S. xylosus* lipase, while at pH values below 5.0, lipolysis was observed only with the *D. hansenii* lipase. A combined effect of temperature, pH, NaCl (5 % w/v) and incubation time (6 days), showed that, with the *S. xylosus* lipase, an increase in temperature caused an increase in predicted lipolysis. Although an increase in temperature generally caused an increase in lipolysis, at low pH the effect of temperature increase was negligible and lipolysis was limited. Sorensen and Samuelsen (1996) found that at a temperature of 10 ^{0}C, pronounced lipolysis only occurred at higher pH values combined with longer incubation periods. Addition of NaCl had a limited effect on lipolysis, although they observed a decrease in lipolysis at concentrations above 5 % w/v. Similar results were reported by Sorensen (1997), who observed that temperature, pH and time of incubation during fermentation were all significant in controlling lipolysis.

Navarro et al. (1997) studied the importance of addition of starter, pre-ripening and temperature, and their two-factor and three factor interactions on fermented sausages. They observed that the increase in FFA depended not only on temperature, but also on pre-ripening, and that a decrease in phospholipids was accompanied by a corresponding increase in FFA throughout ripening. They observed very little change in the phospholipid fraction during fermentation at 24 ^{0}C for 3 days. They ascribed this to the short time temperature exposure, which was too short to produce observable changes in phospholipid concentration. Similar results were reported by Buscailhon et al. (1994), who reported that the phospholipid concentration during ham curing did not change over 2 months at 5 ^{0}C. Temperature was the single factor which produced most changes in the phospholipid concentrations. All samples ripened at 16 ^{0}C had lower concentrations of phospholipid than the one ripened at 8 ^{0}C. The ratio of PUFA concentrations increased throughout ripening irrespective of the fermentation conditions. The increase of the unsaturated fatty acids and the decrease in SFA proportions were observed at both 8 and 16 ^{0}C. Total free fatty acids increased in all samples ripened at 8 and to a much greater extent at 16 ^{0}C. Lipolysis was nevertheless stronger in samples with starter irrespective of the pre-ripening stage. At 16 ^{0}C, they reported maximum lipolysis in non-pre-ripened samples without starter, and minimum lipolysis in pre-ripened samples without starter, whereas samples with starter showed the intermediate effect of the pre-ripening stage.

A rapid decline in pH (5.7 to 5.0) of a sausage during the first 3 days of fermentation was observed and this was followed by a moderate drop during early ripening (4.8 after 14 days). A subsequent increase in pH in the later stages of production (5.1 after 63 days) was reported by Johansson et al. (1994). They observed an increase in FFA throughout production, namely 0.6 % in the raw sausage to reach 6.8 % at the end of storage after 63 days. An observed increase in FFA when the drying temperature was raised was also reported by Navarro et al. (1997), and this has been attributed to the increased activity of meat and microbial lipases. Johansson et al. (1994) reported that, while the raw sausage mix contained only triglyceride and phospholipds, FFA and 1,2 diglycerides were detected after 3 days production, whereas 1,3 diglycerides were observed by the 7th day. Monoglycerides were not detected until the end of storage and a significant decline in the phospholipd fraction was observed between the 7th and the 14th days of production. The FFA composition of the fat showed a slight increase in SFA (38.4 to 39.6 %) and a significant decrease in the unsaturated fatty acid (61.6 to 59.8 %) during the first week of ripening. However they reported a subsequent stabilisation in fatty acid composition during the entire process. Johansson et al (1994) reported a slight increase in the PV during the early stages of processing followed by a decline to values more or less equal to the peroxide content of the raw sausage. The raw sausage had a PV of 3.6, which increased to 5.3 after 7 days and 4.0 in the ripened stored sausage after 63 days. Novelli et al. (1998) reported a similar trend in peroxide values of salame Milano stored for six months, indicating that long-term storage did not significantly affect lipid oxidation as measured by PV.

Wang et al. (1995) reported a linear decrease in PUFA with storage time in vacuum packaged (VP) and modified atmosphere packaging (MAP) Chinese sausages stored at 4 and 15 ^{0}C. However, the amount of decrease in PUFA in the MAP treatment was less than for the VP treatment. There was no difference in PUFA for the MAP stored at 4 and 15 ^{0}C, whereas the PUFA for the sausage stored in VP at 15 ^{0}C decreased significantly ($p < 0.05$) compared with that stored at 4 ^{0}C. PV of all treatments increased progressively ($p < 0.05$) until 3 months storage, after which there was a slight decrease. The PV of the VP treatment was significantly higher than that of the MAP treatment, while lipid peroxidation of sausage stored at 15 ^{0}C was greater than that at 4 ^{0}C storage temperature. The 2-thiobarbituric acid reactive substance (TBARS) values of both VP and MAP treated sausages increased significantly ($p < 0.05$) with storage time. TBARS values of the VP treatment were higher than that of the MAP treatment, implying that the rate of oxidation was higher in the VP treatment. TBARS values were lower at the 4 ^{0}C storage temperature than at the 15 ^{0}C for both treatments. However, lipid oxidation in the sausages packed in MAP and stored at 4 and 15 ^{0}C was not significantly different until after a storage period of one (1) month. PV increases are said to be a result of the catalysis of intracellular compounds, the destruction of the cell structure by NaCl and processing.

Traditionally produced fermented Greek sausages were originally stored in low temperature rooms if they were intended to be consumed within a few weeks, otherwise sausages were preserved by immersion in pots containing melted fat before storage in cool rooms. However, due to the commercialisation of most traditional sausage productions, during summer sausages are air dried for a few hours then stored at 5 to 7 ^{0}C (Papadima and Bloukas, 1990). In South Africa, it's been observed that fermented sausages are stored in various ways, which include, hanging in butcheries, cool room storage, or in display cabinets of retail shops. It is thus important to establish the most suitable storage temperature for South African salami, especially when taking the high ambient summer temperatures in certain regions into consideration.

The aim of this study was therefore to establish the most suitable storage temperature for South African salami.

3.2 MATERIALS AND METHODS

3.2.1 Preparation of a fermented sausage

The salami was formulated according to Table 5.

Table 5: Salami formulation.

Beef 90/10	40.00 %
Pork 90/10	34.73 %
Pork backfat	20.00 %
Curing salt	3.05 %
Spice mixture	2.18 %

Fresh lean meat (beef and pork) and pork backfat obtained from a butchery in the Bloemfontein region were cubed and frozen stored overnight at −18 ^{0}C in non-airtight polythene bags prior to formulation. The frozen beef was placed into a bowl cutter (OKTO 20L Bowl Cutter) and chopped to 10 mm particle size before addition of the spice mixture (45.95 % dextrose, 45.95 % sucrose, 4.60 % white pepper, 2.76 % garlic and 0.75 % nutmeg) and starter culture (0.5 % freeze dried starter culture per kilogram product). The starter culture consisted of *Staphylococcus carnosus* and *Lactobacillus pentosus* (Floracarn SL Hanson) in a ratio of 2:1. After the addition of the starter culture and spice mixture, the beef was chopped to fineness, frozen lean pork added and the mixture further chopped to 20 mm fineness. The lard and curing salt (99.31 % NaCl, 0.30 % nitrite and 0.39 % nitrate) were finally added and the mixture chopped to the required consistency (4-5 mm) at slow bowl cutter speed. The sausage mix was kneaded into a vacuum-filling machine (Trespade) to exclude air and filled into Colpak Fibrous Bak 65/50 casings. Three salamis were removed from the batch, weighed and marked before fermentation (these sample weights were used as initial weights against which to monitor the moisture loss of the rest of the batch throughout the production period). The salamis were transferred to a fermentation room at 22 ^{0}C with RH above 86 % and fermented for 48 hours. After fermentation the salamis were smoked (beech-wood) in a smoking chamber (Crown Mills) at 18-22 ^{0}C for 10 minutes, then transferred to a ripening room at a temperature of 12 ^{0}C and a 75-80 % RH. During production, the marked salamis were weighed every five days to monitor moisture loss, ripening being considered complete when a 20 % minimum loss was attained. The matured salamis were vacuum-packaged and stored at three storage temperatures, namely 4, 12 and 25 ^{0}C for thirty days.

3.2.2 Sampling procedure

Six salamis were drawn from the batch directly after stuffing (raw sausage mix) for day zero, after 48 hours (after fermentation and smoking) and at the end of ripening (after a 20 % minimum weight loss) and immediately transported to the laboratory. After drying, the rest of the salamis were vacuum-packaged and sampled after a subsequent fifteen (15) and thirty (30) days storage period at each of the three storage temperatures referred to above. For the purpose of analysis the casing was removed, the salami thoroughly mixed and stuffed into 4.5 ml Nunc Cryotubes Vials (NUNC Brand Products, Nalgene Nunc International) with stoppers. If not analyzed immediately, the vialed samples were shock frozen in liquid nitrogen before storage at −20 ^{0}C, until needed. Each salami was sampled in duplicate, hence all analyses were done in 12 replicates, except for protein, salt, nitrite and a_w which were only done on the raw sausage mix (day 0 after stuffing) and on the salami before storage (after a 20% minimum weight loss), and presented data in the latter cases are averages of triplicate analysis.

3.2.3 Chemical and physical analyses

All reagents were analytical grade obtained from Merck, unless indicated otherwise.

3.2.3.1 General parameters

3.2.3.1.1 Weight loss

Three salamis were weighed after stuffing (0 day), and the same sausages were re-weighed after 48 hours (fermentation) and every five days until a 20 % weight loss was attained. The differences in weight were expressed as a percentage of the initial weight, and the data presented are means of the three measurements.

3.2.3.1.2. pH Determination.

Homogenates were prepared by blending 10 g of sausage in 100ml distilled water (Koniecko, 1985) using an ULTRA TURRAX T25 (JANKE & KUNKEL, IKA – Labortechnik). A fluted filter paper (Whatman # 1) was used to obtain a filtrate of which the pH was measured with a HANNA instruments, Model HI 8915 pH meter with a glass electrode. The pH meter was standardised using pH 4 and 7 buffer solutions (BDH Laboratory Supplies).

3.2.3.1.3 Protein determination

Protein (% total nitrogen multiplied by a factor of 6.25) was determined in a 0.5 g sample using the Kjeldahl analytical method according to AOAC **39**, 981.10 (1990).

3.2.3.1.4 Salt content (% NaCl)

Salt (% NaCl) was determined in a 2.5 g sample by the AOAC **39**, 935.47 (1990) technique.

3.2.3.1.5 Nitrites ($NaNO_2$)

Nitrite (ppm $NaNO_2$) was determined in a 0.5 g sample using the AOAC **39**, 973.32 (1990) method.

3.2.3.1.6 Water activity (A_w)

A_w was measured in about 10 g sample using a Novasina Model TH 200 water activity meter.

3.2.3.1.7 TBA – mg malonaldehyde/1000 g

Thiobarbituric reactive substances (TBARS) were determined in a 5 g sample using the aqueous acid extraction method of Raharjo et al. (1992).

3.2.3.2 Analytical methods for the analysis of fat

3.2.3.2.1 Lipid extraction

Extraction of total lipids from the sausage (10 g) was performed quantitatively using chloroform and methanol in a ratio of 2:1 according to the method of Folch et al. (1957). Extracts were taken to dryness by concentration under vacuum on a rotary evaporator (BUCHI Rotavapor - R - 114 with a BUCHI water bath B - 480), and further dried in a vacuum oven at 50 ^{0}C for three hours with phosphorus pentoxide as a moisture adsorbent. Total fat content was determined by weighing and expressed as % fat (w/w) per 100 g sample. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g sample. The moisture content of the sample was determined by subtraction (100 – % lipid – % FFDM) and expressed as % moisture (w/w) per 100 g sample. From the total lipid extraction, ± 10 mg and ± 150 mg fat (for methylation and column chromatography respectively) were weighed into glass vial, and these sample and the rest of the total lipid extract (for IV, PV and carbonyl compounds) were stored under a blanket of nitrogen at –20^{0}C until analysed.

The following quality measurements were performed:

3.2.3.2.1.1 Iodine value (g Iodine/100 g lipid)

Iodine value was determined in 0.5g of the extracted lipid by AOAC 41, 920.158 (1990) method.

3.2.3.2.1.2 Peroxide value (milliequivalents/1000 g lipid)

Hydroperoxides were determined in 0.5g of the extracted lipid using the AOAC **41**, 965.33 (1990) technique.

3.2.3.2.1.3 Free fatty acids (% Oleic)

Free fatty acids were determined in 0.5g of the extracted lipid following a method of Pearson (1968).

3.2.3.2.1.4 Carbonyl compounds (mmole/1000 g lipid)

Unsaturated carbonyls (UC), saturated carbonyls (SC) and total carbonyls (TC) were determined in 0.6 g of the extracted lipid using the method of Berry and McKerrigan (1958).

3.2.3.2.1.5 Column chromatography

The extracted lipid (± 150 mg) was subjected to silicic acid column chromatography according to the method of Kendrick and Ratledge (1992). The sample was dissolved in chloroform (5 ml) and fractionated by using a column (25 x 100 mm) of silicic acid with a mesh size of 100-200, obtained from SIGMA (cat. No. SIL-350) and activated by heating at 110 ^{0}C overnight. Successive applications of chloroform (200 ml), acetone (350 ml) and methanol (200 ml) produced fractions containing neutral lipids (triacylglycerols), glycolipids plus sphingolipids and polar lipids (phospholipids) respectively. The extracts were taken to dryness under vacuum in a rotary evaporator and further dried in a vacuum oven at 50 ^{0}C for three hours with phosphorus pentoxide as moisture adsorbent. The weight of each fraction was determined and each fraction expressed as percentage (w/w) per 100 g lipid.

3.2.3.2.1.6 Fatty acid determination

The ± 10 mg total lipid portion was methylated to prepare fatty acid methyl esters (FAME) for gas chromatographic analysis by using the boron-trifluoride methanol complex (CH_4BF_3O) (Slover and Lanza, 1979). Fatty acids were quantified by using a Varian GX 3400 flame ionization gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 μm internal diameter (ID), 0.2 μm film thickness). Column temperatures were 40-230 ^{0}C (hold 2 minutes; 3 ^{0}C/min; hold 10 minutes). Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian Autosampler 8200 CX with a split ratio of 100: 1. The injection port and detector were both maintained at 250 ^{0}C. Hydrogen was used as the carrier gas at 35 psi and nitrogen was the makeup gas. Chromatograms were recorded with Varian Star chromatography software. Identification of sample fatty acids, were made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards SIGMA (cat. no. 189-19). Nonadecanoic acid (C19:0) (SIGMA cat. no. N-5377) was used as internal standard to improve quantitative fatty acid estimation.

Fatty acid datas was used to calculate the percentages of the individual fatty acids including total saturated fatty acids (SFA), total monoenoic fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA).

3.2.4 Statistical analyses

The experiment was considered to consist of two different phases, which were studied separately. The first phase, namely fermentation and ripening, included the study of the changes in the quality characteristics of salami during processing. The second phase, namely the storage, included the study of the effect of temperature on the sausage during further ripening.

Differences in the product parameters during processing and at different storage temperatures were determined by an analysis of variance procedure (ANOVA) using a NCSS 2000 (Cruncher Statistical Systems, Kaysville) statistical package. The Newman-Keuls multiple range test at α = 0.05 was used to identify the differences between means.

3.3 RESULTS AND DISCUSSIONS

An insignificant decline in pH occurred in the salami during fermentation, while a significant decrease was observed throughout the ripening phase (Fig.1). The initial pH of the sausage (5.15), decreased to 5.00 on fermentation and fell even further to 4.84 in the ripened product. This decrease in pH is has been ascribed to an accumulation of organic acids, especially lactic acid from carbohydrate fermentation (Anon, 1990). The pH of the ripened salami compares favourably with pH values of published studies on dry fermented sausages. These studies included German (De Ketelaere et al., 1974), chorizo, saucisson and salami (Chasco et al., 1993) and Greek

(Papadima and Bloukas, 1999) sausages. Samelis et al. (1993) however reported higher final pH values on Greek dry fermented sausages ripened for 28 days compared to pH values observed on this study.

According to Leistner and Roedel (1975), a pH of less than 5.0 is sufficient to ensure the microbiological stability of meat products. It is therefore correct to say that the salami was ripe and ready for consumption at the end of the 27th day of production. This pH value is close to the isoelectric point of the meat proteins (pH 5.4-5.5), which in turn corresponds with the lowest water-holding capacity of the product, hence the more rapid loss of water from the sausage under these conditions (Wismer-Pedersen, 1971). A significant overall loss in moisture content (w/w per 100 g raw sausage) was observed on the salami throughout production, although a slight gain in moisture was observed during fermentation (Fig.2). The increase in moisture content observed during fermentation was not significant. The initial moisture content of the sausages was 56.49 %. This increased to 57.43 % after fermentation and was 44.94 % in the ripened product.

The final moisture content of the salami after ripening was similar to those obtained by other workers on dry fermented sausages (Fernandez et al., 1995). As a result of moisture loss, the sausage lost weight during production (Fig. 2), its a_w declined from 0.941 (raw sausage) to 0.901 (dried product) while the fat content (Fig. 3), fat free dry matter (FFDM) (Fig. 3), nitrite content (Fig. 4), protein content (Fig. 5) and salt content (Fig. 6) increased. The weight loss increased with production time (Fig. 2), a factor that was also observed by other authors (Papadima and Bloukas, 1999). A total weight loss of 20.66 % (w/w per 100 g raw sausage) was observed on the salami after the 27th day of maturation, at which time the sausage was deemed to be fully ripened.

The weight loss observed in the present study was above the minimum required weight loss (20 %), which dictates suitability for consumption (Papadima and Bloukas, 1999), and the typical weight loss (15-20 %) for semi-dry sausages (Lücke, 1985) confirming that the salami was fully matured. The raw sausage had a 21.23 % fat content (w/w per 100 g raw sausage), which increased to 21.31 and 23.92 % during fermentation and ripening respectively. The observed increase in fat content was significant only during the ripening phase (Fig. 3). The fat content of the ripe salami in this study was below the specified 30 to 50 % typical fermented dry sausage fat content (Lücke. 1994). It is nevertheless a true reflection of the amount of fat incorporated in the sausage during formulation (Table 5). The slight increase in moisture content observed during fermentation caused a significant decrease in the FFDM (Fig. 3) component of the sausage during this phase, although there was a significant increase in FFDM on maturation (Fig. 3).

The neutral (Fig. 7) and phospholipid (Fig. 8) fractions of the total lipids decreased throughout fermentation and ripening, and the decrease in these fractions caused a corresponding increase in the glycolipid (Fig. 9) portion of the total lipids. A rapid significant decrease in neutral lipids was observed during fermentation, while the decrease was moderate and insignificant during ripening (Fig. 7). The phospholipid fraction (Fig. 8) decreased rapidly during fermentation and moderately during ripening. The decrease in this fraction was significant only during ripening. The decrease in the phospholipid fraction (Fig. 8) has been explained by the fact that this lipid fraction is more unsaturated compared to the triglyceride fraction (Leseigneur-Meynier and Gandemer, 1991), and thus more susceptible to lipid oxidation. The changes observed in the lipid fractions in this study indicate that, the phospholipid and neutral fractions were hydrolysed mainly during fermentation. A slight decrease in the glycolipid fraction (Fig. 9) was observed during fermentation, however the decrease was not significant. The glycolipid fraction increased significantly during ripening.

The FFA (% oleic acid) was 1.67 % on the raw sausage and increased to 2.46 and 4.30 % in the fermented and ripened sausage respectively (Fig. 10). The increase in % FFA was significant throughout production. These results indicate that the salami had undergone substantial lipolysis. The values observed agree with those reported in most dry fermented sausages (Demeyer et al., 1974; Lois et al., 1987; Dominguez Fernandez and Zumalacarregui Rodriguez, 1991; Zalacain et al., 1995; Quintanilla et al., 1996; Zalacain et al., 1996) and also in French ham (Buscailhon et al., 1994).

The degree of unsaturation of the salami lipid fraction as indicated by the IV (g iodine/100 g lipid), decreased throughout fermentation and ripening (Fig. 11). IV decreased moderately throughout fermentation and drying, although the decrease became significant during ripening. This decrease in IV indicates a decrease in the degree of unsaturation, a factor which can be further interpreted as an indication of preferential release of saturated fatty acids rather than unsaturated ones from the sausage lipids. This does not however agree with what was reported in some fermented sausages. Chasco et al. (1993) observed an increase in IV during fermentation followed by a decrease during ripening of salami, chorizo and saucisson indicating an increase in the degree on unsaturationn during fermentation and a decrease on ripening of these products.

There was a significant accumulation of peroxides, the primary products of auto-oxidation, in the salami throughout processing (Fig. 12). The PV (milliequivalent/1000 g lipid) of the raw sausage was 1.22 and it increased to 2.95 and 10.28 in the fermented and ripened sausage respectively. Increases in PV during dry sausage manufacturing have been reported (Johansson et al. 1994).

The breakdown of peroxides to flavour and aroma compounds e.g. aldehydes, ketones etc. was demonstrated by an accumulation of carbonyl compounds (mmole/1000 g lipid) throughout fermentation and ripening (Fig. 13). Except for a slight decrease in SC during fermentation, there was an increase in UC, SC and TC throughout maturation (Fig. 13). The TC was 19.99 in the raw sausage and 26.34 in the fermented sausage. A final value of 33.26 mmole/kg lipid was observed in the ripened sausage. Similar results were reported by Chasco et al. (1993) who observed an increase in total carbonyl compounds of chorizo, saucisson and salami during fermentation and drying, although a slight but significant decrease in carbonyl value was observed during the ripening phase for some chorizo and salami samples. Quintanilla et al. (1996) reported an increase followed by a decrease to a level relatively similar to the initial content of carbonyl compounds of a dry fermented sausage. The reduction was attributed to possible oxidation of carbonyl compounds giving rise to volatile compounds involved in the aroma of the products.

Unsaturated fatty acids are not only prone to auto-oxidation to form hydroperoxides, but PUFA also form malonaldehydes (TBARS) from oxidation (Chasco et al. 1993; Samelis et al. 1993; Dainty and Blom, 1995). The TBA value (mg malonaldehyde/1000 g meat) is a valid index for lipid oxidation and provides a method of estimation of rancidity that is best correlated with sensory analysis (Raharjo et al., 1993). This correlation should increase on curing, ripening and storage, indicating lipid oxidation. In the current study, the TBA value (Fig. 14) showed a slight decline during fermentation, although this change was not significant. The TBA value of the sausage increased significantly during ripening. However, the final TBA value of the ripened sausage at 0.39 was less than the critical value for rancidity i.e. TBA > 1.0 (Buckley and Connoly, 1980). This implies that, although there was evidence of hydrolysis of FFA from the lipid fraction, and degradation of these fatty acids into peroxides, there was no evidence of development of rancidity in the sausage by the end of ripening. Similar results have been reported in previous studies on dry fermented sausages, chorizo (Dominguez Fernandez and Zumalacarregui Rodriguez, 1991), chorizo, salami and saucisson (Chasco et al., 1993), Spanish sausage (Zalacain et al. 1996).

The changes in the fatty acid composition (% of total lipids) of the salami as presented in Table 6 show an increase in SFA and PUFA during fermentation and ripening, whereas MUFA decreased. The changes in all groups of fatty acids however were significant only during ripening. Myristic acid (C14:0) and stearic acid (C18:0) increased during fermentation and ripening, although significant increases occurred only during ripening. Palmitic acid (C16:0) also increased throughout production, but the changes in this fatty acid were not significant. Palmitoleic acid (C16:1c9) concentration changed marginally throughout production. Oleic acid (C18:1c9) decreased significantly during ripening, while vaccenic acid (C18:1c7) showed a significant increase during ripening. Linoleic (C18: 2c9, 12) and linolenic acid (C18:3c9, 12, 15) increased during fermentation and ripening, with significant increases observed only during ripening. Insignificant trace amounts of arachidonic acid (C20:4c5, 8, 11) were detected throughout production. Trace amounts (< 0.5 %) of other fatty acids were detected (Table 6). In general, for any individual fatty acid, significant changes (positive or negative) were observed only during the ripening phase. The order of magnitude of the principal fatty acids was oleic (43.22 %) > palmitic (22.06 %) > stearic (11.97 %) > linoleic (9.46 %) > palmitoleic (2.65 %) > vaccenic (2.23 %) > eicosenoic (1.45 %) > linolenic (0.77 %). The sequence of release for the homologous fatty acids from the lipid fraction was C16:0 (22.06 %) > C16:1 (2.65 %) and C18:1 (43.22 %) > C!8:0 (11.97 %) > C18:2 (9.46) %, and for the isounsaturated fatty acids C16:0 (22.06 %) > C18:0 (11.97 %), while C18:1 (43.22 %) > C16:1 (2.65 %).

In the current study, oleic acid was the major fatty acid released, and the results indicate preferential release of SFA (palmitic and stearic) from the sausage lipids, instead of the preferential liberation of unsaturated fatty acids with a high concentration of linoleic acid as was the case in some previous studies (Demeyer et al. 1974; Dominguez and Zumalacarregui, 1991). Zalacain et al. (1995) observed a similar release of the FFA, except that they reported a higher proportion of linoleic acid and a lower concentration of myristic acid than in the present study. On the other hand, Samelis et al. (1993) reported a similar release sequence pertaining to the two principal fatty acids (oleic and palmitic acids), although they observed a high proportion of linoleic and a lower proportion of stearic acid than was observed in this study. Demeyer et al. (1974) ascribed this preferential release of the unsaturated fatty acids to the specificity of lipolysis for position 3 of the pork fat triglycerides, where most of the octadecanoic acids (c. 50-60 %) are bound. Cantoni et al. (1967) attributed this preferential release of unsaturated fatty acids to microbial lipases, since certain strains were capable of releasing the unsaturated fatty acids (oleic, linoleic, palmitoleic) more readily than the saturated ones (stearic, palmitic). The lack of specificity for the liberation of unsaturated fatty acids found in this study is further accentuated by the increase of the saturated fatty acid proportion of the lipids throughout the entire processing of the salami.

During storage, the pH of the sausage decreased with time at the 12 and 25 ^{0}C storage temperatures, while there was a slight increase in pH at the 4 ^{0}C storage temperature after 15 and 30 days storage (Table 7). The pH of the salami observed after 15 and 30 days storage at the 4 ^{0}C storage temperature was not significantly different from that of the ripe sausage before storage. The pH observed on the sausage after both 15 and 30 days at the 12 ^{0}C storage temperature, was also not significantly different from the pH of the salami at the end of processing. On the other hand, a significant decrease in pH was observed after 15 and 30 days storage at the 25 ^{0}C storage temperature compared to the pH of the salami at the start of the storage phase. The pH of the salami stored at 25 ^{0}C was significantly lower than the pH of the dry salami and that of the sausages stored at both 4 and 12 ^{0}C storage temperatures throughout the entire storage period. The slight increase in pH observed in the 4 ^{0}C storage temperature salami has been reported in previous studies (Lois et al., 1987; Astiasaran et al., 1990; Samelis et al., 1993; Fernandez et al., 1995). This has been attributed to the accumulation of basic nitrogen compounds, mostly ammonia and amine production as a result of protein breakdown during ripening as well as an increased concentration of buffering substances and the decreased dissociation of the already present electrolytes in the sausage meat (Demeyer et al., 1979; Lücke, 1985).

There was a significant increase in the moisture content of the salami at all storage temperatures after 15 days of storage, however no further increase in moisture content was observed at all storage temperatures after 15 days of storage (Table 7). Due to the increase in the moisture content, there was a significant decrease in the FFDM proportion of the sausages at all storage temperatures with time. The fat content of the salami increased with storage time at 4 and 25 ^{0}C while a decrease in fat content was observed at 12 ^{0}C storage temperature. The sausage at 25 ^{0}C after 15 days had a fat content significantly lower than that of the dry salami before storage and that of the sausages stored for 30 days at 4 and 12 ^{0}C, whereas there was no significant difference in the fat content of this salami and the rest of the stored sausages. There was a slight but evident liquefaction of fat on the surface of the salami stored at 25 ^{0}C. Similar observations were reported on sausages fermented at 30 ^{0}C (Baumgartner et al., 1980).

There was an insignificant decrease in the neutral lipid fraction of the total lipids at all storage temperatures throughout the storage period (Table 7). A slight decrease in the phospholipid fraction was observed at the 4 and 12 ^{0}C storage temperatures with time. The decrease was however significant only in the 30 days stored sausage at 12 ^{0}C. A slight but insignificant increase in this fraction was observed in salami stored at 25 ^{0}C after 30 days storage period. The glycolipid fraction of the total lipids increased significantly with time at all storage temperatures (Table 7). The significant increase in the glycolipid fraction was due to a combined effect of the changes in the neutral and phospholipid fraction, even though the observed decreases in the neutral lipid fraction were not significant for any time-temperature combination. Although its been said that the phospholipid fraction is more unsaturated than the triglyceride fraction and thus more prone to lipid oxidation (Leseigneur-Meynier and Gandemer, 1991), this has not been observed in the current study since the neutral lipid fraction also decreased, though not significantly. The decrease in the phospholipid fraction was also significant only at 12 ^{0}C after 30 days while an insignificant increase was observed in the sausage at 25 ^{0}C.

FFA (% oleic) accumulated significantly between the salami at the beginning of the storage phase and the rest of the stored sausages. A significant increase in the FFA content of the salami was observed at the 4 and 25 ^{0}C storage temperatures throughout the entire storage period (Table 7) whereas the increase observed at the 12 ^{0}C storage temperature was not significant. These results demonstrated the time-temperature effect on lipolysis and lipid degradation. The expected increase in rate of lipolysis with increase in temperature (Johansson et al., 1994; Sorensen and Samuelsen, 1996; Navarro et al., 1997; Sorensen, 1997), was responsible for the FFA accumulation at 25 ^{0}C, although lipid oxidation occurs at an equally high rate at this temperature. On the other hand, the low rate of lipid degradation encountered at low temperatures explain the higher accumulation of FFA after 30 days at 4 ^{0}C, and further confirmed that at 4 ^{0}C the rate of lipid hydrolysis was still significantly high. It can be assumed that hydrolysis and lipid oxidation occurred at similar rates at the 12 ^{0}C storage temperature, hence the moderate yet significant increase in FFA observed at this temperature.

Peroxides have a transitional nature and are primary products intermediate in the formation of hydroxyl and carbonyl compounds. PV decreased at all storage temperatures during the entire storage period (Table 7), however, PV observed after 15 days at the 4 ^{0}C storage temperature was not significantly different from the PV of the dry salami at the beginning of the storage period. This further emphasises the importance of the time-temperature effect on lipid changes, indicating that the 15 days storage at 4 ^{0}C (time-temperature combination) was not sufficient to bring about significant changes in the peroxide value. Johansson et al. (1994) observed a rise and fall in peroxide value of fermented sausage during maturation, drying and subsequent storage at 4 ^{0}C over a 63-day production period. The low peroxide values observed during ripening has been attributed to either no fat oxidation occurring due to the rapid development of anaerobic environment in the sausage (Roedel et al. 1992), to the antioxidative effect of smoking (Potthast and Lowe, 1988) or the decomposition of peroxides (Johansson et al., 1994).

TBA value increased at all storage temperatures throughout the storage period (Table 7). Significant increases in TBA value were observed in sausages stored for 15 days at 25 ^{0}C and in those stored for 30 days at both 12 and 25 ^{0}C storage temperatures. The TBA value of the sausages stored for 15 days at 4 and 12 ^{0}C and the 30 days stored salami at 4 ^{0}C were below the critical TBA value for oxidative rancidity (TBA > 1.0), indicating that there was no evidence of oxidative rancidity on these products. On the other hand, the TBA value of salami stored at 25 ^{0}C for 15 days and that of sausages at 12 and 25 ^{0}C for 30 days had TBA values of above 1.0, indicating the possible development of rancidity of these sausages resulting in the sausage becoming unacceptable. Wang et al. (1995) reported similar results in Chinese-style sausages vacuum packaged and stored at 4 and 15 ^{0}C. They observed TBARS values (2-thiobarbituric acid reactive substances) of above 1.0 in sausages stored for 2 months at both temperatures, and the TBARS values of sausages stored at 15 ^{0}C were significantly higher than that of the sausages stored at 4 ^{0}C.

IV of the salami increased at all storage temperatures during the entire storage period (Table 7). The most significant increase in IV was observed at the 12 ^{0}C storage temperature for both 15 and 30 day storage periods, although these IV were significantly different from that of the sausage before storage and that of the 30 days 4 ^{0}C stored salami only. This increase in IV indicates an increase in the degree of unsaturation in the liberated fatty acids. The increase in the degree of unsaturation observed during storage confirms that unsaturated fatty acids are released more readily than saturated ones from the lipid fractions. The liberation and accumulation of FFA are temperature dependent (Johansson et al., 1994; Wang et al., 1995; Navarro et al., 1997). It would seem correct to therefore postulate that although lipid hydrolysis is higher at 25 ^{0}C, the rate of decomposition of the liberated fatty acids into short-chain fatty acids, peroxides, aldehydes, ketones and other products of auto-oxidation is high and more rapid than the rate of lipid hydrolysis. On the other hand, the rate of breakdown of unsaturated fatty acids at 12 ^{0}C is moderate and slower than at 25 ^{0}C, resulting in the significant increase of unsaturated fatty acids as measured by the IV at this temperature.

UC (mmole/1000 g lipid) increased in sausages stored for 30 days at 12 and 25 ^{0}C, although the increase was significant only on the 25 ^{0}C stored sausages (Table 7). A significant decrease in UC was observed in the salami stored for 30 days at the 4 ^{0}C storage temperature. SC (mmole/1000 g lipid) increased significantly on salami stored for 30 days at 25 ^{0}C, while a decrease in SC was observed in sausages stored at 4 and 12 ^{0}C after a 30 day storage period (Table 7). The decrease in SC was significant only in the 30 days, 4 ^{0}C stored salami. TC increased significantly in the salami stored for 30 days at the 25 ^{0}C storage temperature. TC decreased in sausages stored for 30 days at both 4 and 12 ^{0}C storage temperatures, although the decrease in this component was significant only in the salami stored at 4 ^{0}C (Table 7). The observed decreases in PV and the accumulation of carbonyl compounds with increases in time and temperature confirmed the intermediary nature of the peroxides and their breakdown into flavour and aroma compounds during salami ripening.

The fatty acid composition of the salami during storage (Table 8) shows a decrease in myristic acid (C14:0) concentration at all storage temperatures during the entire storage period, indicating hydrolysis of this fatty acid. The decrease in this fatty acid was not significantly different between the salami at the start of storage and all the sausages stored for 15 days at all storage treatments, including the sausage stored at 4 ^{0}C for 30 days. There was also no significant differences between the 15 and 30 days stored sausages at all storage temperatures. This implies that, at any of the studied storage periods, the changes in myristic acid were not significantly affected by a variation in storage temperature. Palmitic acid (C16:0) was hydrolysed with time at all storage temperatures. The decrease in concentration of this fatty acid was more pronounced at the 4 ^{0}C storage temperature only, irrespective of the storage period (Table 8). However, except for the sausage at the beginning of storage, the palmitic acid content at of the sausages at 4 ^{0}C after 15 and 30 days of storage was not significantly different from that of the rest of the sausages.

Stearic acid (C18:0) increased in concentration with storage time at all storage temperatures, although the concentration of stearic acid observed at 12 ^{0}C for both storage periods was not significantly different from the content of this fatty acid on the dry salami before storage (Table 8). Although traces ($x < 0.5$ %) of C20:0 were observed throughout storage at all temperatures, a significant increase of this fatty acid was observed only in salami stored for 30 days at 12 and 25 ^{0}C (Table 8). Margaric acid C17:0 incurred slight but significant hydrolysis during storage after 30 days at 12 and 25 ^{0}C storage temperatures (Table 8).

Palmitoleic acid (C16:1c9) decreased in concentration at all storage temperatures with time. The degree of hydrolysis of this acid was significantly different between the salami at beginning of the storage period and the stored sausages (Table 8). A slight insignificant increase in concentration was observed in oleic acid (C18:1c9) throughout storage at all storage temperatures, whereas vaccenic acid (C18:1c7) was found to decrease in concentration. This was significant only in sausages stored for 30 days at 12 and 25 ^{0}C. Eicosenoic (C20:1c11) increased at all temperatures throughout storage, although the concentrations observed at 12 and 25 ^{0}C storage temperatures after 15 days were not significantly different from the content of this fatty acid in the ripened salami. Linoleic acid (C18:2c9, 12) showed marginal changes in concentration at all storage temperatures with time, except in the sausage stored for 30 days at 25 ^{0}C in which a significant decrease was observed. Linolenic acid (C18:3c9, 12,15) decreased throughout storage at all temperatures, and its content in the salami after 30 days at 25 ^{0}C was significantly lower than that of all the other sausages except the salami kept at 12 ^{0}C throughout the storage period. SFA decreased with time at all storage temperatures (Table 8). The content of this fraction in the sausage stored at 4 ^{0}C for both

15 and 30 days was however significantly different from the SFA content of the salami before storage, but similar to that of salami stored for 15 days at 12 and 25 ^{0}C. A significant difference in MUFA was observed between the sausages stored for 15 days at 4 and 12 ^{0}C, while changes observed in MUFA were not significant at all other storage temperatures with time (Table 8). There were no significant changes in the PUFA content of the salami at all storage temperatures throughout the entire storage period (Table 8).

This study has demonstrated the complex pattern of reactions occurring during processing and storage of a fermented sausage (salami). Temperature and time have been shown to have direct implications on the changes in the lipid fraction of salami. Of the storage temperatures used in the current study, 4 ^{0}C was the best, 12 ^{0}C was satisfactory and the most practical, while 25 ^{0}C was unacceptable in controlling lipid stability of a fermented sausage.

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CHAPTER 4

4: THE EFFECT OF DIETARY POLYUNSATURATED FATTY ACID ENRICHMENT ON THE LIPID STABILITY OF A FERMENTED SAUSAGE.

ABSTRACT

The suitability of meat and backfat high in PUFA for salami manufacturing was determined. The PUFA gradient was obtained by feeding one group of pigs a control diet and another group the control diet plus 3 % sunflower oil. Lipolysis measured as % FFA (% oleic acid), hydroperoxide build-up (PV) and their degradation (saturated-, unsaturated- and total carbonyl values) and the oxidative stability of the salami (TBA value) was significantly affected by the PUFA content. The results have shown that sunflower oil enriched feed employed in the present study had PUFA levels of 57.88 ± 0.65 %. This feeding regime led to a PUFA level of 30.03 ± 4.53 % in backfat. The latter level in the backfat used for processing purposes is likely to negatively affect the keeping quality of South African salami.

4.1 INTRODUCTION

The addition of highly unsaturated oil to animal feed affects the fatty acid composition of the tissue lipids in meat animals (Marchello et al., 1983; Ajuyah et al., 1993; Miller et al., 1993; Kouba and Mourot, 1999), especially in simple stomach animals because, unless the fatty acids are utilised for energy little modification of the fat occurs (Koch et al., 1968). Saturated fats are implicated in raising the plasma low density lipoprotein (LDL)-cholesterol in man (Mattson and Grundy, 1985) and the resulting increased LDL-cholesterol has been correlated with coronary heart disease. Consequently, the reduction of saturated fat intake has developed as a primary diet and health issue with regard to red meat consumption (Miller et al., 1993). Pork fat and-meat are thought to possess a relatively high content of SFA and is therefore considered to be unhealthy (Warnants et al., 1996). Pork fat however also contains large amounts of MUFA, in the form of oleic acid, which plays a neutral to positive role in cardiovascular disease (Mensink et al., 1990; Zevenbergen, 1993; Warnants et al., 1998). Furthermore, unlike other SFA; stearic acid occurring abundantly in animal fat, is not hypercholesterolemic (Bonanome and Grundy, 1988; Warnants et al., 1998). Pork containing an increased proportion of unsaturated fatty acids and less palmitic acid would, from a human nutritional point of view, be better for health (Mattson and Grundy, 1985), provided there is no concomitant increase in fat and cholesterol intake from other dietary sources (Kouba and Mourot, 1999).

According to Lücke (1985), for the production of fermented sausages, especially dry sausages for long-term storage, the fat must have a high melting point and a low unsaturated fatty acid content. The use of soft fatty tissue e.g. backfat from pigs fed a diet rich in unsaturated fatty acids, may cause colour and flavour defects, interference with the drying process and an overall reduction in the shelf life of the sausages due to autoxidative fat deterioration.

Miller et al. (1993) reported that, the storage stability of fresh pork sausage formulated with meat/ fat from animals fed a grower-finishing diet that contained 10 % safflower or sunflower oil were comparable to the control. The same level of pork fat originating from animals fed canola oil in the diet however resulted in the sausage having undesirable storage characteristics. According to Kouba and Mourot (1999), diets with a high linoleic acid content increased the linoleic acid content of pig adipose tissue and muscles and decreases the oleic acid content of pig adipose tissue. Marchello et al. (1983) observed similar results with an overall decrease in the SFA content of the control compared with sunflower seed added treatments. Significant differences were observed in the latter study for stearic and palmitic acid, however this was in exception of stearic acid in the intramuscular fat and outer backfat. At all sites with the exception of intramuscular fat, the control diet yielded greater proportions of palmitoleic, oleic and linoleic acids in the final product as compared with the sunflower seed dietary treatments. A significant increase in linoleic at all locations (lean fat, flank fat, inner backfat, outer backfat and intramuscular fat) was observed when comparing the control and the dietary regimes containing sunflower seed, suggesting that there was a direct deposition of linoleic acid in the pork fat tissues. Marchello et al. (1983) suggested that, because of the deleterious resultant carcass effects, diets for growing-finishing swine should contain less than 13 % sunflower oil seed (approximately 5 % oil). Otherwise, it would lead to a soft oily-type carcass that is unacceptable to consumers and processors. A practical limit of 10 % of sunflower seed in the diet was recommended. Kouba and Mourot (1999) reported that, diets high in linoleic acid lead to the production of pork with increased linoleic acid content without any concomitant increase in intramuscular fat content. This resulted in an acceptable level of cholesterol from a human nutritional point of view. On the other hand they emphasised that the technological qualities of such carcasses were poor, having a soft backfat leading to a decrease in storage stability of the product. They recommended that the meat should be consumed fresh, and not subjected to further processing.

Houben and Krol (1980) reported that, except for cervelat-type sausages, the use of raw materials with increased PUFA levels (up to about 30 % linoleic acid in the backfat) did not present any major problems in the preparation of a variety of products. Pork resulting from 10 and 20 % dietary canola oil treatments had no significant effect on the fatty acid composition and oxidative rancidity of frankfurters (St John et al, 1986; Shackelton et al., 1989), whereas Rhee et al. (1988) reported that pigs fed higher levels of canola oil produced fresh cuts that tended to undergo greater oxidative deterioration. Based on the backfat colour, consistency and storage stability measurements combined with sensory evaluation results of grilled chops, for fresh meat production, the PUFA content of the feed can be as high as 18 g PUFA/kg feed, corresponding to 22 % PUFA in the outer backfat layer of gilts and 18 % in the case of barrows (Van Oeckel et al., 1996). Problems could however occur for cured meat processing since the influence of PUFA are not only exerted through backfat, but also through the intramuscular fat in meat (Warnants et al., 1996). They recommended a PUFA level (C18:2 as the major PUFA) not higher than 18 g PUFA/kg feed resulting in a maximum of 22 % PUFA in backfat, at least if the pork is to be used for direct consumption. Feed PUFA contents of 26 g/kg corresponding to 21 % PUFA in the backfat was specified as a limit to be used for dry sausage manufacture, especially to avoid drying and consistency problems. Backfat containing 25 % PUFA was regarded to be totally unsuitable for dry sausage production (Stiebing et al., 1993). They recommended that, no more than 14 % PUFA in backfat should be used in dry sausage manufacture. According to Warnants et al. (1998), 28 g PUFA/kg feed which was equivalent to 24 to 26 % PUFA in backfat and 18 % PUFA in salami was beyond the limit for a typical Belgian lean fattening pig based on sensory panel and instrumental evaluations. Apparently, 25 g PUFA/kg feed resulting in 23 and 15 % PUFA in backfat and salami respectively seems to be more acceptable in view of the suitability for salami manufacture and salami taste. A PUFA content of less than 21 g/kg feed (resulting in 20 and 14 % PUFA in the backfat and salami respectively) is recommended. They also emphasised that an improvement in salami firmness might be achieved by changing the process parameters or by adding firmer lard, whereas an aberrant taste cannot be rectified. On the other hand, they suggested that feed supplementation of 25 g PUFA/kg (predominantly C18:2) corresponding to a PUFA: SFA ratio of 0.38 in the salami will not cause any real taste problems.

Modification of the dietary regime of meat-producing monogastric animals is the most practical way of increasing the monounsaturates in human diet (Miller et al., 1993) and fermented sausages e.g salami, being a high fat content (30-50 %) meat product (Lois et al., 1987; Lücke, 1994), would benefit in nutritional quality from an increase of PUFA at the expense of the SFA in pork backfat.

The aim of this study was to establish the effect of incorporation of highly PUFA e.g. 3 % sunflower oil, in pig feed on the lipid stability of a South African salami.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Animals were fed one of two diets, a typical South African finishing diet (control), and the control diet supplemented with 3 % sunflower oil. The aim of this dietary supplementation was basically to induce two kinds of backfat, namely one with a more normal PUFA level and the other with a slightly elevated PUFA content.

Two groups of large white gilts were used. Each group consisted of six (6) animals. Pigs were slaughtered at Bloemfontein abattoir under commercial slaughtering conditions at an average weight of 110 kg for each group.

4.2.2 Preparation of the fermented sausages

Two salami batches were formulated according to Table 5 in chapter 3, p. 37 of this thesis and salami was produced using only pork ingredients from the specific diet group. Sausages were prepared according to the procedure (3.2.1) described in chapter 3, p. 38 of this thesis, however the matured salamis were vacuum-packaged and stored at 12 ^{0}C only.

4.2.3 Sampling procedure

Sampling was done according to the technique (3.2.2) outlined in chapter 3, p. 38 of this thesis, although the matured vacuum-packaged salamis were sampled after 30 days storage only.

4.2.4 Chemical and physical analyses

All reagents were analytical grade obtained from Merck, unless indicated otherwise.

4.2.4.1 General parameters

4.2.4.1.1 Weight loss

The weight loss of the salami batches was monitored as in (3.2.3.1.1) chapter 3, p. 39 of this thesis.

4.2.4.1.2 pH determination

pH was determined according to the procedure described in (3.2.3.1.2) chapter 3, p. 39 of this thesis.

4.2.4.1.3 Protein determination

Protein content was determined according to the procedure described (3.2.3.1.3) chapter 3, p. 39 of this thesis

4.2.4.1.4 Salt content (% NaCl)

Salt (% NaCl) was determined according to the technique described in (3.2.3.1.4) chapter 3, p. 39 of this thesis.

4.2.4.1.5 Nitrites ($NaNO_2$)

Nitrites (ppm $NaNO_2$) were determined according to the technique described in (3.2.3.1.5) chapter 3, p. 39 of this thesis.

4.2.4.1.6 Water activity (A_w)

A_w was measured using the procedure described in (3.2.3.1.6) chapter 3, p. 40 of this thesis.

4.2.4.1.7 TBA – mg malonaldehyde/1000g meat

Thiobarbituric acid reactive substances (TBARS) were determined according to the procedure outlined in (3.2.3.1.7) chapter 3, p.40 of this thesis.

4.2.4.2 Analytical methods for the analyses of fat

4.2.4.2.1 Lipid extraction

4.2.4.2.1.1 Feeds

Lipids were extracted from the feeds according to the method outlined in (3.2.3.2.1) chapter 3, p. 40 of this thesis. The extracted fat was used to determine the fatty acid composition of the feed.

4.2.4.2.1.2 Backfat

Lipids were extracted from the backfat according to the method outlined in (3.2.3.2.1) chapter 3, p. 40 of this thesis. The extracted lipids were used for determining the IV and fatty acid composition of the pork backfat. The fatty acid data was used to calculate the percentages of the individual fatty acids including DBI = Σ % unsaturated fatty acids x number of double bonds of each unsaturated fatty acid (Alam and Alam, 1986).

4.2.4.2.1.3 Salami

Lipids were extracted according to the procedure described in (3.2.3.2.1) chapter 3, p 40 of this thesis.

4.2.4.2.1.4 Iodine value (g iodine/100 g lipid)

IV were determined on the pork backfat and salami samples according to the technique described in (3.2.3.2.1.1) chapter 3, p. 40 of this thesis.

4.2.4.2.1.5 Peroxide value (milliequivalents/1000 g lipid)

PV were determined according to the procedure outlined in (3.2.3.2.1.2) chapter 3, p. 41 of this thesis.

4.2.4.2.1.6 Free fatty acids (% oleic)

FFA were determined according to procedure (3.2.3.2.1.3) in chapter 3, p. 41 of this thesis.

4.2.4.2.1.7 Carbonyl compounds (mmole/1000 g lipid)

UC, SC and TC were determined according to the procedure described in (3.2.3.2.1.4) chapter 3, p. 41 of this thesis.

4.2.4.2.1.8 Fatty acids determination

Fatty acid composition was determined according to the procedure described in (3.2.3.2.1.7) chapter 3, p. 41 of this thesis.

4.2.5 Statistical analyses

Differences in salami were determined by ANOVA using a NCSS 2000 (Cruncher Statistical Systems, Kaysville) statistical package. Newman and Keuls multiple range test at $\alpha = 0.05$ was used to identify differences between means.

4.3 RESULTS AND DISCUSSIONS

The feeding regimes consisting of a typical South African finishing diet (control) and the control diet supplemented with 3 % sunflower oil (sunflower) differed in fatty acid composition (Table 9). The control diet was rich in palmitic (C16:0), oleic (C18:1c9), linolenic (C18:3c9, 12, 15), SFA and MUFA, and the proportion of these fatty acids was significantly higher than in the sunflower oil supplemented feed treatment. The sunflower oil supplemented treatment on the other hand had significantly higher proportions of stearic (C18:0), linoleic (C18:2c9, 12) and PUFA than the control diet (Table 9). Dietary treatment was well reflected in the backfat fatty acid composition (Table 10). The control diet backfat had significantly higher quantities of palmitic, palmitoleic, oleic, vaccenic (C18:1c7), linolenic, SFA and MUFA than the sunflower oil supplemented treatment , while linoleic, eicosadienoic (C20:2c11, 14) and PUFA were significantly higher in the sunflower oil supplemented treatment backfat than the control backfat (Table 10). There were however no significant differences in the C10:0 and stearic acid content of the backfat, although the proportions of these fatty acids were higher in the control than in the sunflower oil supplemented treatment backfat (Table 10). Dietary treatment was also reflected in the muscle lipids (Table 11), although the effects were not significant. Myristic, palmitic, palmitoleic, oleic, vaccenic, SFA and MUFA were higher in muscle from animals fed the control diet while stearic, linoleic, arachidonic and PUFA were higher in muscle from the sunflower oil supplemented diet regime (Table 11).

These results agree with previous reports that, diets with a high linoleic acid content increases the linoleic acid content of pig adipose tissue and decreases the oleic acid content of pig adipose tissue (Marchello et al., 1983; Kouba and Mourot, 1999) and backfat (Warnants et al., 1996; Warnants et al., 1998). The observed decreases in SFA and MUFA especially an overall decrease in SFA from a sunflower-seed supplemented diet and an increase in PUFA have also been reported (Marchello et al., 1983). Marchello et al. (1983) recommended that, to avoid soft-oily carcasses that are unacceptable for consumers and processors alike, diets for growing-finishing swine should contain less than 13 % sunflower seed (approximately 5 % oil). For this reason the sunflower-oil supplemented diet used in this study was within this limit. The PUFA content of the backfat from the sunflower oil supplemented diet in this study (Table 10) was above the recommended limits for cured meat processing, namely 22 % PUFA in the outer backfat of gilts and 18 % for barrows (Van Oeckel et al., 1996), 22 % PUFA in the backfat (Warnants et al., 1996), 21 % PUFA backfat (Stiebing et al., 1993). According to Stiebing et al. (1993), backfat containing 25 % PUFA is unsuitable for dry sausage production and no more than 14 % PUFA in the backfat should be used in dry sausage manufacture. Warnants et al. (1998) recommended 21 % PUFA/kg feed resulting in 20 and 14 % PUFA in the backfat and salami respectively as the most suitable limit for salami manufacture and taste.

The suitability of backfat for salami manufacture can also be determined according to its IV (g iodine/100 g lipid) and double bond index (DBI) (Alam and Alam, 1986). Where the DBI = $\sum$ (mono-double bonds x 1) + ($\sum$ (di-double bonds x 2) + $\sum$ (tri-double bonds x3) +...$\sum$ (N-double bonds x N), where N is the highest number of double bonds in the fatty acids that make up the lipid fraction of the substrate. According to Prabucki (1991), the DBI of good quality fat should be less than 80. An IV of 70 g/100 g lipid has been recommended as a critical value for good quality fat (Barton-Gade, 1987) and good quality adipose tissue (Houben and Krol, 1983). The IV of the backfat were 71.91 ± 5.06 and 82.00 ± 5.26 g/100 g lipid, while the DBI values were 92.77 ± 5.94 and 105.30 ± 7.72 for the control and sunflower oil supplemented treatments respectively (Table 10). Although the PUFA content (30.03 ± 4.53), IV (82.00 ± 5.26 g iodine/100 g lipid) and DBI (105.30 ± 7.72) of the sunflower oil supplemented treatment backfat were above the recommended limits for good quality fat (Table 10), there were no processing problems encountered in the salami manufactured from this treatment. The weight losses were similar, namely 21.56 and 22.04 % after 21 days production for the control and sunflower oil supplemented treatment salami respectively (Table 12), and no fat exudate was observed on the salami from the sunflower oil supplemented diet.

A decline in pH was observed within each dietary treatment throughout production, although there was no significant difference in pH of the salamis between the control and the sunflower oil supplemented treatment throughout the entire processing period (Fig. 15). This implied that PUFA level had no significant effect on the pH decline of the salami (Fig. 15). There were no significant differences in the moisture (% w/w per 100 g raw sausage), fat (% w/w per 100 g raw sausage), and fat free dry matter (% w/w per 100 g raw sausage) of the sunflower oil supplemented treatment and control salamis throughout the entire production period (Table 12). Due to drying the moisture content decreased, while the fat, FFDM, protein, salt (% NaCl) and nitrite content (ppm $NaNO_2$) increased (Table 12). As the sausages dried, their a_w decreased from 0.954 to 0.926 and 0.942 to 0.916 for the control and sunflower oil supplemented diet treatment salamis respectively.

There were no significant differences in the FFA content (% oleic) of the raw control and sunflower oil supplemented treatment salamis, although the FFA content of the sunflower oil supplemented treatment salami was higher than that of the control (Fig. 16). FFA increased with time in both treatments (Fig. 16). The FFA content of sunflower oil supplemented salami was significantly higher than that of the control after fermentation and ripening, whereas there were no significant differences in the FFA content of the salamis after storage. The sunflower oil supplemented treatment salami had the highest FFA content after storage. This implied that, lipolysis was more pronounced in the sunflower oil supplemented treatment salami (Fig. 16). Warnants et al. (1998) reported an increase in FFA (g oleic/100 g total fatty acids) especially in salami batches prepared with fat from gilts compared with that from barrows. Lipolysis was not however affected by dietary treatment.

Lipid oxidation was progressive in both salami treatments throughout the entire production period, although there was a slight decline in PV of the sunflower oil supplemented treatment salami after ripening (Fig. 17). The PV (milliequivalent/1000 g lipid) of the sunflower oil supplemented treatment salami was significantly higher than that of the control throughout production, implying that PUFA in the diet had a significant effect on lipid oxidation (Fig. 17). Rapid lipid oxidation in the form of peroxide numbers and *p*-anisidine was observed during storage of cervelat sausages and back bacon (Houben and Krol, 1980). There was an increase in TBA value (mg malonaldehyde/1000 g meat) for both salami treatments throughout production, although the TBA values of the raw salamis were not significantly different (Fig. 18). The TBA value of the control salami was less than the critical TBA value for rancidity (TBA < 1.0) until after ripening, while only raw and fermented sunflower oil supplemented treatment salami had TBA values lower than the critical value (Buckley and Connoly, 1980). There were significant differences in TBA values of the salamis after fermentation, ripening and storage, while the TBA values of the ripened sunflower oil supplemented treatment salami and for both treatments after storage were above the critical TBA for rancidity (TBA < 1.0). This implied that oxidative rancidity in salami was affected by PUFA levels in the diet. Accordingly, the sunflower oil supplemented treatment salami was not acceptable after ripening (TBA > 1), and both types of salami were unacceptable after 30 days storage.

Warnants et al. (1998) reported that oxidative stability decreased with increasing ripening time and PUFA content in the backfat. UC (mmole/1000 g lipid) increased with production time until it reached a plateau on storage in the sunflower oil supplemented treatment salami, whereas a slight decline in UC was observed in the control after ripening (Fig. 19). The UC of the sunflower oil supplemented treatment were significantly higher than those of the control salami in the raw sausage, after ripening and storage, however, there were no significant differences in the UC of the salamis after fermentation (Fig. 19). A slight decline in UC was observed on the control salami after ripening. While this decline was not statistically significant, the UC of the fermented sunflower oil supplemented treatment salami was higher than that of the control after fermentation (Fig. 19).

SC (mmole/1000 g lipid) increased progressively with time for all treatments, although a slight decline in this proportion was observed in the sunflower oil supplemented treatment salami after ripening (Fig.20). Except on the fermented sausages where SC content of the sunflower oil supplemented treatment salami was significantly higher than that of the control, there were no significant differences in the SC of the sunflower oil supplemented treatment and control salamis throughout production (Fig. 20). The SC content of the sunflower oil supplemented treatment salami were however higher than that of the control salami throughout the entire production period (Fig. 20). TC (mmole/1000 g lipid) of the sunflower oil supplemented treatment salami was significantly higher than that of the control in the raw sausage and after fermentation, whereas no significant differences were observed in the TC of the sausages after ripening and storage (Fig. 21). TC of the sunflower oil supplemented treatment salami (Fig. 21) were similar to the UC (Fig. 19) and SC (Fig. 20), but higher than those of the control salami for the entire production period. This implied that dietary PUFA content affected the liberation of carbonyl compounds from the hydroperoxides, the primary products of lipid oxidation.

Since the main source of lipids in salami is backfat, the composition of the fat extracted from salami should reflect that of the backfat and thus the dietary treatment (Warnants et al., 1998). In the current study, the effect of PUFA level was observed in the fatty acid composition of the salami (Table 13). Palmitic (C16:0), palmitoleic (C16:1c9), oleic (C18:1c9), vaccenic (C18:1c7), linolenic (C18:3c9, 12, 15), SFA and MUFA were significantly higher in the control salami than the sunflower oil supplemented treatment sausage (Table 13). This reflected the backfat fatty acid composition (Table 10). On the other hand, the sunflower oil supplemented treatment salami was significantly higher in linoleic (C18:2c9, 12) and PUFA than the control salami, whereas there was no significant difference in the myristic (C14:0) acid content of the salamis. While the stearic acid (C18:0) content of the sunflower oil supplemented treatment was lower than that of the control, except for the fermented control salami, there were no significant differences in the stearic acid content of the salamis. A similar trend was observed with eicosadienoic (C20:2c11, 12) in the sunflower oil supplemented treatment salami, where the raw sunflower oil supplemented treatment salami had a significantly higher eicosadienoic acid content. No significant differences were observed in the rest of the salamis (Table 13).

Comparing the raw and stored salami fatty acid contents, stearic acid increased in both treatments, while oleic acid declined in both treatments. An increase in linoleic acid was observed in the control while this fatty acid decreased in concentration in the sunflower oil supplemented treatment salami (Table 13). SFA decreased in the control salami and increased in the sunflower oil supplemented treatment salami. MUFA decreased in both treatments, whereas an increase in PUFA was observed in the control salami. PUFA decreased in the sunflower oil supplemented treatment sausages (Table 13).

These changes can be interpreted as confirmation of susceptibility of PUFA to oxidation. In the high PUFA content sunflower oil supplemented treatment salami, PUFA (linoleic, eicosadienoic and PUFA) were significantly oxidised. The hydrolysis of these fatty acids was more pronounced than their oxidation in the control salami. It is clear from these results that dietary PUFA affected changes in the fatty acid composition of salami. Warnants et al. (1998) reported that contrary to what could be expected on the basis of their susceptibility to oxidation, PUFA linoleic and linoleic acid were not oxidised during Belgian salami ripening.

The incorporation of PUFA (3 % sunflower oil) in pig feed increased the rate of lipid hydrolysis, hydroperoxide formation and their degradation and as a result decreased the oxidative stability of salami. The oxidative stability of the control salami however was also questionable at the end of storage (TBA > 1.0). It may therefore be concluded that the feed and backfat PUFA levels used in this study are likely to compromise the keeping quality of South African salami.

CHAPTER 5

5: THE EFFECT OF REPLACING PORK BACKFAT WITH OSTRICH AND SHEEPTAIL FAT ON THE LIPID STABILITY OF A FERMENTED MEAT PRODUCT (SALAMI).

ABSTRACT

The viability of replacing pork backfat with other unconventional fat sources like ostrich and sheeptail fat in the manufacture of a fermented dry sausage was determined by studying the changes in the lipid fraction of salami during drying and subsequent storage at 12 ^{0}C. A decline in pH was observed within each treatment and the final pH of the sheeptail fat salami was significantly higher than that of the pork backfat and ostrich fat salamis. The sheeptail fat salami had a significantly higher FFA content (% oleic) than the pork backfat and ostrich fat sausages throughout production. There were no significant differences in PV values of the salami treatment, although there was an accumulation of peroxides within each treatment. The TBA values of the sausages were not significantly different until after ripening when the TBA value of salami manufactured from pork backfat was significantly higher than that of salami manufactured from ostrich and sheeptail fat. At the end of ripening, pork backfat salami had a significantly higher TC, while the final TC of the sausages were significantly different. The ostrich fat salami lipids were rich in PUFA, the sheeptail fat salami lipids were highly saturated, while those of the pork backfat salami had an intermediary fatty acid composition. The ostrich fat salami had significantly higher redness value (a) and higher yellowness value (b*) than the pork backfat and sheeptail fat sausages. There were no significant differences in the lightness values (L*) of the sausages. The keeping quality of the sausages were similar. With regard to sensory properties the pork backfat manufactured salami was the most preferred while the ostrich fat salami was the least preferred by a sensory panel.*

5.1 INTRODUCTION

Meat lipids vary in quantity and composition within the variety of avian, aquatic and mammalian muscle foods. The content and composition of meat lipids differ within an animal depending upon the muscle function, and in addition to differences in lipid content between muscles and species, the fatty acid composition of meat has some distinct differences between species. These differences represent one of the most significant variables in determining the processing, palatability, and storage characteristics of different muscle foods (Allen and Foegeding, 1981).

Fermented sausages are one type of product in which texture traits and shelf life are susceptible to differences in pork fat quality. Since the main fat source in salami is pork backfat, the composition of the fat extracted from salami should reflect that of the backfat and the corresponding dietary treatments and gender variations (Warnants et al. 1998). The fatty acid profile of the pork backfat is one of the factors that determine the oxidative stability of a fermented meat product. The rate and extent of lipid oxidation in muscle tissue in turn is governed by a number of factors, the most important being the level of PUFA present in the particular muscle system (Buckley et al. 1995). From the point of view of high processing quality and product stability, firm lard, low in PUFA or high in oxidative stability is needed especially in raw cured products such as dry fermented sausages (Scheeder et al. 1998). According to Barton-Gade (1984) fats with an IV exceeding 70 g iodine/100 g lipids are very soft in consistency, and a critical IV of 70 g iodine/100 g lipid for good quality fat is recommended (Barton-Gade, 1987).

An adipose tissue of good quality contains less than 15 % PUFA, more than 12 % stearic acid and should have an IV of lower than 70 g iodine/100 g lipids (Houben and Krol, 1983). Warnants et al. (1996) proposed a maximum PUFA limit of 15 % and a maximum IV of 70 g iodine/100 g lipid for good quality backfat. Using a too soft fatty tissue in fermented sausages can cause problems like smearing, poor water release and fading of the cured pigment colour (Fischer, 1989). Fatty tissue with high linoleic acid content in turn is totally unsuitable for fermented raw sausage manufacture (Houben and Krol, 1983).

According to Houben and Krol (1980, cervelat-type summer sausage manufactured using pork backfat with a linoleic acid content of 26.3 % and an IV of 69.4 g iodine/100 g lipid was unacceptable because part of the fat entered the liquefying phase during the early stages of the cutter/chopper operation. Houben and Krol (1980, 1983) demonstrated that fermented sausages can not be manufactured from raw material with a PUFA content of 30 % in the backfat. The reasons given are that the polyunsaturated lipid content in this range caused the formation of a fat exudate during ripening, an end product with bad texture and a tendency to early rancidity. To avoid problems arising from the use of unsuitable raw materials in the manufacture of fermented raw sausage, it is recommended that backfat employed should have an IV of no more than 60 g iodine/100 g lipid (Fischer, 1989), whereas Barton-Gade (1987) suggested an IV of 70 g iodine/100 g lipid as the critical limit. In terms of PUFA levels, feed PUFA contents of 26 g/kg corresponding with up to 21 % PUFA in backfat is suggested as a critical limit for fermented sausage manufacture (Warnants et al., 1998). The latter value is a limit to be used in dry sausage manufacture, since the risk of drying and consistency problems are highly possible (Warnants et al., 1998). Backfat containing 25 % PUFA is totally unsuitable for dry sausage production and the use of not more than 14 % PUFA in backfat for dry sausage manufacture is recommended (Stiebing et al., 1993). According to Prabucki (1991), the DBI of good quality fat should be below 80. The DBI = Σ (mono-double bonds x 1) + Σ (di-double bonds x 2) + Σ (tri-double bonds x 3) + ...Σ (N-double bonds x N), N being the highest number of double bonds in the fatty acids that make up the lipid fraction of the substrate (Alam and Alam, 1986).

Warnants et al. (1998) reported that contrary to what could be expected on the basis of their susceptibility to oxidation, PUFA, linolenic and linoleic acid did not decrease during the ripening of salami manufactured from backfat with elevated PUFA dietary levels. Although salami lipolysis was not affected by dietary treatment, Warnants et al. (1998) reported an increase in FFA (g oleic/100 g lipid) until the levels reached a plateau after 7 weeks of storage. FFA dropped slightly in the 11th week, a factor attributed to probable FFA degradation. Oxidative stability in the form of TBA decreased with an increase in ripening time and PUFA content in the backfat. This was explained by the Buege and Aust (1978) hypothesis. This hypothesis postulates that only PUFA with three and more double bonds can form malonaldehyde. For this reason variation in malonaldehyde production may be a reflection of the fatty acid composition rather than of susceptibility to lipid oxidation. Houben and Krol (1980) also reported rapid oxidation during storage of cervelat sausages and back bacon, while using peroxide and *p*-anisidine values to assess oxidation. Higher PV were observed in fresh back bacon of soyabean oil fed pigs compared to tallow fed pigs (Houben and Krol, 1980). These differences increased during storage. This was a factor that further confirmed that differences in oxidative stability as measured by the TBA test between meat products with varying PUFA levels becomes more pronounced with increasing storage time (Stiebing et al. 1993). PUFA levels also had an effect on backfat consistency. Too high PUFA levels resulted in a softer salami (Warnants et al., 1998), although significant differences in salami firmness were observed between day 3 of production and the rest of the production time. According to Warnants et al (1998) high PUFA levels can be responsible for the discolouration of the fat and hence of the meat product. These authors reported a decrease in the Lab L* value (colour brightness) as the salami darkened after 3 weeks storage, probably due to drying, while the red colour (Lab a*) remained constant throughout processing for all treatments. This factor was attributed to the probable colour stabilisation effect of the added nitrite. Apparently, the Lab b* value (yellow colour) was higher for the two higher PUFA level salamis, although there was no evidence of yellow discolouration in the carcass fat. Warnants et al (1998) mentioned that this was in agreement with the findings that the use of high PUFA backfat (maximum of 21 % PUFA) led to an increase in yellow colour for sausages. They reported that the highest Lab b* value was observed at the beginning of ripening, followed by a decline and a leveling off to constant values after 3 weeks of ripening. This phenomenon was explained as a possible degradation of heme pigments contributing to the yellow colour compound. A consumer taste panel apparently identified odd flavours and textural differences, e.g. oilier and fishy taints in some treatments. It was not however, established whether this was due to the oxidative spoilage of the fat or of the compounds inherent to linseed which was incorporated in the backfat.

Enser et al. (1995) reported a higher oleic and palmitic acid content in pork than in lamb muscle, whereas stearic acid was present in the highest concentration in lamb muscle. The linoleic acid concentration was greater in pork than in lamb muscle. On the other hand the oleic and palmitic acid content of the adipose tissue were similar across the species, while stearic acid was almost twice as high in lamb adipose tissue as that in pork. Lamb adipose tissue had a higher concentration of myristic and C18:1 trans, while linoleic and α-linolenic acids respectively were 10-fold and almost 40 % more in pork adipose tissue than in lamb adipose tissue.

According to Paleari et al. (1998), ostrich meat has a high protein content, low fat content, and a low fat to protein ratio. Its cholesterol level is lower than that of turkey and even more so than that of bovine meat. It is rich in unsaturated fatty acids, especially PUFA, and its tenderness is similar to that of turkey. The low collagen content relative to protein makes the meat more digestible than beef. The pH of ostrich meat is however relatively high, creating the perception that it has a limited shelf life (Sales and Mellet, 1996). Due to its tenderness, low fat content and low cholesterol levels, ostrich meat is, in accordance with modern-day nutritional principles, a valid alternative to other kinds of meat (Paleari et al., 1998). The main obstacle in marketing ostrich meat is the need for consumer information about this meat that is dark in colour compared to beef. There is also the fact than there are neither corresponding cuts nor a commercialised classification system that can facilitate its use (Morris et al. 1995a,b). In spite of the latter constraints the use of ostrich meat in processed meat products can offer an alternative option for marketing non-prime cuts, in this way improving overall product quality. Undesirable mutton or lamb characteristics maybe overcome through fermentation, addition of spices, and microbial enzymatic reactions (Bartholomew et al., 1984). Economically, their use in processed meat could lower the costs of the products while increasing profitability of the mutton industry (Wu et al., 1991).

The aim of this study was to establish the possibility of replacing pork backfat with more unconventional fats like ostrich and sheeptail fat in fermented dry sausage manufacture.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of fermented sausages

Three salami batches differing in fat sources, namely pork backfat, ostrich and sheeptail fat were formulated in accordance with Table 5 chapter 3, p. 37 of this thesis. Lean beef, lean pork, pork backfat and sheeptail fat were purchased from a butchery while ostrich fat was obtained from an ostrich abattoir in the Bloemfontein region. Sausages were prepared according to the procedure (3.2.1) described in chapter 3, p. 38 of this thesis, however the matured salamis were vacuum-packaged and stored at 12 ^{0}C only.

5.2.2 Sampling procedure

Sampling was done according to the technique (3.2.2) outlined in chapter 3, p. 38 of this thesis, although the matured vacuum-packaged salamis were sampled after 30 days storage only.

5.2.3 Chemical and physical analyses

All reagents were analytical grade obtained from Merck, unless indicated otherwise.

5.2.3.1 General parameters

5.2.3.1.1 Weight loss

The weight loss of the salami batches was monitored as in (3.2.3.1.1) chapter 3, p. 39 of this thesis.

5.2.3.1.2 pH determination

pH was determined according to the procedure described in (3.2.3.1.2) chapter 3, p. 39 of this thesis.

5.2.3.1.3 Protein determination

Protein content was done in accordance with procedure (3.2.3.1.3) in chapter 3, p. 39 of this thesis

5.2.3.1.4 Salt content (% NaCl)

Salt (% NaCl) was determined according to the technique described in (3.2.3.1.4) chapter 3, p. 39 of this thesis.

5.2.3.1.5 Nitrites ($NaNO_2$)

Nitrites (ppm $NaNO_2$) were determined according to the technique described in (3.2.3.1.5) chapter 3, p. 39 of this thesis.

5.2.3.1.6 Water activity (A_w)

A_w was measured using the procedure described in (3.2.3.1.6) chapter 3, p. 40 of this thesis.

5.2.3.1.7 TBA – mg malonaldehyde/1000 g meat

Thiobarbituric acid reactive substances (TBARS) were determined according to the procedure outlined in (3.2.3.1.7) chapter 3, p.40 of this thesis.

5.2.3.1.8 L-hydroxyproline content

The L-hydroxyproline content was determined using a colorimetric technique based on the reaction of oxidised hydroxyproline (i. e. as a pyrrole) with p-dimethyloaminobenzaldehyde (ISO, 1978).

5.2.3.1.9 Colour measurement

The lightness (L*), redness (a*) and yellowness (b*) colour co-ordinates were measured using a MINOLTA Chroma Meter type CR-2000, (MINOLTA, JAPAN). Two measurements were taken on the surface of six (6), 25mm thick slices of salami. Data presented are means of 12 measurements.

5.2.3.2 Analytical methods for the analysis of fat

5.2.3.2.1 Lipid extraction

5.2.3.2.1.1 Fat

Lipids were extracted from the pork backfat, ostrich and sheeptail fat according to the method outlined in (3.2.3.2.1) chapter 3, p. 40 of this thesis. The fatty acid data was used to calculate the percentages of the individual fatty acids including DBI = Σ % unsaturated fatty acids x number of double bonds of each unsaturated fatty acid (Alam and Alam, 1986).

5.2.3.2.1.2 Salami

Lipids were extracted according to the procedure described in (3.2.3.2.1) chapter 3, p 40 of this thesis.

5.2.3.2.1.3 Peroxide value (milliequivalents/1000 g lipid)

PV were determined according to the procedure outlined in (3.2.3.2.1.2) chapter 3, p. 41 of this thesis.

5.2.3.2.1.4 Free fatty acids (% oleic)

FFA (% oleic) were determined according to the procedure described in (3.2.3.2.1.3) chapter 3, p. 41 of this thesis.

5.2.3.2.1.5 Carbonyl compounds (mmole/1000 g lipid)

UC, SC and TC were determined according to the procedure described in (3.2.3.2.1.4) chapter 3, p. 41 of this thesis.

5.2.3.2.1.6 Iodine value (g Iodine/100 g lipid)

IV were determined on the pork backfat and salami samples according to the technique described in (3.2.3.2.1.1) chapter 3, p. 40 of this thesis.

5.2.3.2.1.7 Fatty acids determination

Fatty acid composition was determined according to the procedure described in (3.2.3.2.1.6) chapter 3, p. 41 of this thesis.

5.2.4 Statistical analyses

Differences in salami manufactured from the different fat sources were determined according to the procedure described in (4.2.5) chapter 4, p. 57 of this thesis.

5.2.5 Sensory analysis

Sensory characteristics of the salami were evaluated using preference ranking (Basker, 1988). Sixty-six (66) panelists (17-62 years of age) were recruited for the panel session from students (15) and staff (51) of the Faculty of Agriculture, University of the Orange Free State (35 females: 31 males). The salami was cut into thin slices, folded and secured with a toothpick. For evaluation the samples were presented on white polystyrene trays. Panelists assigned ranks by using a scale with 1 = most preferred sample and 3 = least preferred sample. Tap water at room temperature was provided for rinsing between samples during taste sessions. Thinly sliced apples were also available to clean the palate from excessive fat-buildup. Three salami samples (ostrich, sheep, pork) were evaluated by panelists. Samples were coded using three-digit numbers picked from a table of random numbers. Evaluations were performed at room temperature (20-22 ^{0}C), in individual testing booths under red light in the Sensory Evaluation Laboratory, Department of Food Science, University of the Orange Free State.

5.3 RESULTS AND DISCUSSIONS

Table 14 shows the results of the chemical analysis of the three fat sources. The pork backfat had the highest moisture content (w/w per 100 g raw sausage), highest FFDM and protein content, while the sheeptail fat values were the lowest. Ostrich fat proximate values were intermediary. The L-hydroxyproline content of the ostrich and sheeptail fat were similar and about half that of the pork backfat. Collagen is assumed to consist of 14 % L-hydroxyproline (Bailey and Light, 1989), therefore the low L-hydroxyproline content of ostrich and sheeptail fat imply that both are tender when compared to pork backfat. The sheeptail fat had the highest PV of 14.54 milliequivalents/1000 g lipid, while the ostrich fat had the lowest PV (7.68) of the three. The IV (g iodine/100 g lipid) of the three fats were less than 70, this being the critical value for good quality fat (Barton-Gade, 1987; Warnants et al., 1996). The sheeptail fat had the lowest IV of 48.27 ± 1.64 g iodine/100 g lipid, while the ostrich fat's IV was the highest at 67.43 ± 2.21 g iodine/100 g lipid. This implies that the ostrich fat is rich in PUFA while the sheeptail fat is the most saturated of the three. The SC and TC (mmole/1000 g lipid) of the pork backfat were higher than for the other fats, with the exception of the UC, which were highest in the sheeptail fat.

The three fats had distinct differences in their fatty acid composition. The sheeptail fat tended to have a higher proportion of saturated individual fatty acids (% total lipid) e.g. myristic acid (C14:0) 6.42 ± 0.02 %, heptadecanoic (C17:0) 1.80 ± 0.14 %, and traces of decanoic (C10:0) and lauric acid (C12:0) which were not detected in the other fats. The ostrich fat had the highest palmitic acid (C16:0) content, while the pork backfat had the highest stearic acid (C18:0) content. The high degree of saturation of the sheeptail fat was further reflected in the overall SFA content of this fat which was the highest at 47.57 ± 0.88, compared to 42.18 ± 0.52 and 37.40 ± 0.33 % for pork backfat and ostrich fat respectively. On the other hand the ostrich fat was rich in unsaturated fatty acids, especially palmitoleic acid (C16:1c9) at 8.20 ± 0.24, and linoleic acid (C18:2c 9, 12) which was 15.05 ± 0.22 %, whereas the pork backfat had the highest concentration of oleic acid (C18:1c 9). The PUFA content of ostrich fat was the highest at 17.60 ± 0.40, pork backfat had 11.52 ± 0.34 % PUFA, while the sheeptail fat had the lowest PUFA concentration at 5.53 ± 2.32 %. The pork backfat had the highest proportion of MUFA (42.87 ± 0.43), while its SFA and PUFA content were intermediary between the ostrich and sheeptail fat. The DBI of the fat was 71.71 ± 0.73, 81.78 ± 0.38, and 54.70 ± 2.29 for pork backfat, ostrich and sheeptail fat respectively (Table 14). Except for the ostrich fat, the DBI of the fat types were below 80, this being the critical DBI for good quality fat (Prabucki, 1991).

There was a decline in pH in all the sausage types (Fig. 22) throughout the production period. The initial pH of the control batch and ostrich fat sausage were not significantly different, but the two were significantly higher than that of the sheeptail fat salami. After fermentation, the ostrich fat sausage had the highest pH, the sheeptail fat salami the lowest and the pH of the sausages were significantly different (Fig. 22). The pH of the sheeptail fat salami was significantly higher than that of the pork backfat and ostrich fat sausages after ripening and storage (Fig. 22).

The salamis lost weight as they dried (Table 15) and their a_w decreased from 0.940 - 0.892, 0.910 - 0.893 and 0.910 - 0.879 for the pork backfat, ostrich and sheeptail fat salami respectively. The moisture content increased slightly on fermentation and decreased throughout the entire production period for each treatment (Table 15). There were no differences in moisture content between the treatments until storage when the moisture content of the sheeptail fat salami was significantly higher than for the ostrich fat salami (Table 15). As a result of the slight increase in the moisture content during fermentation, the FFDM proportion of the salamis declined, although the fat (Table 15) and FFDM content (Table 17) of all treatments increased throughout the entire production period. The changes in these parameters were not significantly different. The initial moisture content of the three salami types ranged between 58.20 and 58.63 % and it decreased to 46.91 to 48.06 %, while the fat content ranged from 16.42 to 17.78 % in the raw sausage and increased to 24.00 to 23.57 % in the ripened salamis after storage. The initial protein content of the ostrich fat salami was slightly higher than that of the pork backfat and sheeptail fat sausages and as a result of drying the protein content of all treatments increased throughout the entire production period (Table 15). The pork backfat salami had the highest protein content in the ripened and stored sausage while the protein content of the sheeptail fat salami remained the lowest throughout production (Table 15). As the sausages dried, the nitrite (Table 15) and salt (Table 15) content increased, although the differences in salt and nitrite content of the treatments were marginal.

The FFA content (% oleic acid) of the raw sheeptail fat salami was significantly higher than that of the pork backfat and ostrich fat sausages (Fig. 23). The sheeptail fat salami accumulated a higher proportion of FFA during fermentation, and there was a significant difference between the FFA content of this treatment and that of the control and ostrich fat treatments (Fig. 23). An increase in FFA content was observed in all sausage types during ripening. The FFA content of the pork backfat and sheeptail fat sausages were however significantly higher than that of the ostrich fat salami after ripening (Fig. 23). The FFA content of the salami treatments were significantly different at the end of storage (Fig. 23), the FFA content of the sheeptail fat salami was the highest while the FFA content of the ostrich fat salami was the lowest (Fig. 23).

There were no significant differences in the PV of the raw sausages (Fig. 24), although the control batch had the highest initial PV and the PV of the sheeptail fat salami was the lowest. A build-up of peroxides was observed in all salami types during fermentation, but there were no significant differences in PV between the salami types (Fig. 24). The PV of the control batch was the highest (Fig. 24). Hydroperoxides continued to increase within individual salami types during ripening, although there were no significant differences in PV between the sausage types after ripening (Fig. 24). The ostrich fat salami had the highest PV after this phase. There were still no significant differences in the PV of the salami types at the end of storage (Fig. 24). The PV of the ostrich fat salami was the highest while the lowest PV was observed on the sheeptail fat salami after storage (Fig. 24). The TBA value (mg malonaldehyde/1000 g meat) of the raw pork backfat salami was significantly higher than that of the ostrich and sheeptail fat sausages (Fig. 25). There were no significant differences in the TBA value of the sausages after fermentation (Fig. 25). There was an increase in TBA value for all sausage treatments and the TBA values of all treatments were superior to the critical TBA value (TBA > 1.0), indicating the possible development of rancidity (Buckley and Connoly, 1980). The control batch had a significantly higher TBA value than the ostrich and sheeptail fat sausages at the end of the ripening period, whereas a drastic decline in the TBA value of the sheeptail fat salami was observed during this phase (Fig. 25). A slight decline was observed on the TBA value of the control batch, while the TBA value of the ostrich fat salami increased. The TBA values of these sausages were nevertheless still above the critical TBA for rancidity (TBA > 1.0) at the end of the storage phase (Fig. 25). The TBA value of the control and ostrich fat salamis were significantly higher than that of the sheeptail fat sausage which had declined to a value similar to that of the raw sausage by the end of storage (Fig. 25).

UC of the raw and fermented salami treatments were similar and there were no significant differences in UC of the sausages (Fig. 26). The UC of ripened pork backfat salami was significantly higher than that of the sheeptail fat sausage while the ostrich fat salami had an UC content similar to both treatments after ripening (Fig. 26). The ostrich and sheeptail salami UC increased while the UC of the pork backfat salami decreased on storage. The UC of the ostrich fat salami was significantly higher than that of the control and sheeptail fat salami after storage (Fig. 26). The raw and fermented sausage SC (mmole/1000 g lipid) were similar and there were no significant differences in SC after these phases (Fig.27). SC of the pork backfat salami were significantly higher than that of the ostrich and sheeptail fat sausages after ripening, while the pork backfat and ostrich fat sausages had significantly higher SC than the sheeptail fat after storage (Fig. 27). SC decreased on fermentation, increased during ripening and increased again in the stored pork backfat salami, while a decrease in SC during fermentation through ripening and followed by an increase during storage was observed in the ostrich fat salami. SC of the sheeptail fat salami decreased gradually during the entire production period (Fig. 27). There were no significant differences in the TC (mmole/1000 g lipid) of the raw sausages, although the ostrich fat had the lowest initial TC content (Fig. 28). Minimal changes were observed in TC of the individual salami types during fermentation (Fig. 28), and the changes in TC between treatments were not significant. The TC content of the control batch was significantly higher than that of the ostrich and sheeptail fat salamis after ripening (Fig. 28). Significant differences were observed in the TC content of the salamis after storage with the ostrich fat salami presenting the highest TC value (Fig. 28).

The ostrich fat was rich in PUFA (Table 14), while the sheeptail fat was highly saturated, and therefore one would expect a higher FFA content (% oleic) in the ostrich fat salami due to the preferential liberation of long-chain PUFA from the lipid body. However, in the current study there was a more significant buildup of FFA in the sheeptail fat sausage than in the ostrich fat salami. On the other hand, the low concentration of FFA in the ostrich fat salami (Fig. 23) was accompanied by a high concentration of hydroperoxides (Fig. 24) after ripening and storage and a significantly high concentration of TC in the stored product (Fig. 28). It is correct to postulate that although the ostrich fat was highly unsaturated, the PUFA liberated from its lipid body were oxidised into hydroperoxides. These were themselves broken down into carbonyl compounds as indicated by the build up of hydroperoxides and carbonyl compounds (UC, SC and TC). The preferential release of long-chain PUFA which otherwise would be confirmed by a higher concentration of FFA in the ostrich fat salami was not observed because the rate of oxidation of these long-chain fatty acids was higher than their hydrolysis from the lipid body. The high FFA content observed in the sheeptail fat salami was not accompanied by high PV (Fig. 24) and TC (Fig. 28) because sheeptail fat is rich in saturated fatty acids which are less susceptible to oxidation than PUFA.

The myristic acid (C14:0) content of the sausage types were significantly different throughout production, with the highest content observed in the sheeptail fat salami while the ostrich fat salami had the lowest (Table16). There was a decline in myristic acid of the sheeptail fat salami throughout production, and the decline was marginally significant between the processing phases (Table 16). There were very little changes in this fatty acid in the pork backfat and ostrich fat salamis throughout production. There were significant differences in the palmitic (C16:0) acid content of the sausages (Table 16). The palmitic acid content of the sausages decreased significantly in the order, ostrich fat salami > pork backfat sausage > sheeptail fat salami, which reflected the concentration of this fatty acid in the fat sources (Table 14). With the exception of the differences ascribed to species, there were no significant changes in the stearic (C18:0) acid content of the sausages (Table 16). The ostrich fat salami had significantly high proportions of palmitoleic (C16:1c9) acid while the pork backfat sausages were the poorest in this fatty acid (Table 16). Except for the ostrich fat salami where changes in palmitoleic acid were significant between the phases, there were no significant changes in this fatty acid within any of the other treatments (Table 16).

There were significant differences in oleic (C18:1c9) acid between the treatments with the highest concentrations observed in the control salami and the lowest in the ostrich fat salami (Table 16). The oleic acid decreased during fermentation and ripening and increased on storage in the pork backfat salami, while a decrease during fermentation and ripening was followed by an increase during storage in the ostrich and sheeptail fat manufactured sausages.

Linoleic (C18:2c9, 12) acid increase during fermentation, decreased during ripening and increased again during storage in the case of the ostrich fat salami, these changes were however not significant (Table 16). A significant decrease during ripening and storage was observed for linoleic acid in the pork backfat salami, while an increase during fermentation and ripening and a decrease during storage was observed in the sheeptail fat salami. These changes were however not significant (Table 16). The linoleic content of the raw and fermented pork backfat salami were significantly higher than that of the other treatments (Table 16).

The SFA content of the sheeptail fat salami was significantly higher than that of the pork backfat and ostrich fat sausages and there were differences in the SFA content of the sheeptail fat throughout processing (Table 16). The SFA content of the sheeptail fat salami decreased throughout the entire processing, while increases and decreases were observed in the pork backfat and ostrich fat sausages. The MUFA contents of the three sausage types were similar and not significantly different. The ostrich fat salami had a significantly higher PUFA content than the pork backfat and ostrich fat sausages (Table 16). The pork backfat salami PUFA however decreased during fermentation and increased during ripening and storage, while a decrease in PUFA during fermentation and ripening followed by an increase on storage was observed in the ostrich fat salami (Table 16). These changes were not significant. PUFA increased during fermentation, decreased during ripening and increased again during storage in the sheeptail fat salami (Table 16).

The type of fat (Table 17) significantly affected the redness (a*) and yellowness (b*) of the salami, while the differences in the lightness (L*) of the sausages were not significant ($p < 0.005$). The degree of lightness (L*) decreased in the order pork backfat > sheeptail fat > ostrich fat salami, implying that the salami manufactured with the pork backfat (50.74 ± 4.40) was lighter than that made from sheeptail (47.90 ± 2.18) and ostrich fat (47.47 ± 3.79) (Table 17). The ostrich fat was the most red (a*) of the three salami types. The degree of redness (a*) of the ostrich fat salami (14.89 ± 2.14) was significantly higher than that of the pork backfat salami (12.76 ± 1.89), while the sheeptail fat salami redness (13.83 ± 1.42) was not significantly different from that of either of the other two (Table 17). The ostrich fat salami (11.27 ± 1.29) was significantly more yellow (b*) than the pork backfat (9.35 ± 1.09) and ostrich fat (9.33 ± 0.40) salamis, whereas the degree of yellowness of the pork backfat and sheeptail fat was similar and not significantly different (Table

17). There were no problems with the sausage appearance, separation of salami from casing, no evidence of fat exudation under the casing (Bloukas et al., 1997) nor were there any sliceability problems (Bloukas et al., 1997; Warnants et al., 1998).

A panel of 66 assessors ranked the three salami samples and the following results were obtained:

Table 18: Salami ranking in the sensory evaluation assessment

Sample		A (OSTRICH)	B (SHEEP)	C (PORK)
Rank sum		172	131	93
Difference vs	A	NA	41	79
	B	NA	NA	38

NA., not applicable.

According to (Basker, 1988), the p = 0.05 significance level is attained when the rank sum differences are greater than or equal to 26.9, and the p = 0.01 significance level is attained when the rank sum differences are greater than or equal to 33.5

Table 19: Significance levels and critical differences for salami sensory evaluation

Significance level		**p = 0.01**
Critical difference		33.5
Sample	C (pork)	a
	B (sheep)	b
	A (ostrich)	c

a, b, c., at 99% (p = 0.01) confidence levels, the sensory panel identified significant differences in the salami (A., ostrich; B., sheep; C., pork).

Product C (pork backfat salami) was significantly preferred (p = 0.05 and 0.01) over products B (sheeptail fat salami) and A (ostrich fat salami), and product A was the least preferred. There were no overlapping ranges of preferences; and the panel therefore evaluated them as being different in taste.

In the present study, lipid oxidation judged according to the TBA value was observed in all treatments (TBA > 1) after fermentation (Fig. 25) although the sheeptail fat salami showed a decline in TBA value after ripening and storage. The sensory evaluation panel however accepted the pork backfat salami and rejected the ostrich fat sausage in spite of the two treatments having similar TBA values. These values were not significantly different after storage. Miller et al. (1993) reported high indices of lipid oxidation in the form of TBA number although the sausages similarly proved to be sensorically acceptable. This can be taken to be a confirmation of the hypothesis that, variations in malonaldehyde production may be more a reflection of the fatty acid composition than of susceptibility to lipid oxidation (Buege and Aust, 1978)

The study has demonstrated that the changes and overall stability of the lipid fraction of salami are highly dependent on the raw material fat quality, especially the fatty acid composition. The sausages presented similar changes in terms of FFA buildup, peroxide deterioration, carbonyl value and TBA accumulation, indicating that all treatments incurred some degree of hydrolysis and lipid oxidation. Although there were differences in the magnitude of these parameters as a result of the varying degrees of saturation or unsaturation of the fat sources, there were no gross differences in the quality of the final products. Nevertheless the sensory evaluation results indicated that consumers preferred the pork backfat while the ostrich fat salami was the least preferred Table 20 and Table 21). It is therefore correct to conclude that pork backfat can be replaced with sheeptail fat in salami manufacture since fermentation successfully reduces the characteristic objectionable flavour, toughness and waxy fat of mutton and lamb (Bartholomew et al. 1984). On the other hand, replacement of pork backfat with ostrich fat in salami manufacture may result in a rejection of the product by consumers.

CHAPTER 6

6: GENERAL CONCLUSIONS

6.1 Background

Over and above the preservative effect that fermentation has on a number of commodities, fermented foods are a delicacy due to their unique flavour and aroma. Furthermore, fermented foods have been enjoyed for many years because of the health aspects usually attributed to them (Zeuthen, 1995). According to Huis in't Veld et al. (1990), health aspects related to fermented foods include, increased digestibility, therapeutic properties such as ability to reduce the level of serum cholesterol, antitumour activity, effects on the immune system, and also bacteriocin and probiotic activity. Fermented meats do not usually offer this wide range of benefits, although fermenting microorganisms have been known to be inhibitory to competing pathogens (Anon, 1990; Lawrie, 1995), a factor which can be credited for prevention of food poisoning outbreaks due to fermented meat consumption (Zeuthen, 1995). On the other hand, ingestion of fermented foods exposes the consumer to the risk of ingesting mycotoxins and amines, possible by-products of fermentations (Zeuthen, 1995). Fermented sausages e.g. salami have a high fat content 30-50 % (Lois et al., 1987; Lücke., 1994). Pork meat has also been blamed to be too high in fat, especially in saturated fat and consequently should be avoided by those who suffer from elevated serum cholesterol and hypertension (Warnants et al., 1998).

According to Allen and Foedeging (1981), intramuscular lipids are involved in many quality traits of meat products such as, nutritional value (energy, fatty acid and cholesterol supply), sensory attributes (tenderness, juiciness, colour and flavour) and technological properties (shelf life). Lipolysis and oxidation are the two main biochemical processes that take place in the lipid fraction during dry sausage ripening, while the distinct dry fermented sausage flavour is related to the hydrolytic and oxidative changes of the lipid fraction (Roca and Incze, 1990). According to the literature, lipid hydrolysis and oxidation are influenced by factors such as, processing (heating, mincing, mixing); storage conditions (temperature, time); pH; a_w; lipid composition; metals; haem compounds; nitrite; salt and additives (Demeyer et al., 1974; Quintanilla et al., 1996; Chizzolini et al., 1998; Novelli et al., 1998).

An increase in PUFA at the expense of the SFA in pork fat can lead to better agreement with the dietary guidelines of 10 energy % of each of the fatty acids: SFA, MUFA and PUFA in the human diet and consequently to a better image of pork to the consumer (Warnants et al., 1998). On the other hand, polyunsaturated fats are known to be highly digestible (Eeckhout et De Paepe, 1988), and polyunsaturated fats can improve the digestibility of saturated fats due to their role as emulsifying agents (Bayley and Lewis, 1965). Nevertheless the beneficial effect of PUFA with regard to cardiovascular diseases is accompanied by inferior fat consistency and storage stability of the product (Houben and Krol, 1980). Replacement of pork backfat with other unconventional fat sources will influence the fatty acid profile of salami and hence its keeping quality. It also provides an alternative to pork fat in the light of its SFA, MUFA and PUFA levels.

An investigation into the effect of temperature on the lipid stability of a South African salami was aimed at establishing a suitable storage temperature relative to lipid stability. A further aim was to use that storage temperature to determine the viability of dietary PUFA enrichment and replacement of pork backfat in salami manufacture with other locally available fat sources.

6.2 Storage temperature

The abuse of temperature can be detrimental to the keeping quality of salami because lipid hydrolysis and oxidation are strongly influenced by temperature (Johansson et al., 1994; Wang et al., 1995; Sorensen and Samuelsen, 1996; Navarro et al., 1997; Sorensen, 1997). In the current study, lipid hydrolysis was demonstrated by an increase in FFA (% oleic acid) throughout processing. Significant increases were observed with increases in storage temperature and time. There was an increase in peroxides during fermentation and ripening, although peroxides decreased significantly on storage. The time-temperature effect on the changes in the lipid fraction was further confirmed by a decrease in peroxide, significant only at the 12 and 25 ^{0}C storage temperatures. There was also a significant build-up of carbonyl values at the 25 ^{0}C storage temperature after 30 days. SFA and PUFA increased during fermentation and ripening. MUFA in turn decreased, significant changes being observed during ripening. During storage, SFA and PUFA increased and MUFA decreased while increases and decreases were observed in individual fatty acids. Significantly higher changes were observed at 25 ^{0}C indicating that temperature has a significant effect on the changes in the lipid fraction of salami. There was no evidence of oxidative rancidity in the salami during processing and storage after 15 and 30 days at 4 ^{0}C and 15 days at 12 ^{0}C, since their TBA < 1.0, the critical TBA value for oxidative rancidity (Buckley and Connoly, 1980). On the other hand, the salamis stored for 30 days at 12 ^{0}C and for 15 and 30 days at 25 ^{0}C had TBA > 1.0, indicating possible development of rancidity on these sausages.

Of the temperatures studied 4 ^{0}C proved to be the best, 25 ^{0}C was unacceptable while 12 ^{0}C was satisfactory and the most practical temperature for storage of salami under South African climatic conditions.

6.3 Effect of high dietary PUFA

Modification of the dietary regimen of meat-producing monogastric animals is the most practical way of increasing the monounsaturates in the human diet (Miller et al., 1993). The increase of PUFA at the expense of SFA in pork fat can lead to a better agreement with the dietary guidelines of 10 energy % of each of the fatty acids: SFA, MUFA and PUFA in the human diet and consequently to a better image of pork with the consumer (Warnants et al., 1998). An increase of PUFA in meat can however adversely affect the shelf life of meat and meat products, because PUFA are highly susceptible to oxidation (Chizzolini et al., 1998; Gandemer, 1998).

PUFA levels of 57.88 ± 0.65 and 30.03 ± 4.53 % in pig feed and pork backfat respectively were used to establish the effect of dietary PUFA enrichment on the lipid stability of South African salami. PUFA levels significantly increased hydrolysis, measured by % FFA (% oleic), hydroperoxide accumulation (indicated by an increase in PV) and their degradation, illustrated by significant increases in SC, UC and TC. PUFA levels also significantly affected the oxidative stability of the salami since the TBA values of sunflower oil supplemented treatment salami after ripening and storage were higher than the critical TBA for oxidative rancidity (TBA > 1.0), indicating possible development of rancidity. Such a possibility was however also shown in the control salami with its TBA value of 1.87 ± 0.26 after storage.

It can be concluded therefore, that feed and pork backfat PUFA levels used in the current study were likely to compromise the keeping quality of South African salami.

6.4 Replacement of pork backfat

According to Bohme et al. (1996), ostrich meat is a well-known delicacy, usually served cooked, grilled or dried (biltong). Ostrich breeding similarly is a strongly developed industry in South Africa (Paleari et al., 1998). Due however to insufficient consumer information about ostrich meat and the fact that there are neither corresponding cuts nor a commercial classification that would facilitate marketing (Morris et al., 1995a,b), ostrich meat has not infiltrated the market. On the other hand, there are a number of indigenous fat tailed breeds of sheep (Damara, Persian, Afrikaner etc.) available in South Africa, which are discriminated against by the meat grading system. For this reason this study investigated the possibility of using ostrich and sheeptail fat to replace pork backfat in salami manufacture, with the view to offering an alternative way of marketing ostrich and sheep meat products and adding value.

Ostrich fat is highly unsaturated while sheeptail fat is rich in saturated fatty acids, a variation that greatly influenced the chemical changes in the lipid fraction of ostrich and sheeptail fat manufactured salamis. Although an increase in FFA (% oleic) was observed in all treatments, the sheeptail fat salami FFA was significantly higher than that of pork backfat and ostrich fat salami throughout production. Lipid oxidation measured as PV, was not significantly different between the treatments, and the TBA values of the salamis were only significantly different after ripening when the high PUFA salami (ostrich fat salami) had a significantly higher TBA value. The influence of high PUFA levels was also reflected in redness (a*), and yellowness (b*) of the sausages, the ostrich fat having significantly higher redness and yellowness values than the control and sheeptail fat salamis. However, there was no significant differences in the lightness value (L*) of the sausages. Although there were no gross differences in the keeping quality of the salamis, the pork backfat manufactured salami was sensorically the most preferred, while the ostrich fat salami was the least preferred by a sensory panel.

For these reasons it was concluded that sheeptail fat can be used to replace pork backfat in salami manufacture with minimum problems, whereas replacing pork backfat with ostrich fat in salami manufacture could result in the salami being rejected by consumers.

6.5 Future research

Dietary cholesterol is not reflected directly in the plasma cholesterol, and the major risk factor for coronary heart diseases (CHD) has been proven not to be hypercholesterolemia but rather the high n-6/n-3 ratio of foods (Okuyama et al., 1997). Apparently, hypocholesterolemic drugs (HMG-CoA reductase inhibitor) reduced the risk of CHD by 30 % (Okuyama, 1997). Decreasing the n-6/n-3 ratio by dietary means on the other hand reduced it by 70 % in clinical studies (de Lorgeril et al., 1994). Cholesterol oxidation products have however been reported to have a wide range of adverse biological effects such as atherogenesis, cytotoxicity, mutagenesis, and carcinogenesis (Guardiola et al., 1996). According to Lee et al. (1999), the oxidation of cholesterol occurs readily in foods including meat, poultry and their products since cholesterol oxidation occurs through chemical processes similar to that of unsaturated fatty acid oxidation. Oxidation of cholesterol in foods is affected by the environment surrounding cholesterol, especially closely related unsaturated lipids (Gray et al., 1996). Prolonged storage, application of heat, and exposure to light and irradiation also promote the oxidation of cholesterol (Paniangvait et al., 1995).

Linoleic acid (LA, 18:2n-6) and a-linolenic acid (a-LNA or α - LNA, n-3) are reported to be synthesised in plants but not in animals. When ingested, LA is desaturated and elongated to form dihomo-λ-linolenic acid (DHA) and arachidonic acid (AA), while aLNA is metabolised to eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA). No conversion however occurs in animals in the n-6 and n-3 series (Okuyama and Ikemoto, 1999). They report further to say that, grasses are rich in aLNA while grains are enriched with LA, therefore tissue PUFA composition (n-6/n-3 ratio) varies depending on the choice of animal food. LA is reported to be essential for the maintenance of growth and reproductive physiology (± 1 % is enough), while α - LNA is essential for the maintenance of brain and retinal function. Essential amounts are in the order of ± 1% (Okuyama and Ikemoto, 1999). It is recommended that, the n-6/n-3 ratio of human foods should be decreased to below 2 (Okuyama, 1997), or to the nutritionally acceptable 4-5 (Wood et al., 1999), in order to prevent the diseases that are expected to prevail in the early 21st century. Increasing n-3 PUFA levels in meat however could reduce shelf life and adversely affect flavour because n-3 PUFA are highly susceptible to lipid oxidation (Wood et al., 1999).

Conjugated linoleic acid (CLA) is a collective term for positional and geometrical isomers of linoleic acid. While linoleic acid has double bonds between the 9th and the 10th carbons and the 12th and the 13th carbons, CLA has double bonds at carbon atom 10 and 12 or 9 and 11, with possible cis and trans isomers (Cassens, 1999). CLA are said to antioxidative (Lee et al., 1999), anticarcinogenic, antiatherogenic, and are capable of inducing a decrease in body fat levels with an increase in protein content (Cassens, 1999). Dietary conjugated linoleic acid (CLA) has been reported to be the only source of elevation of CLA in pork muscle and an effective way of controlling lipid oxidation of pork (Lee et al., 1999).

The is therefore a need for further research with the view to improving the status of meats in terms of n-6/n-3 ratio, and to establish the effect of dietary conjugated linoleic acid (CLA) on the lipid changes and stability. This includes the organic (short-chain fatty acids) and cholesterol oxidative products (COP) of meat and meat products.

CHAPTER 7

SUMMARY

Salami is a high fat content (30-50 %) meat delicacy produced by different sectors of the economy, i.e. household, cottage industries, butcheries and big enterprises. The composition of the product is varied, and salami usually hangs in butcheries or is stored in display cabinets. Because of the high fat content of the product, salami is as a result prone to spoilage due to temperature abuse. The lack of uniformity in storage conditions of salami has necessitated a study to establish the most suitable storage temperature for South African salami, based on the detectable changes in the lipid fraction of the product.

A typical South African salami was manufactured, processed to maturation and stored at three temperatures, namely 4, 12 and 25 ^{0}C for 15 to 30 days. Lipid hydrolysis and oxidation were significantly high at the 25 ^{0}C, moderate at 12 ^{0}C and minimal at the 4 ^{0}C. This implied that, based on the changes in the lipid fraction of salami, 4 ^{0}C is an excellent temperature, 12 ^{0}C is satisfactory and the most practical, while 25 ^{0}C in unacceptable for storage of South African salami.

The 12 ^{0}C storage temperature was used to establish the effect of high dietary PUFA on the lipid stability of salami. Higher PUFA levels adversely affected lipid hydrolysis and oxidation, implying that typical South African pig finishing diet supplemented with 3 % sunflower oil (corresponding to 57.88 ± 0.65 and 30.03 ± 4.53 PUFA levels for feed and backfat respectively) may compromise the keeping quality of salami.

In an effort to diversify salami production, the viability of replacing pork backfat with ostrich and sheeptail fat in salami manufacture was assessed. Ostrich fat is rich in PUFA; sheeptail fat is more saturated while pork backfat fatty acid composition is intermediate between the two, a factor that was reflected in the salami manufactured from these fat sources. Lipid hydrolysis was significantly higher in the sheeptail fat salami, although no significant differences were observed in the build-up of peroxides. The pork backfat and ostrich fat salami in turn had significantly higher TBA values than the sheeptail fat salami during storage. The keeping quality of the salamis were similar, a sensory evaluation panel preferred the pork backfat salami while the ostrich fat salami was the least preferred.

Keywords: fermentation, sausage, salami, fatty acids, oxidative stability, storage temperature, PUFA levels, pork, ostrich, sheep.

SAMEVATTING

Salami is 'n hoë vetinhoud (30-50 %) vleislekkerny wat deur verskillende sektore van die ekonomie, nl. huishoudings, tuisbedrywe, slaghuise en groot ondernemings, geproduseer word. Die samestelling van die produk varieer en salami hang gewoonlik in slaghuise of word in vertoonkabinette uitgestal. Met hoë temperatuur opberging kan salami, as gevolg van die hoë vetinhoud, bederf. Die gebrek aan eevormige bergingstoestande by Suid-Afrikaanse salami, het 'n studie genoodsaak, sodat die beste bergingstemperatuur vasgestel kon word. Dit berus op die waarneembare veranderinge in die lipiedfraksie van die produk.

'n Tipiese Suid-Afrikaanse salami is vervaardig, by drie temperature, nl. 4, 12 en 25 ^{0}C gestoor vir 15-30 dae. By 25 ^{0}C is betekenisvolle hoë lipiedhidrolise en oksidasie waargeneem, terwyl dit by 12 ^{0}C gematigd en minimaal by 4 ^{0}C was. Met die veranderinge in die lipiedfraksie van die salami as uitgangspunt het dit geimpliseer dat 4 ^{0}C 'n uitstekende temperatuur, 12 ^{0}C 'n bevredigende en mees praktiese temperatuur en 25 ^{0}C 'n onaanvaarbare temperatuur vir opberging van Suid-Afrikaanse salami was.

Die 12 ^{0}C bergingstemperatuur is gebruik om die effek van hoë polionversadigde vetsuur (POVS) inhoud op die lipiedstabiliteit van die salami vas te stel. Hoër POVS vlakke het lipiedhidrolise en oksidasie benadeel, wat impliseer dat 'n tipiese Suid-Afrikaanse vark afrondingsrantsoen, wat aangeuul is met 3 % sonneblomolie (wat ooreenstem' met 57.88 ± 0.65 en 30.03 ± 4.53 POVS vlakke vir voer en rugvet onderskeidelik), die goedhouvermoë van salami kan benadeel.

In 'n poging om salamiproduksie te diversifiseer is die lewensvatbaarheid van volstruis en skaap sterkvet as 'n plaasvervanger vir vark rugvet in salamivervaardiging gebruik. Volstruisvet is ryk in POVS, daarteenoor is skaap stertvet meer versadig, terwyl vark rugvet 'n intermediêre vetsuursamestelling het, soos gereflekteer in die salami vervaardig vanuit die onderskeie vetbronne. Lipiedhidrolise was betekenisvol hoër in skaap stetvet salami hoewel geen betekenisvolle verskille in die opbou van peroksiede waargeneem is nie. Die vark rugvetsalami en volstruisvet salami het weer betekenisvolle hoër TBA – waardes as die skaap stetvet salami tydens opberging getoon. Die goedhouvermoë van al drie salamis was dieselfde. 'n Sensoriese evaluering het getoon dat vark rugvet salami voorrang geniet, terwyl volstruisvet salami die minste verkies is.

CHAPTER 8

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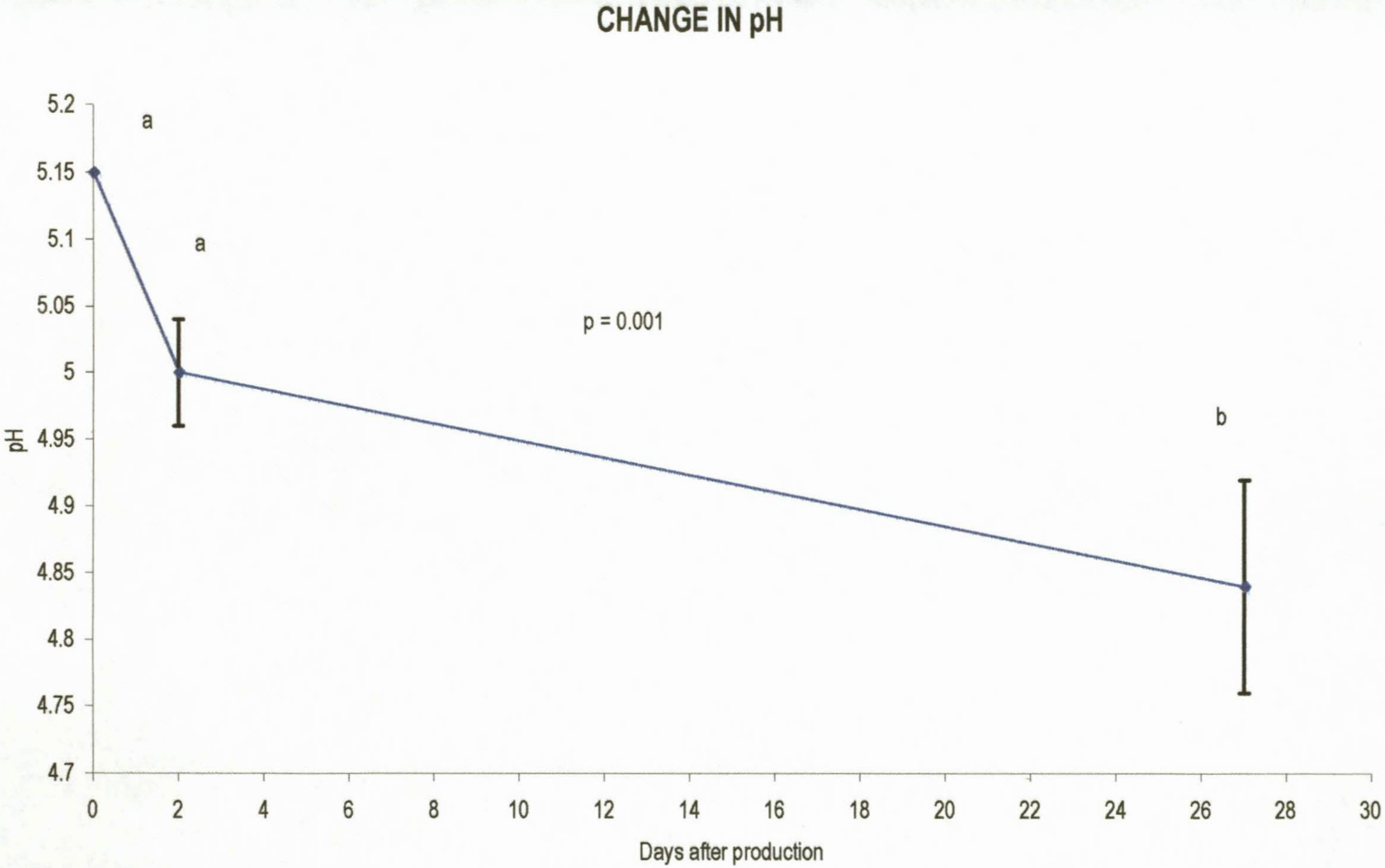
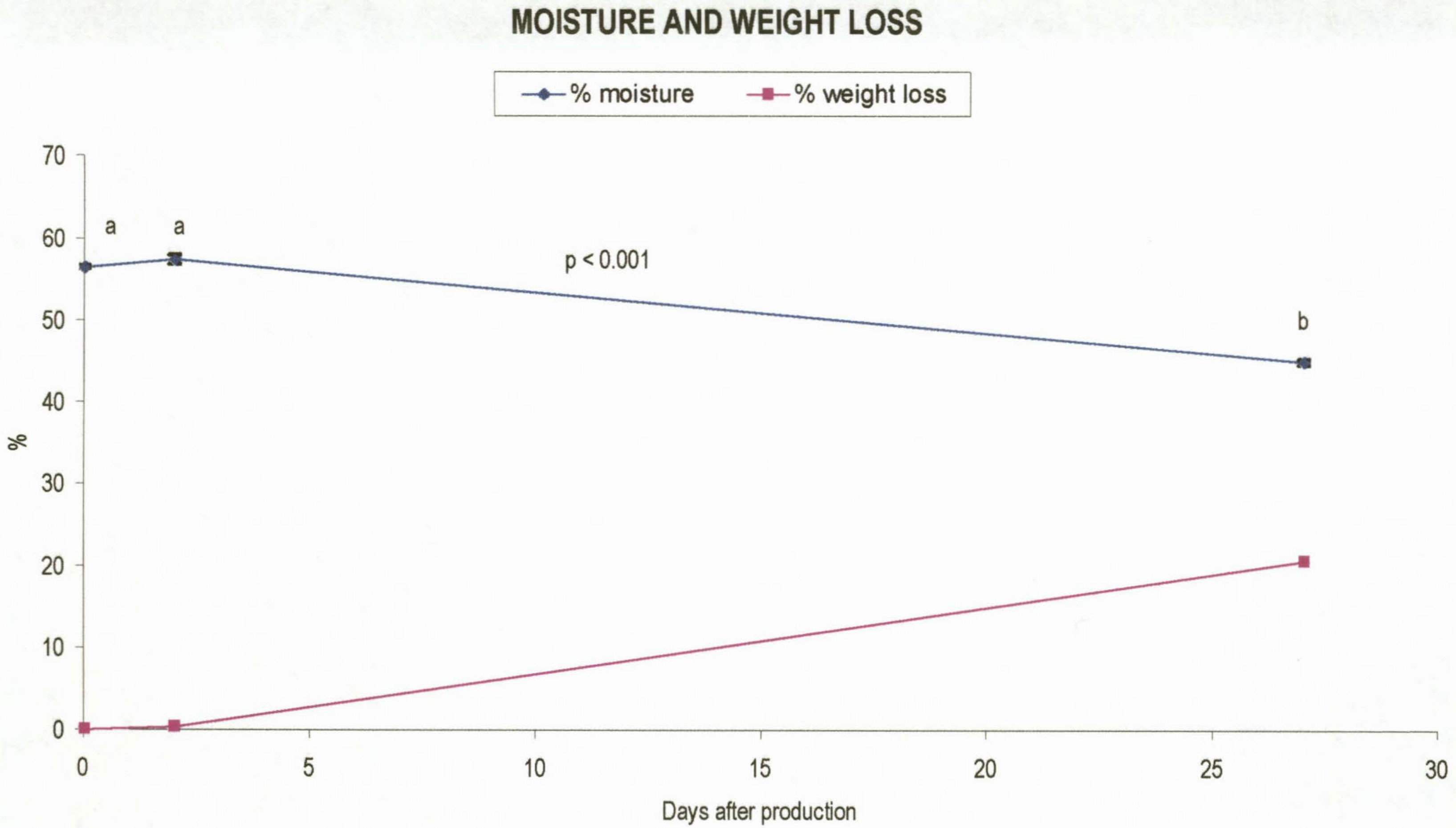


(means with the same superscript letter do not differ significantly)

Fig. 1. Changes in pH of salami during fermentation and ripening.



(^{a,b} on the same graph, means with the same superscript do not differ significantly)

Fig. 2. Moisture content and weight loss of salami during fermentation and ripening.

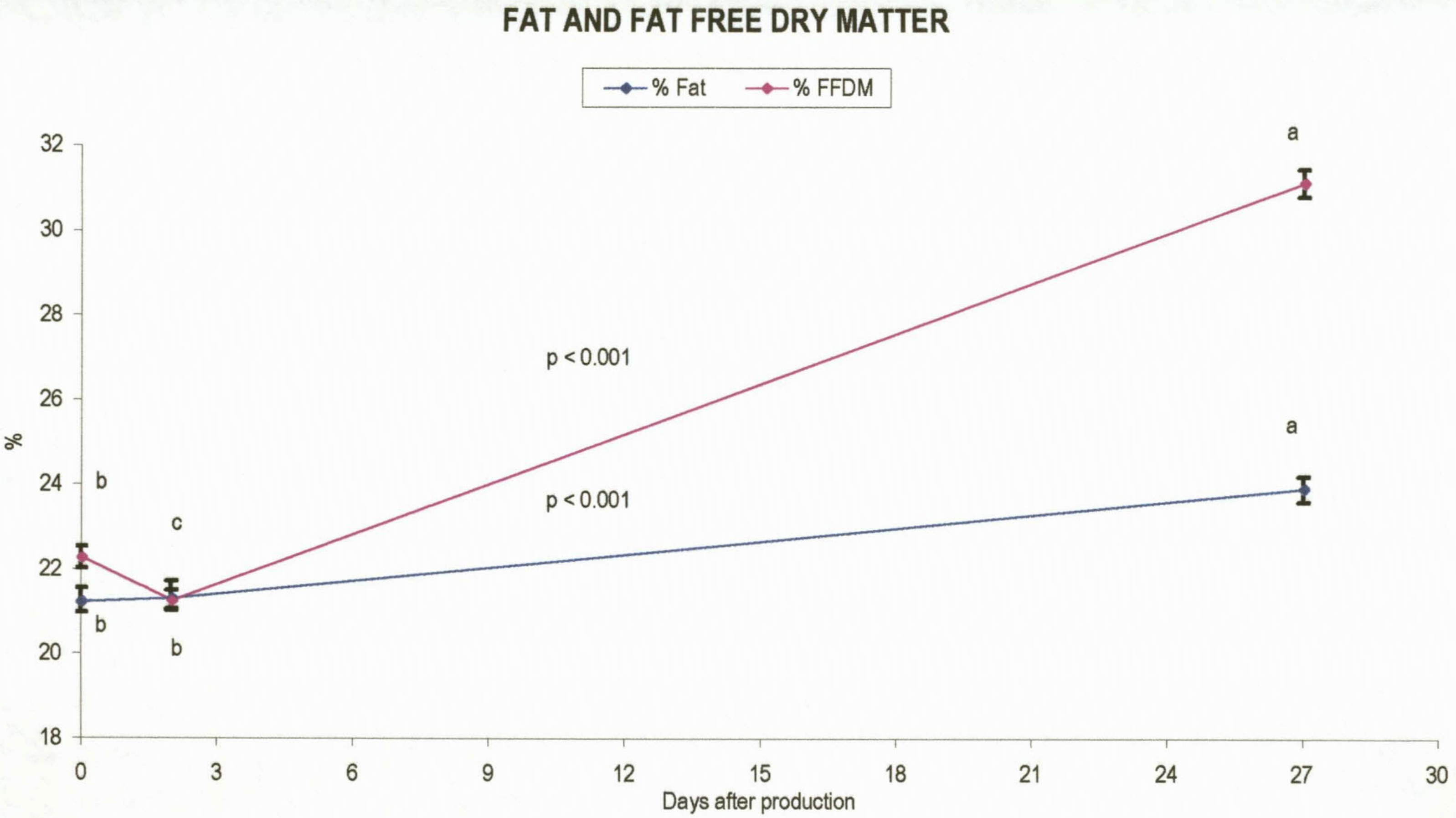


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Fig. 3. Fat and fat free dry matter of salami during fermentation and ripening.

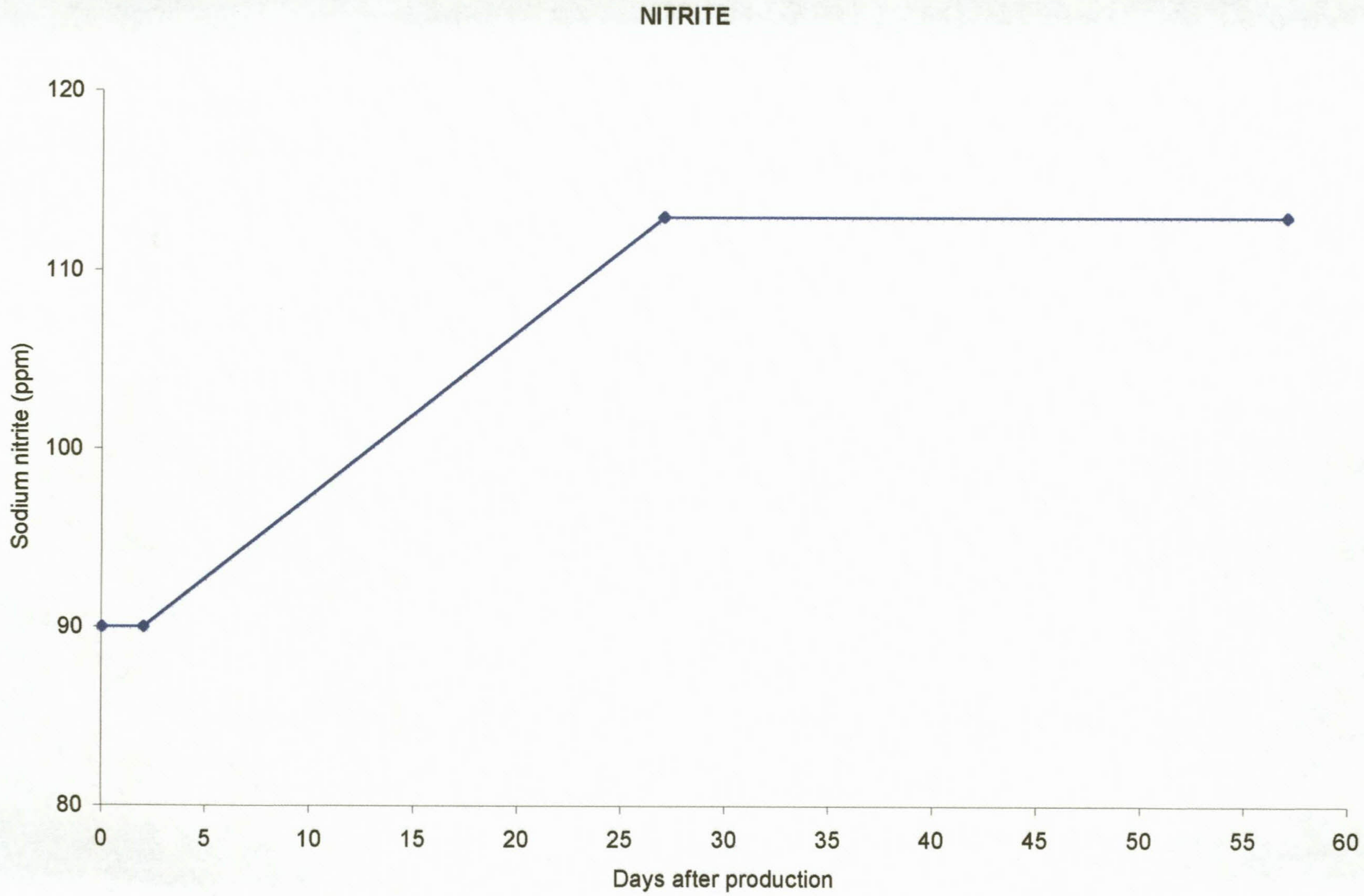


Fig. 4. Nitrite content of salami during fermentation, ripening and storage.

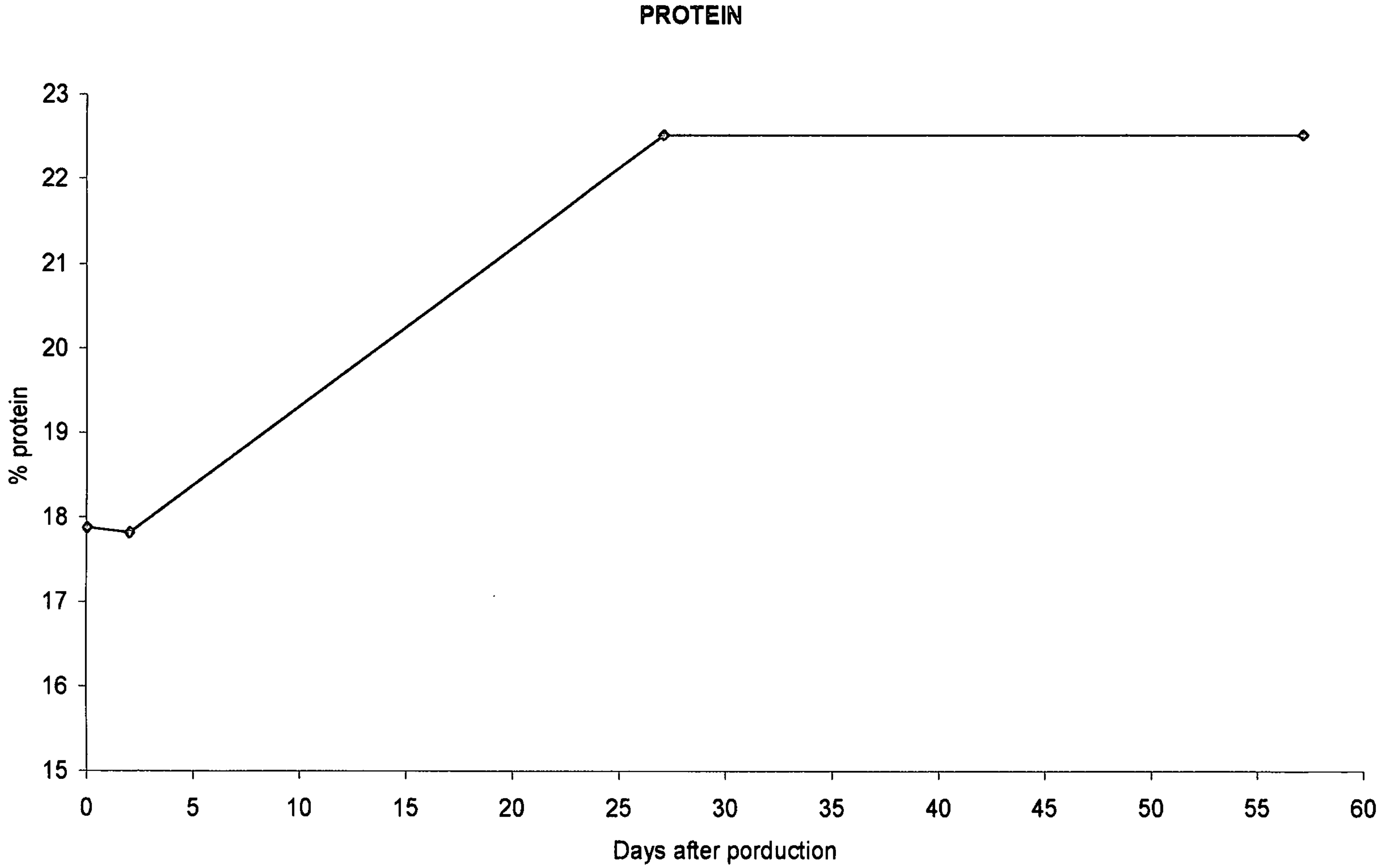


Fig.5. Protein content (% protein) of salami during fermentation, ripening and storage.

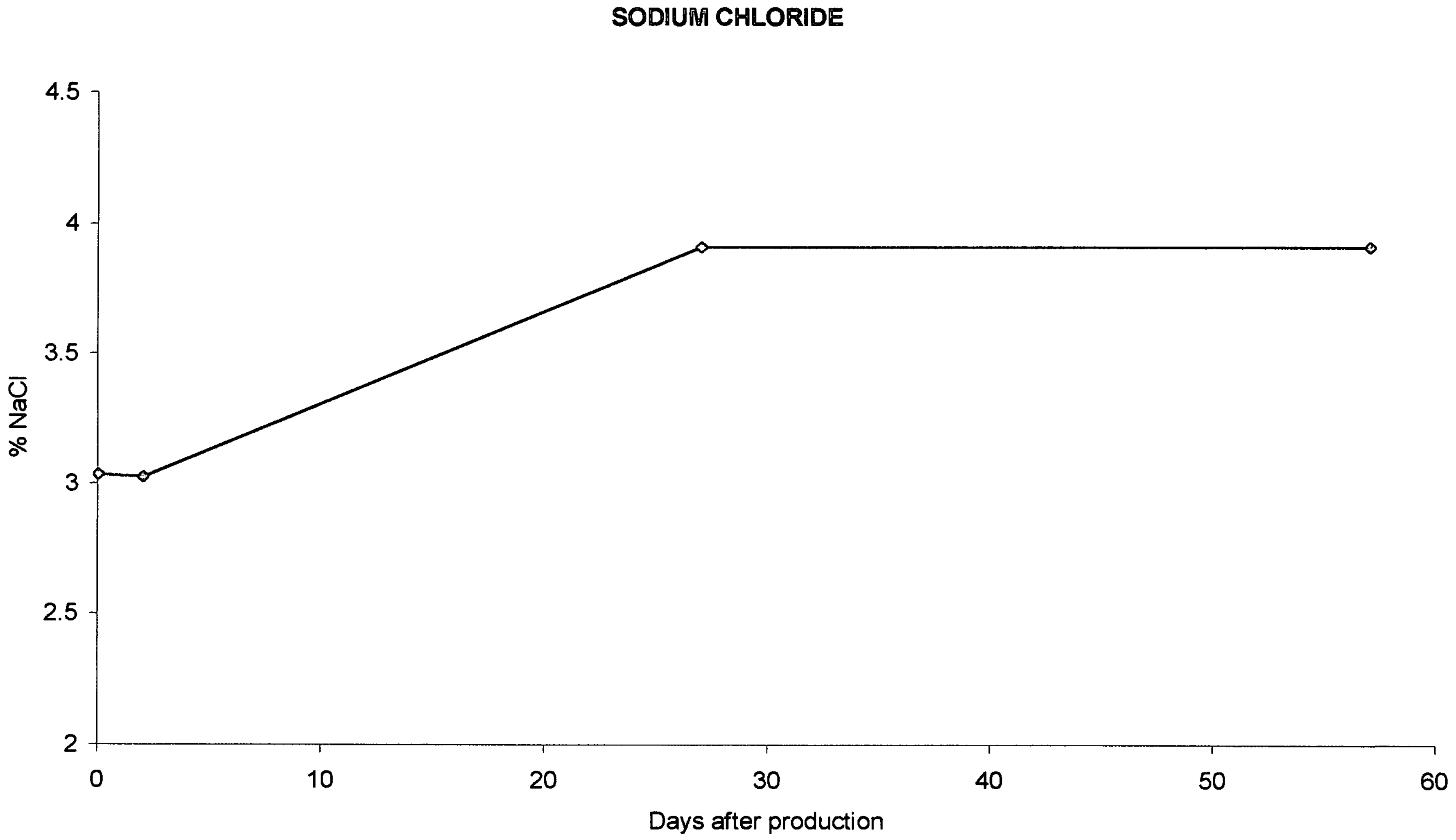
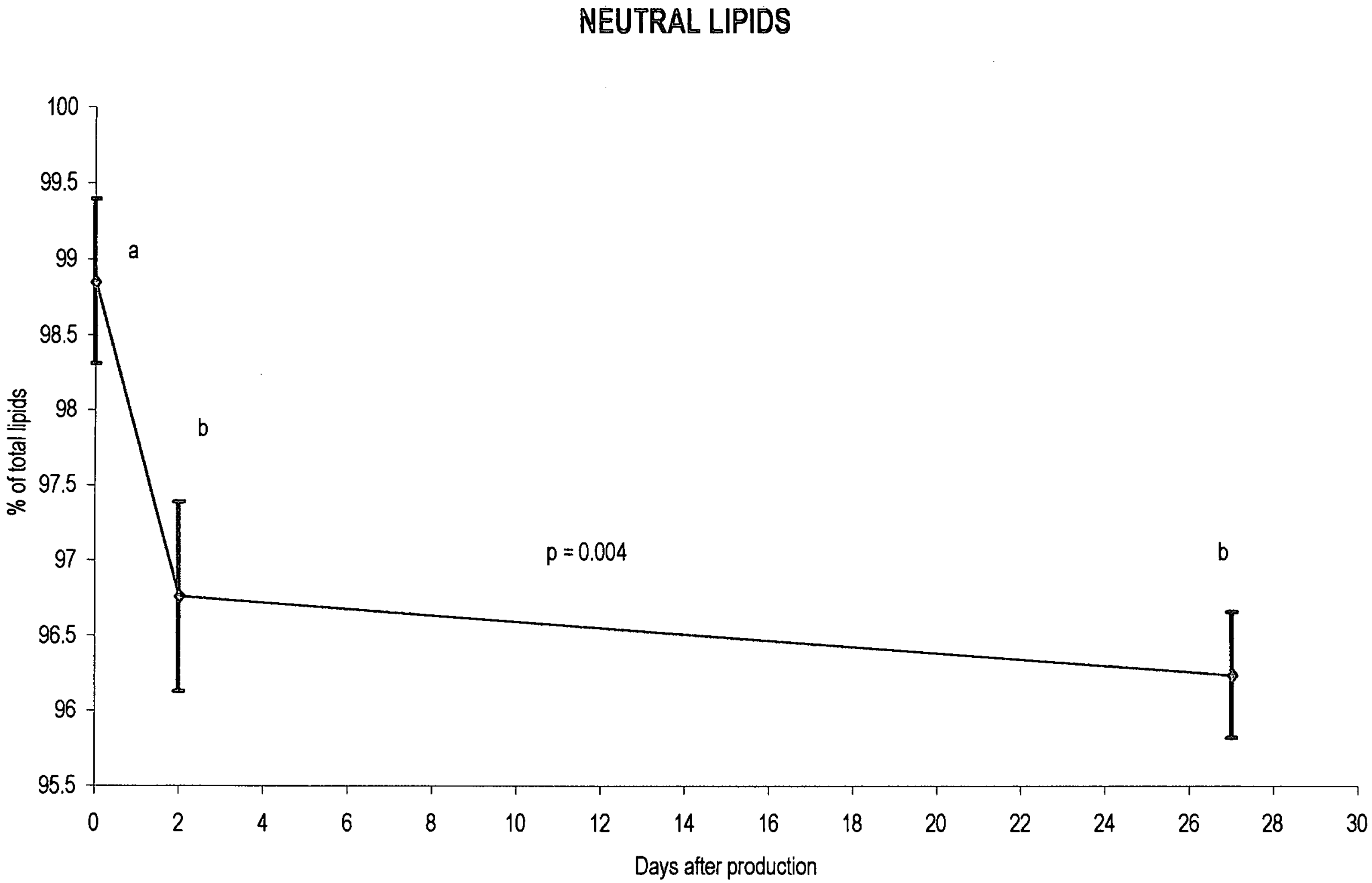
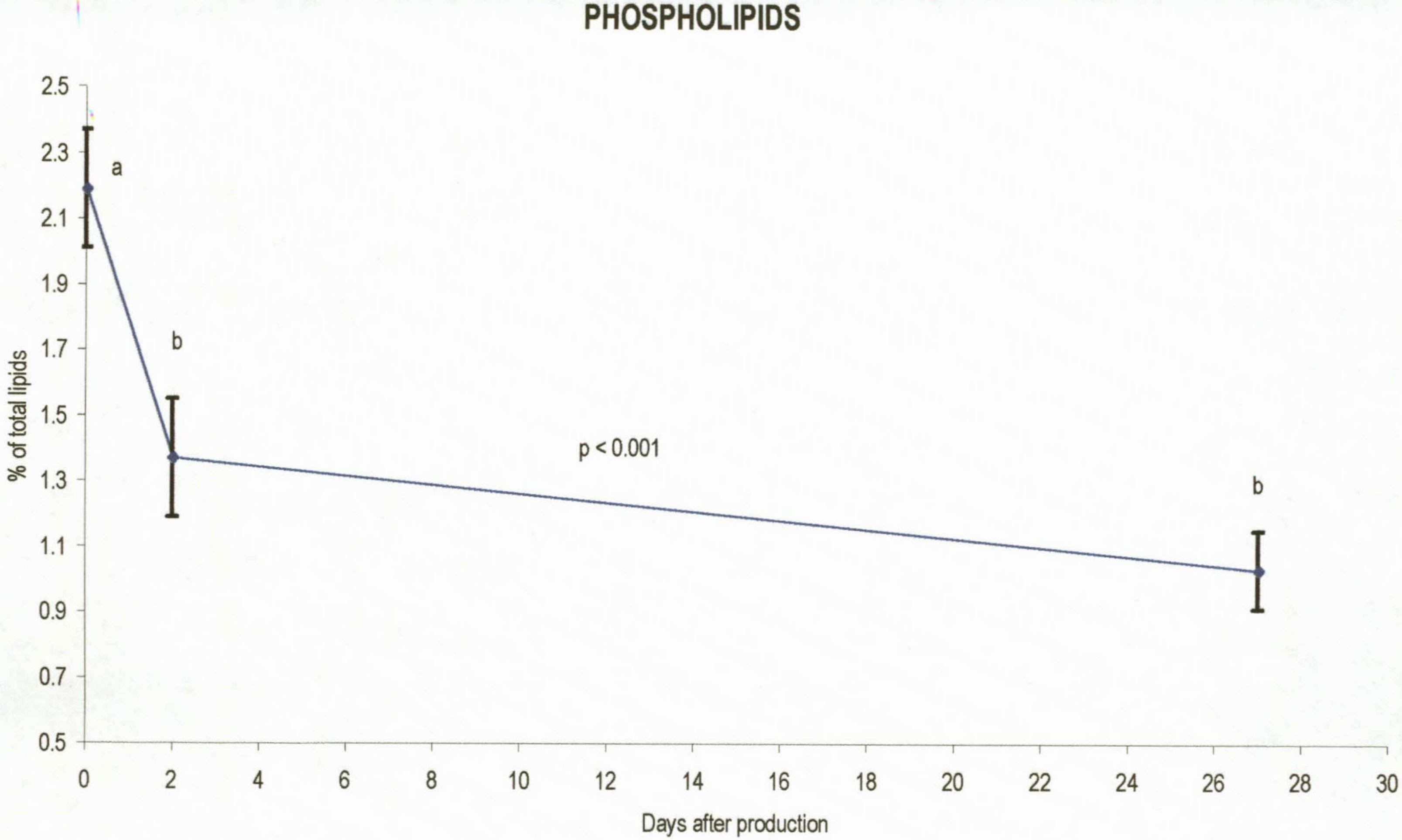


Fig. 6. Sodium chloride content (% NaCl) of salami during fermentation, ripening and storage



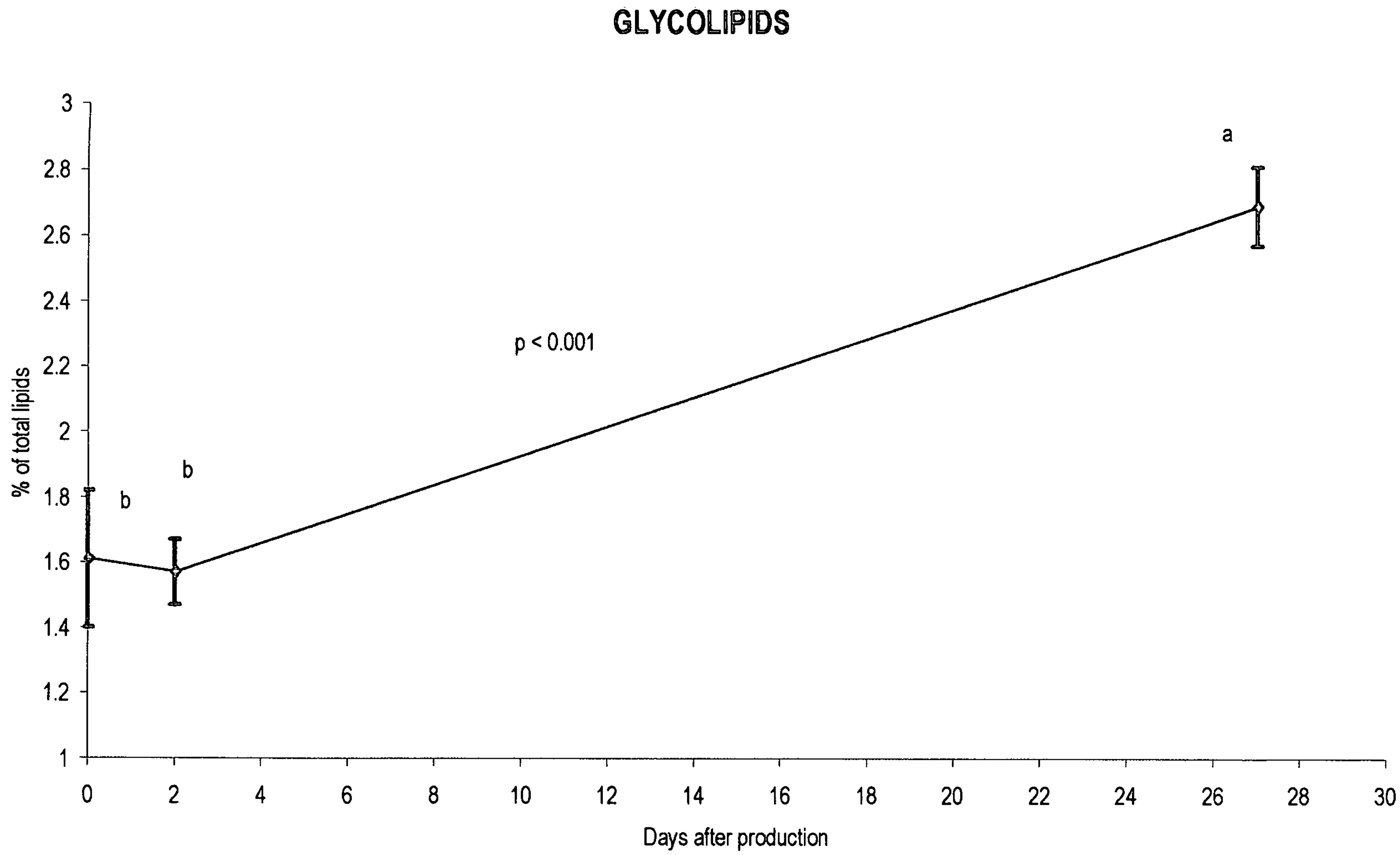
(^{a,b} means with the same superscript letter do not differ significantly)

Fig. 7. Neutral lipid content of salami during fermentation and ripening.



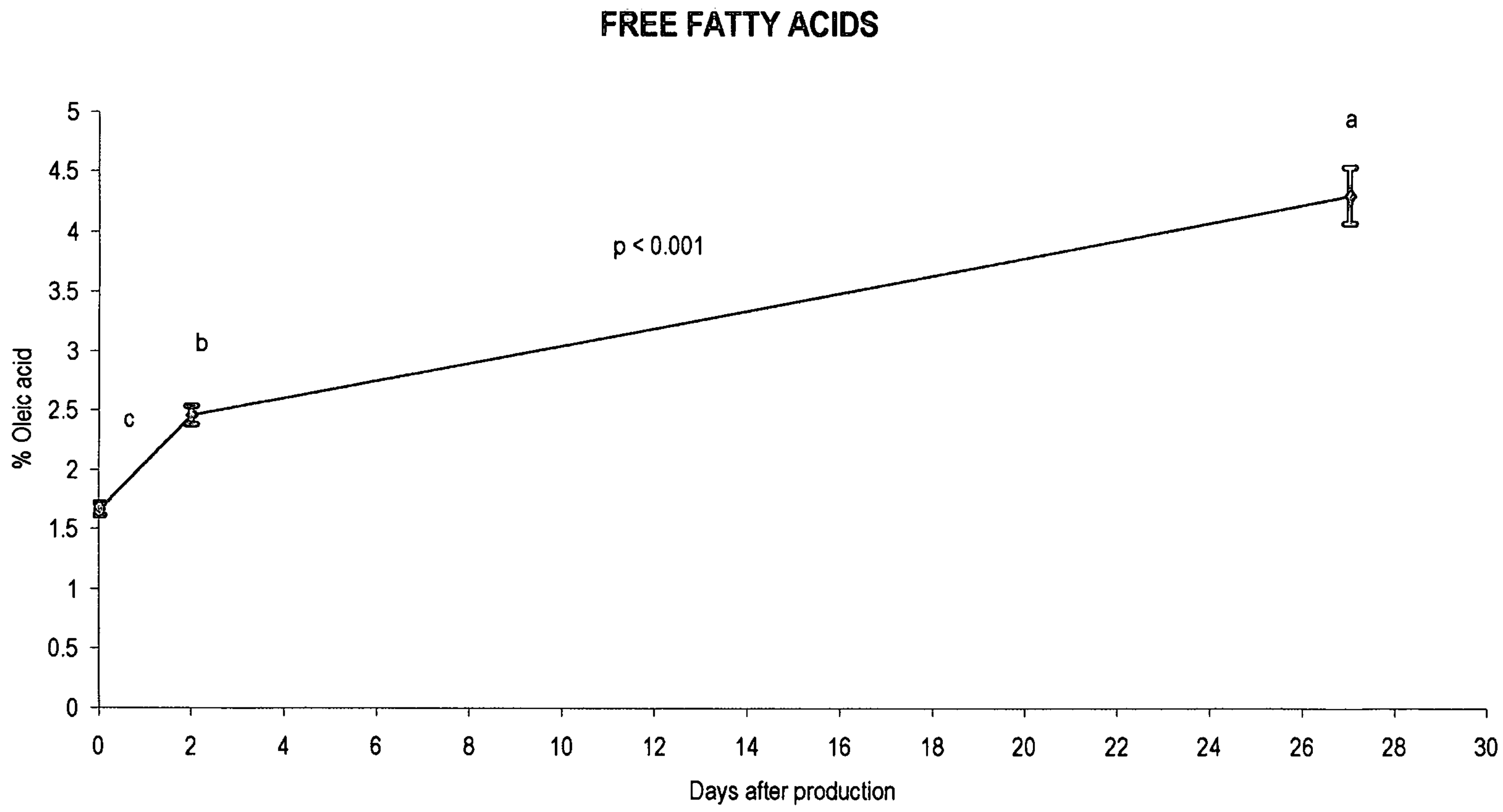
([a,b] means with the same superscript letter do not differ significantly)

Fig. 8. Phospholipid content of salami during fermentation and ripening.



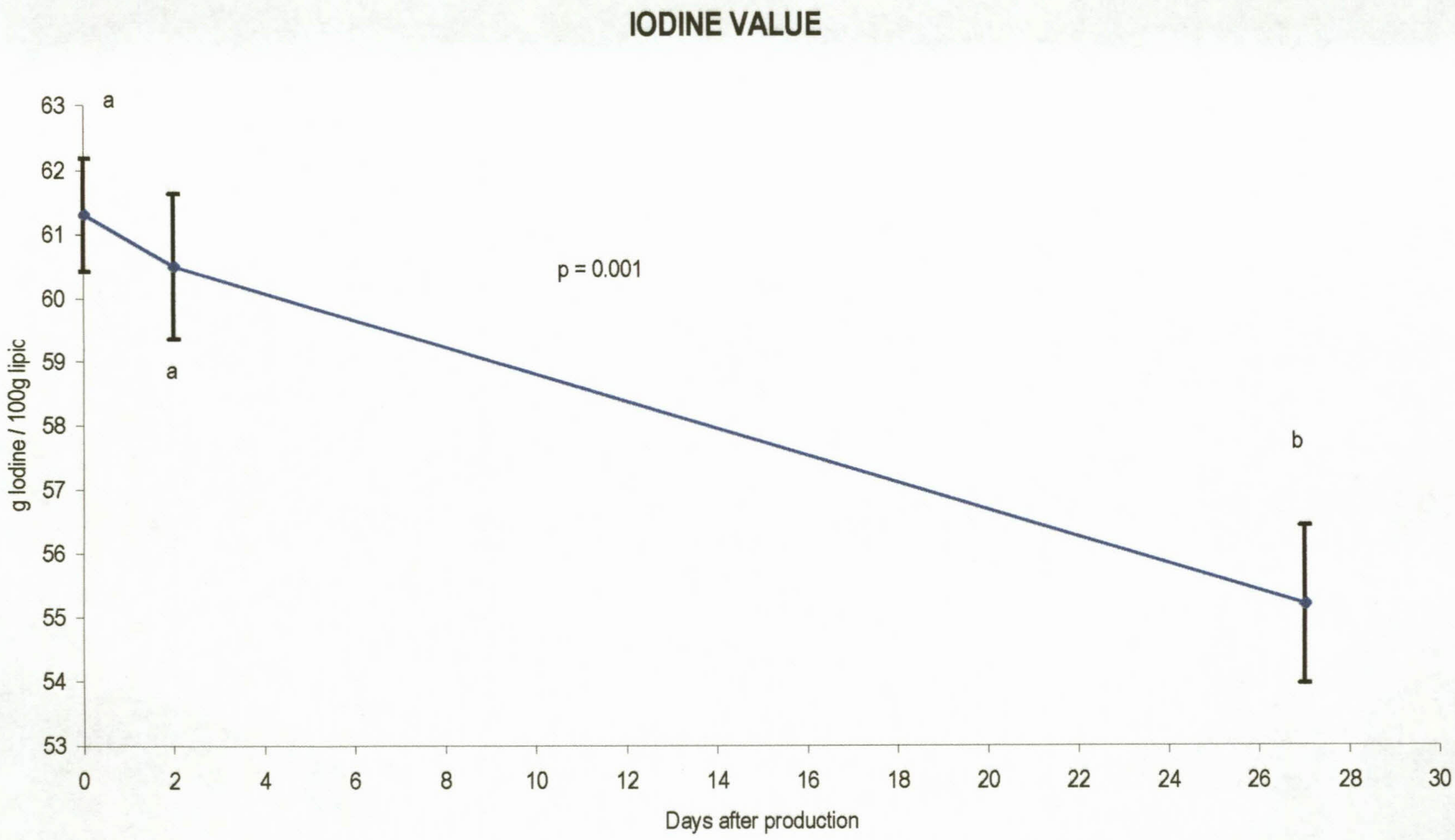
(^{a,b} means with the same superscript letter do not differ significantly)

Fig. 9. Glycolipid content of salami during fermentation and ripening.



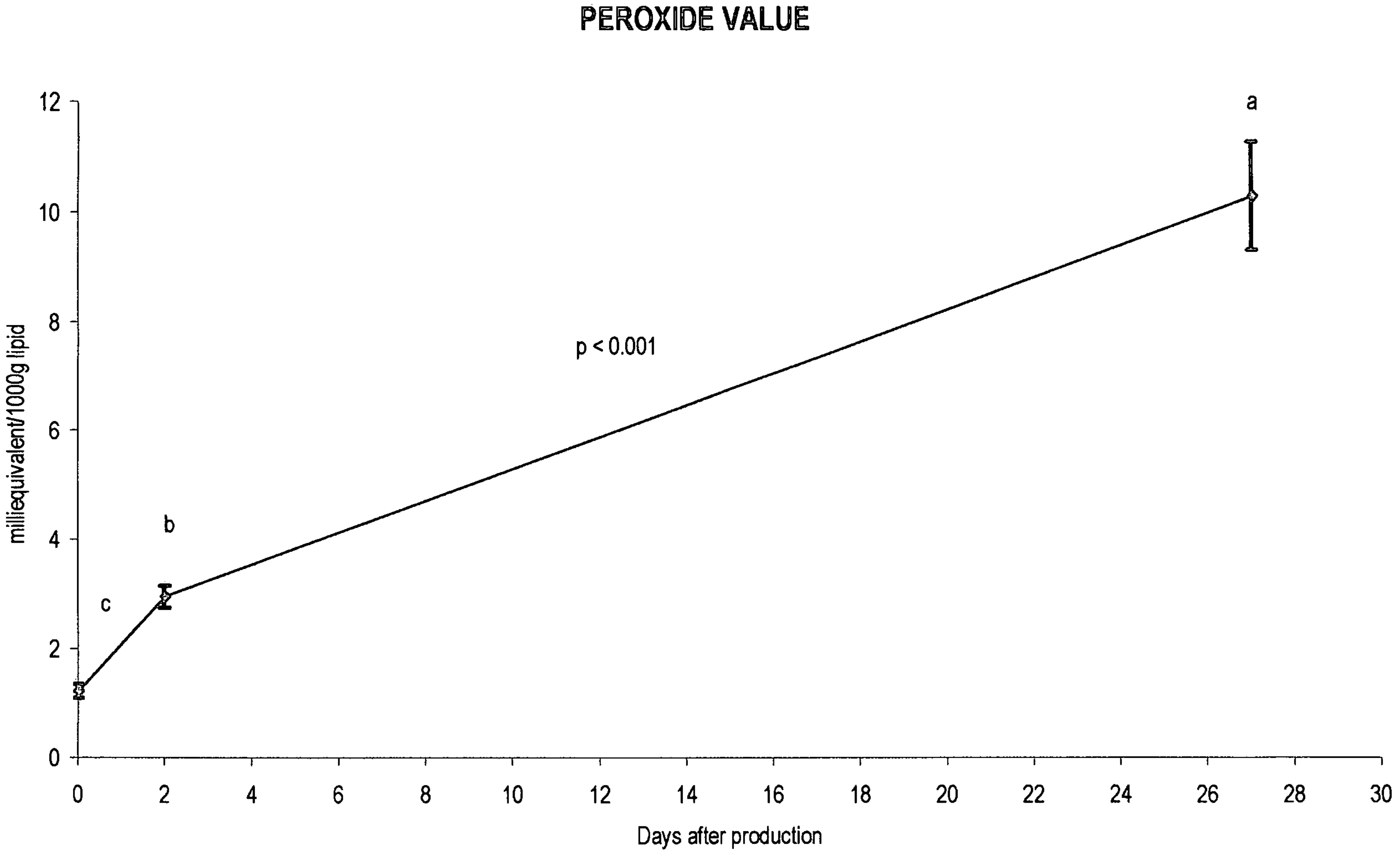
(^{a,b} means with the same superscript letter do not differ significantly)

Fig. 10. Free fatty acid (% oleic) of salami during fermentation and ripening.



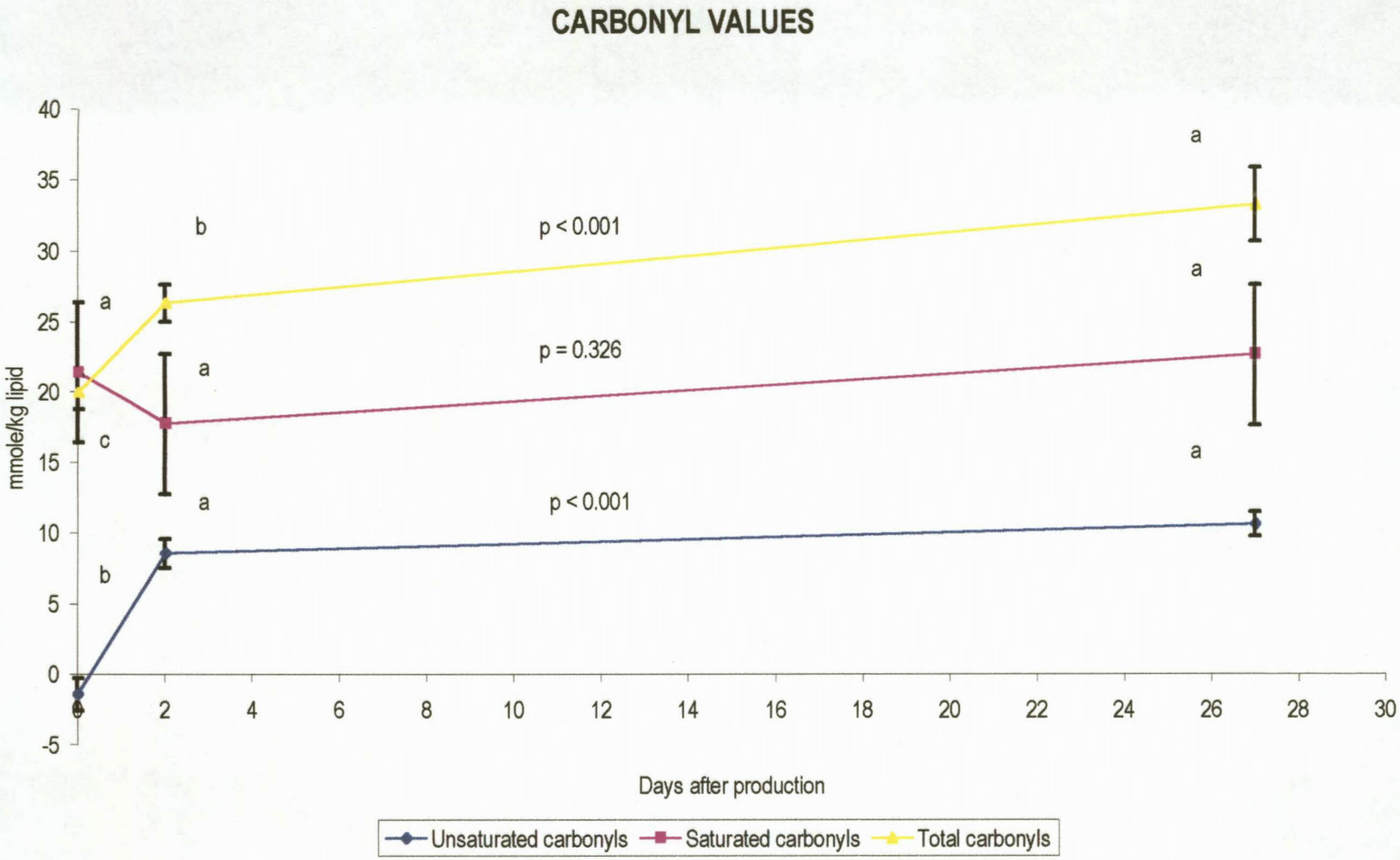
(^{a,b} means with the same superscript letter do not differ significantly)

Fig. 11. Iodine value of salami during fermentation and ripening.



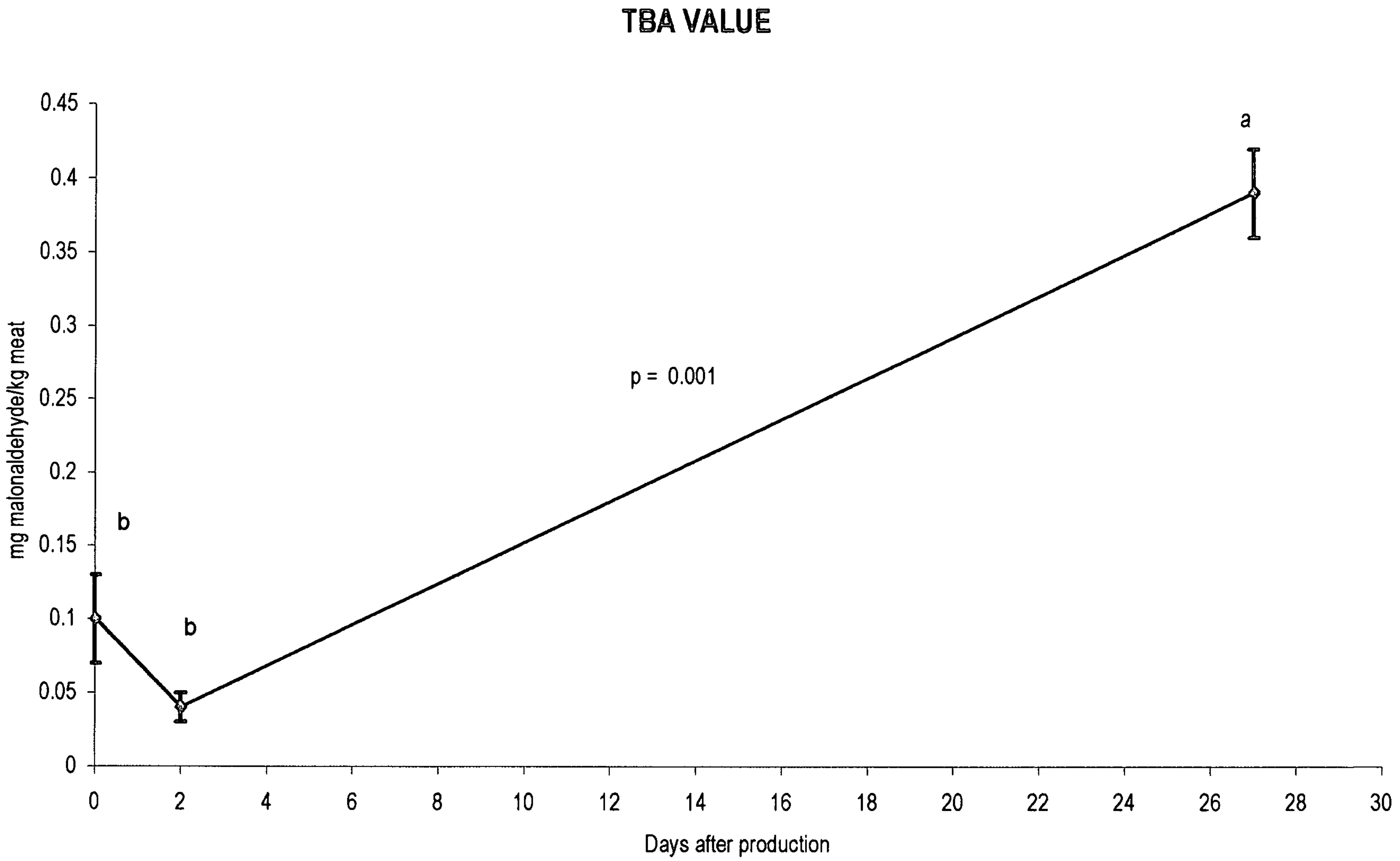
(^{a,b,c} means with the same superscript letter do not differ significantly)

Fig. 12. Peroxide value of salami during fermentation and ripening.



(^{a,b,c} on the same graph, means with the same superscript letter do not differ significantly)

Fig.13. Carbonyl values of salami during fermentation and ripening.



(^{a,b} means with the same superscript letter do not differ significantly)

Fig. 14. TBA value of salami during fermentation and ripening.

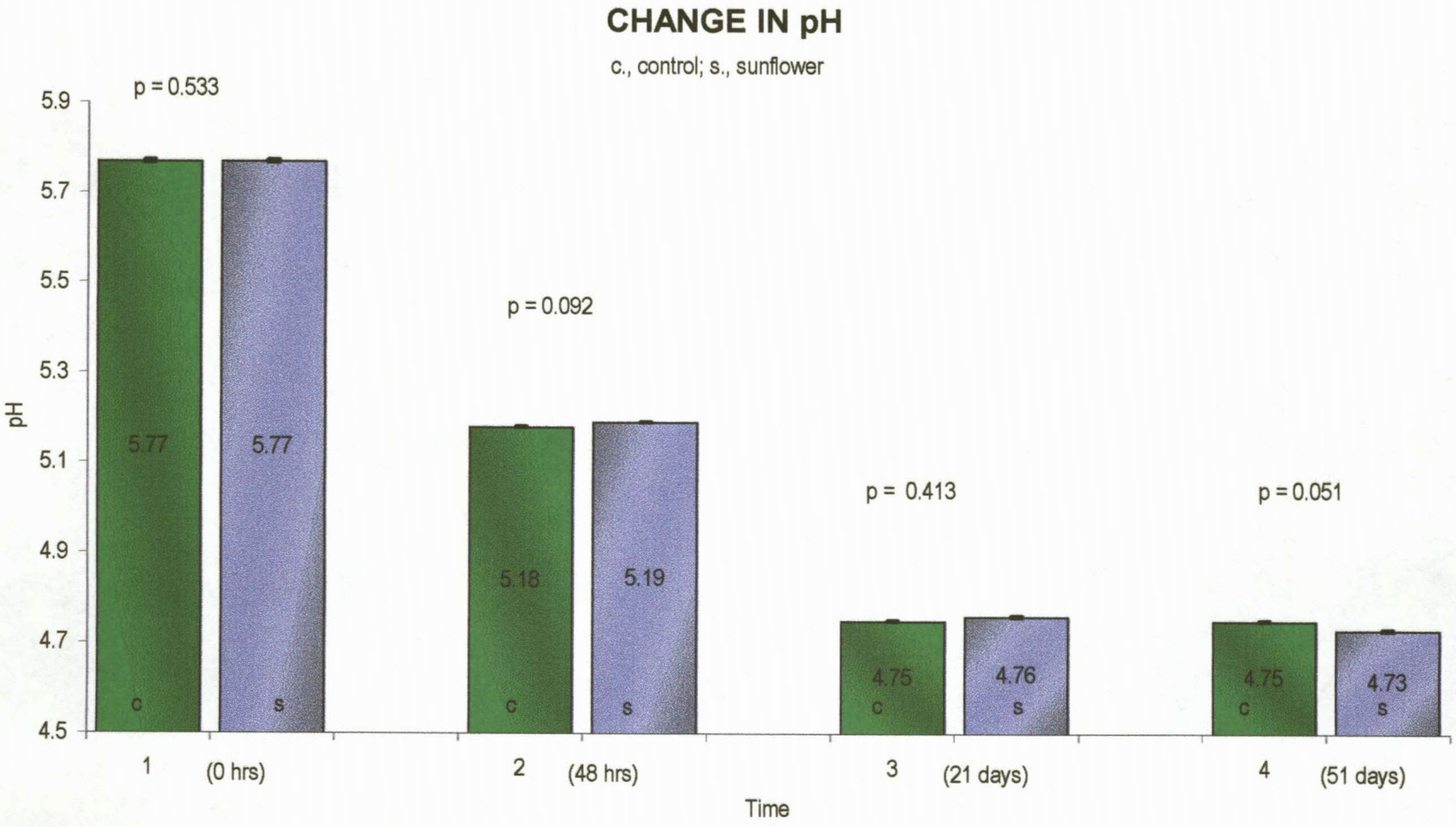
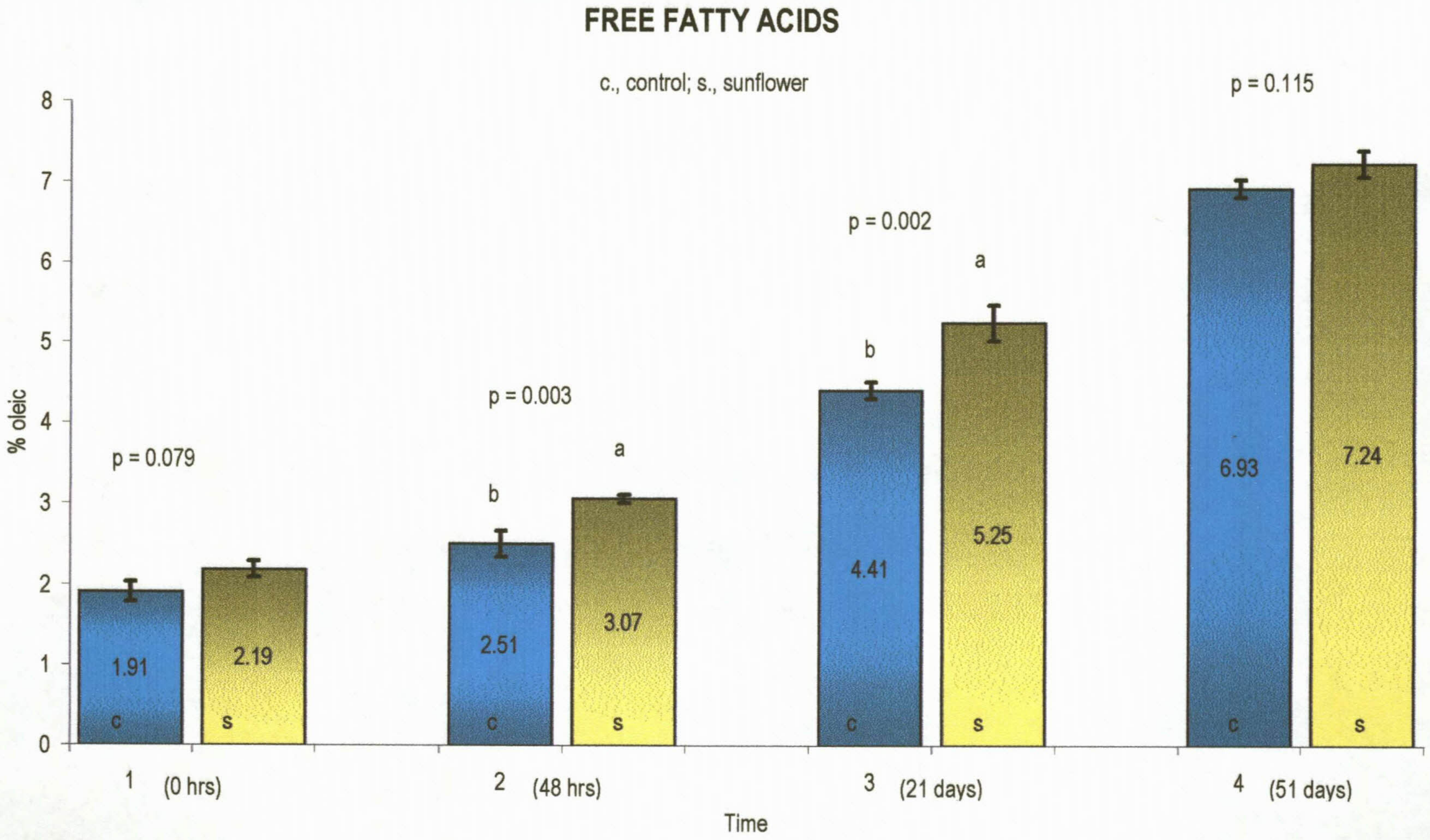
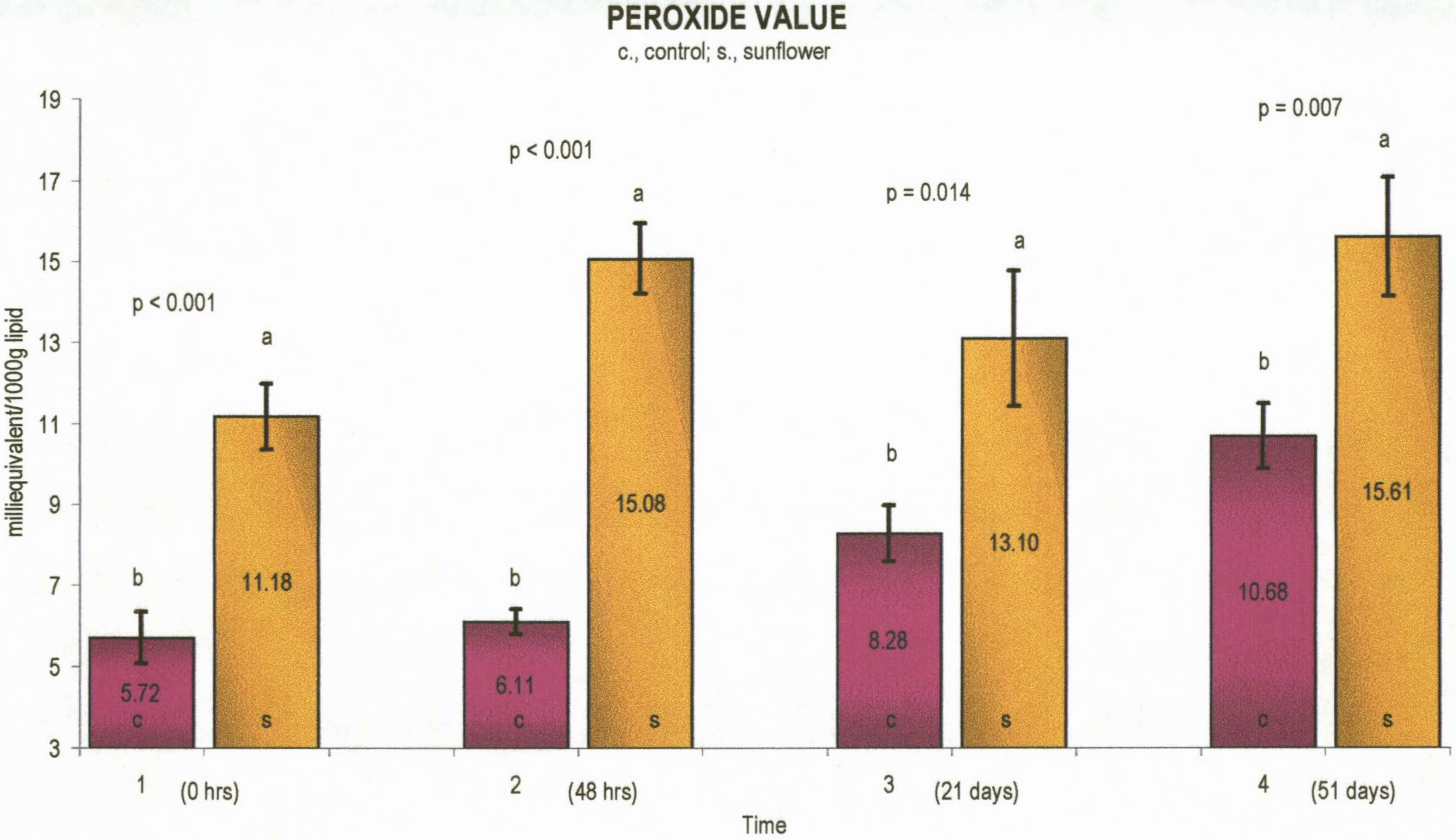


Fig 15. Changes in pH of salami during fermentation, ripening and storage.



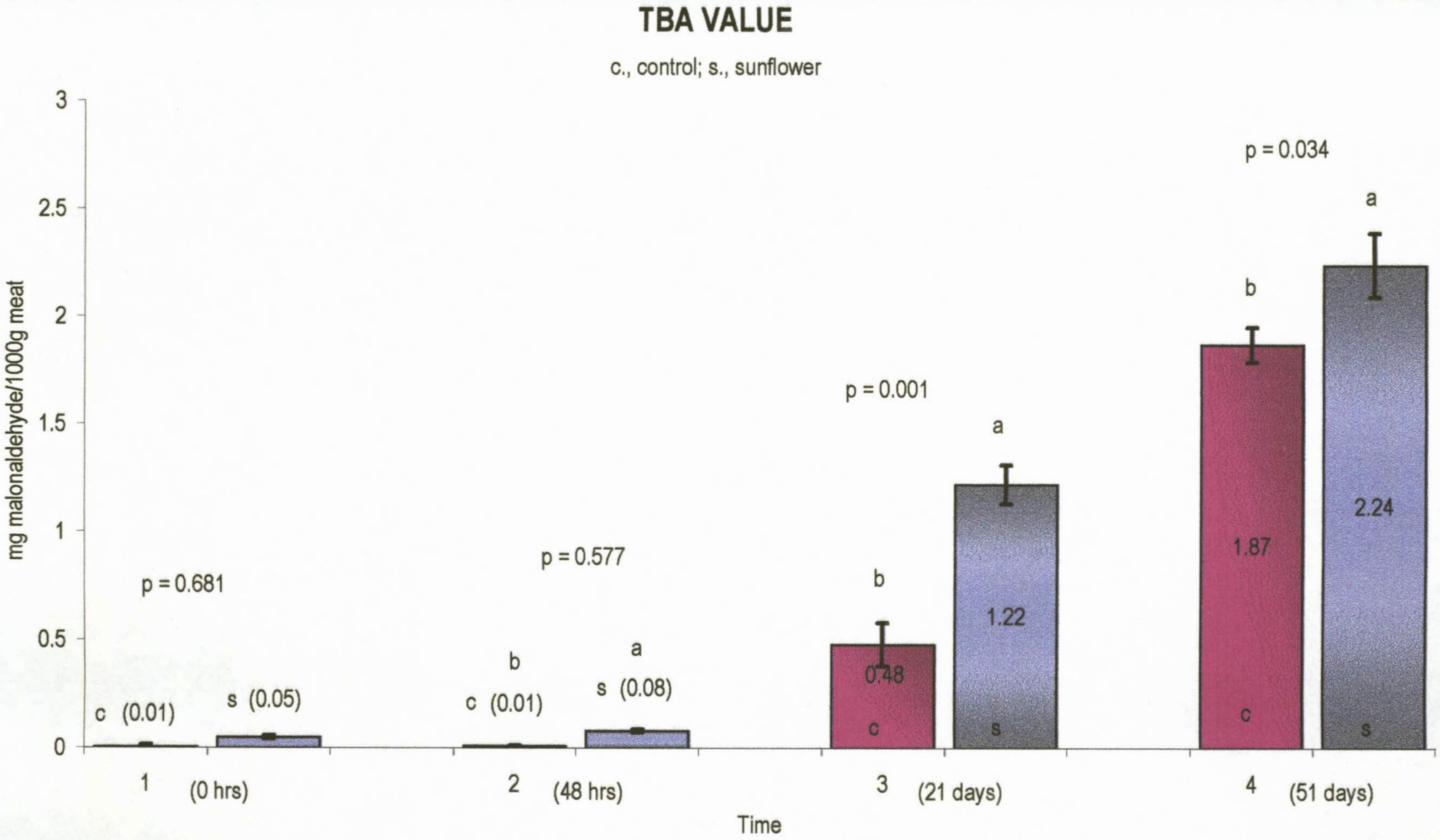
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 16. Free fatty acid contents of salami during fermentation, ripening and storage.



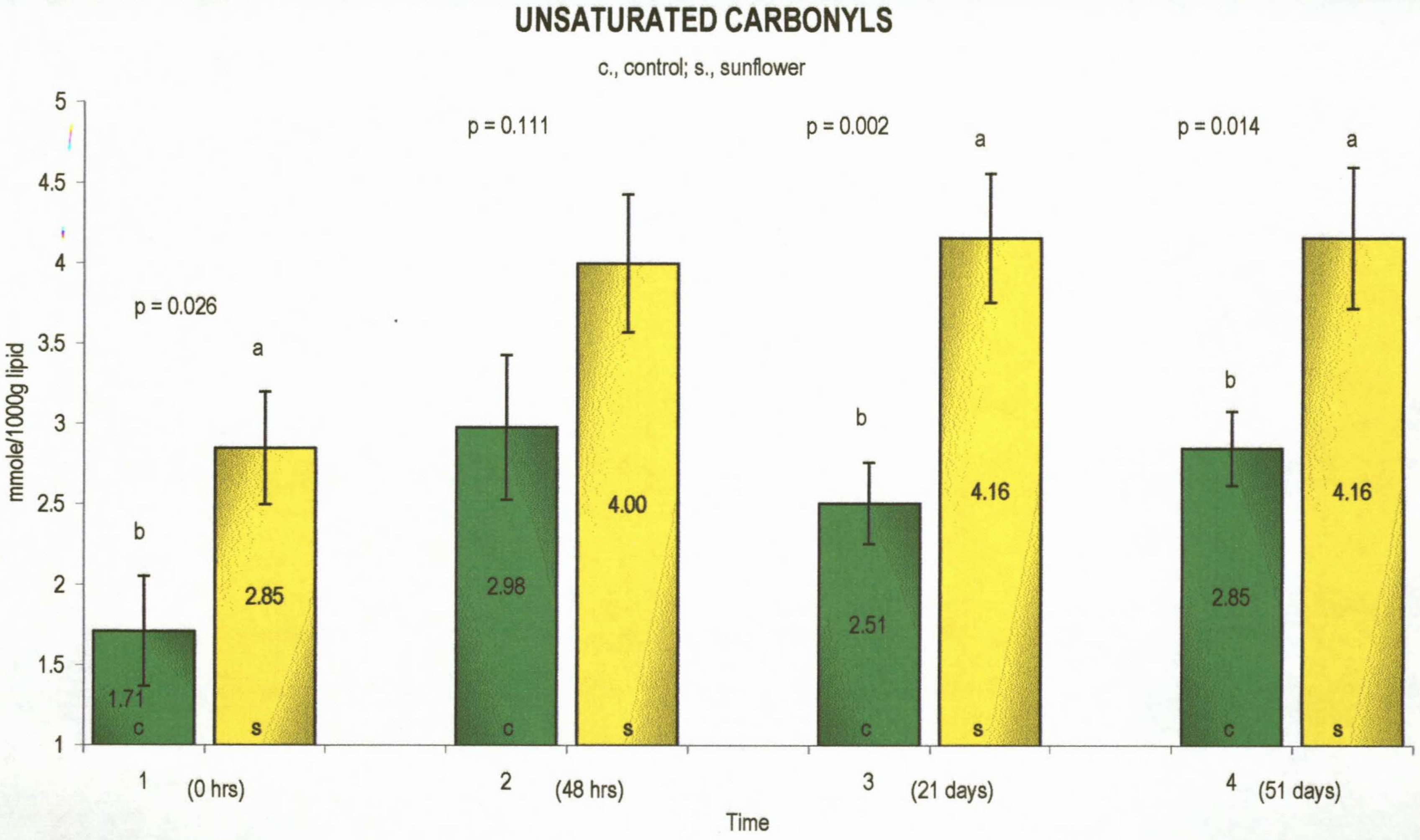
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 17. Peroxide values of salami during fermentation, ripening and storage.



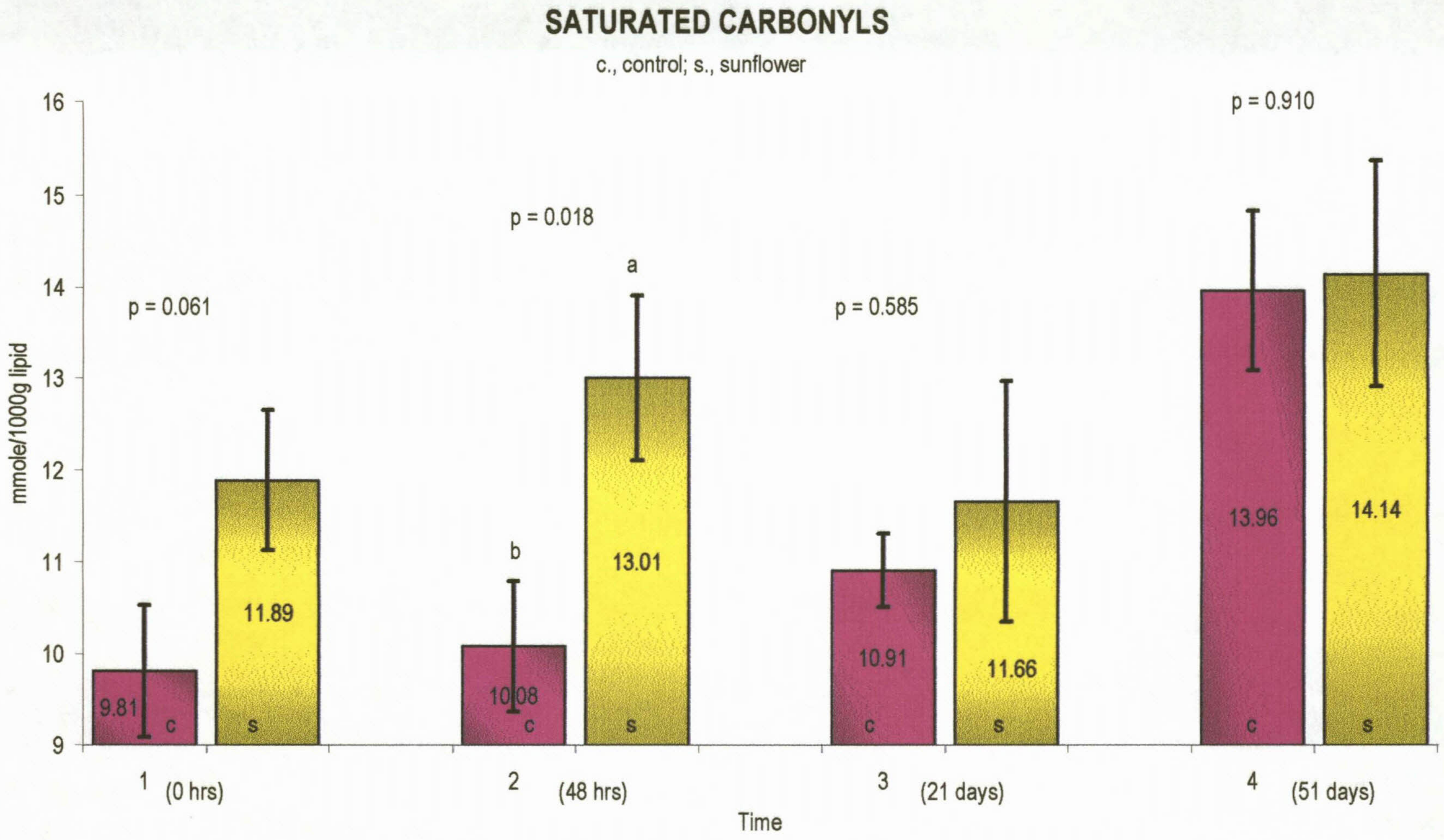
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 18. TBA values of salami during fermentation, ripening and storage.



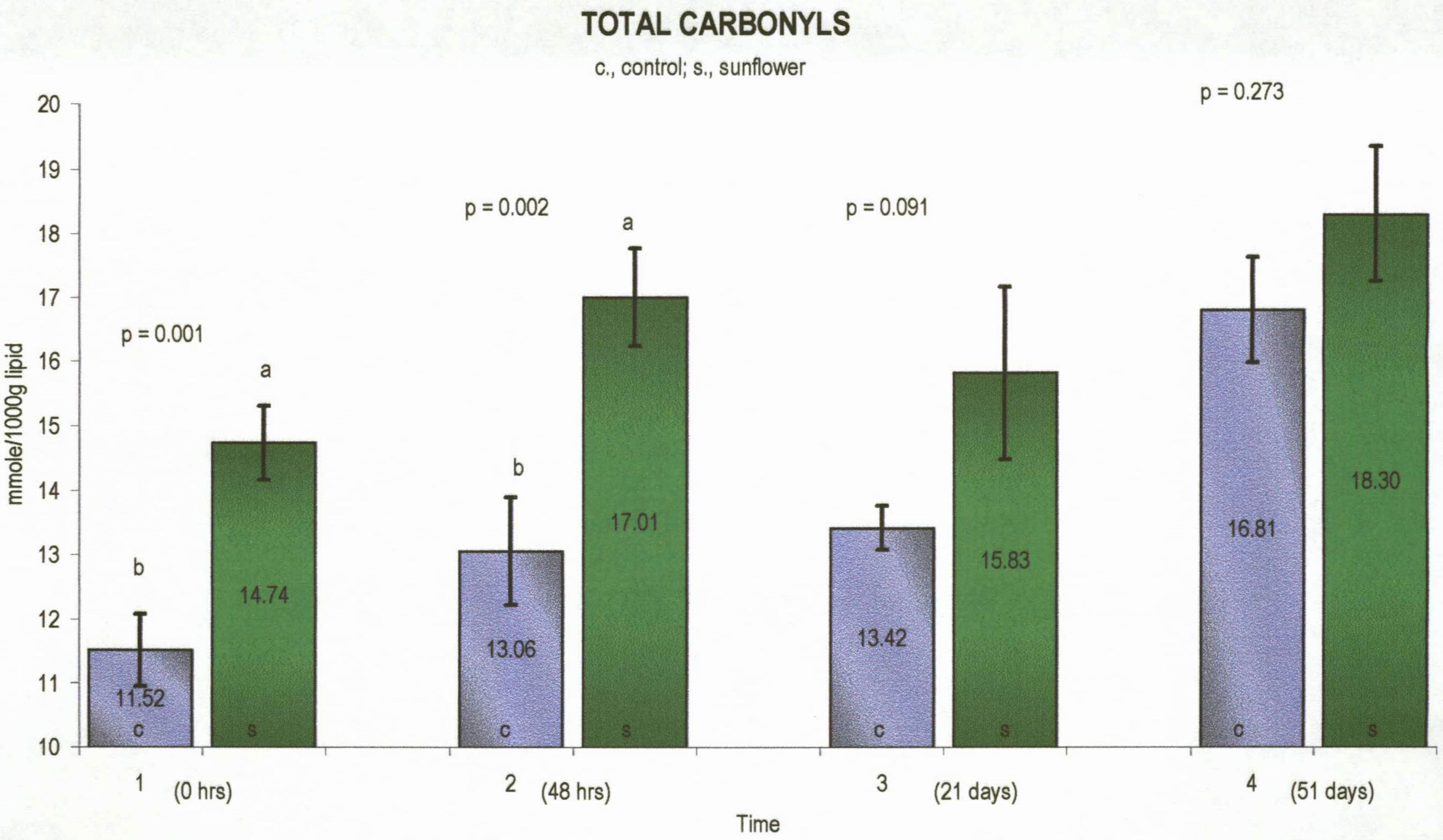
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 19. Unsaturated carbonyl values of salami during fermentation, ripening and storage.



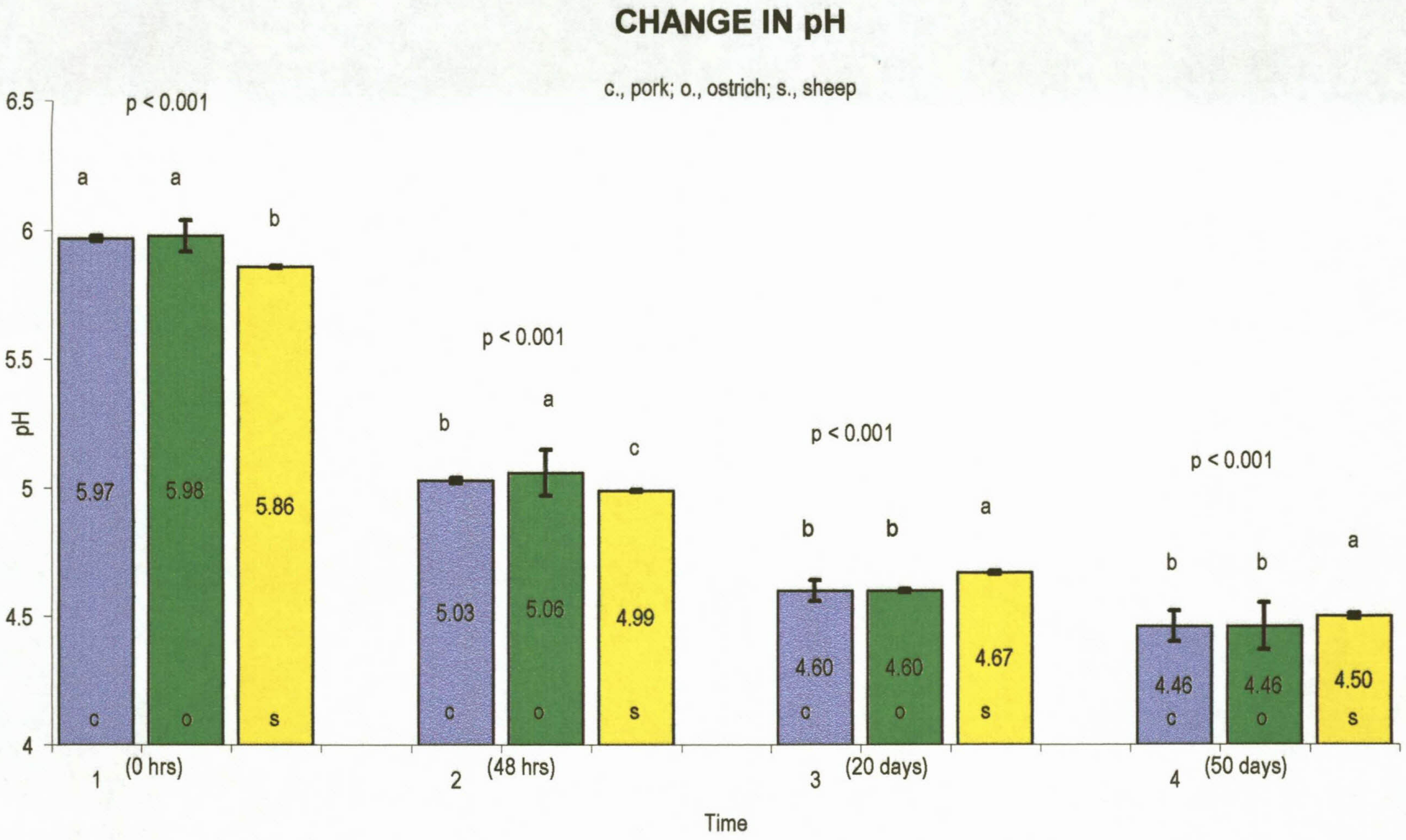
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 20. Saturated carbonyl values of salami during fermentation, ripening and storage.



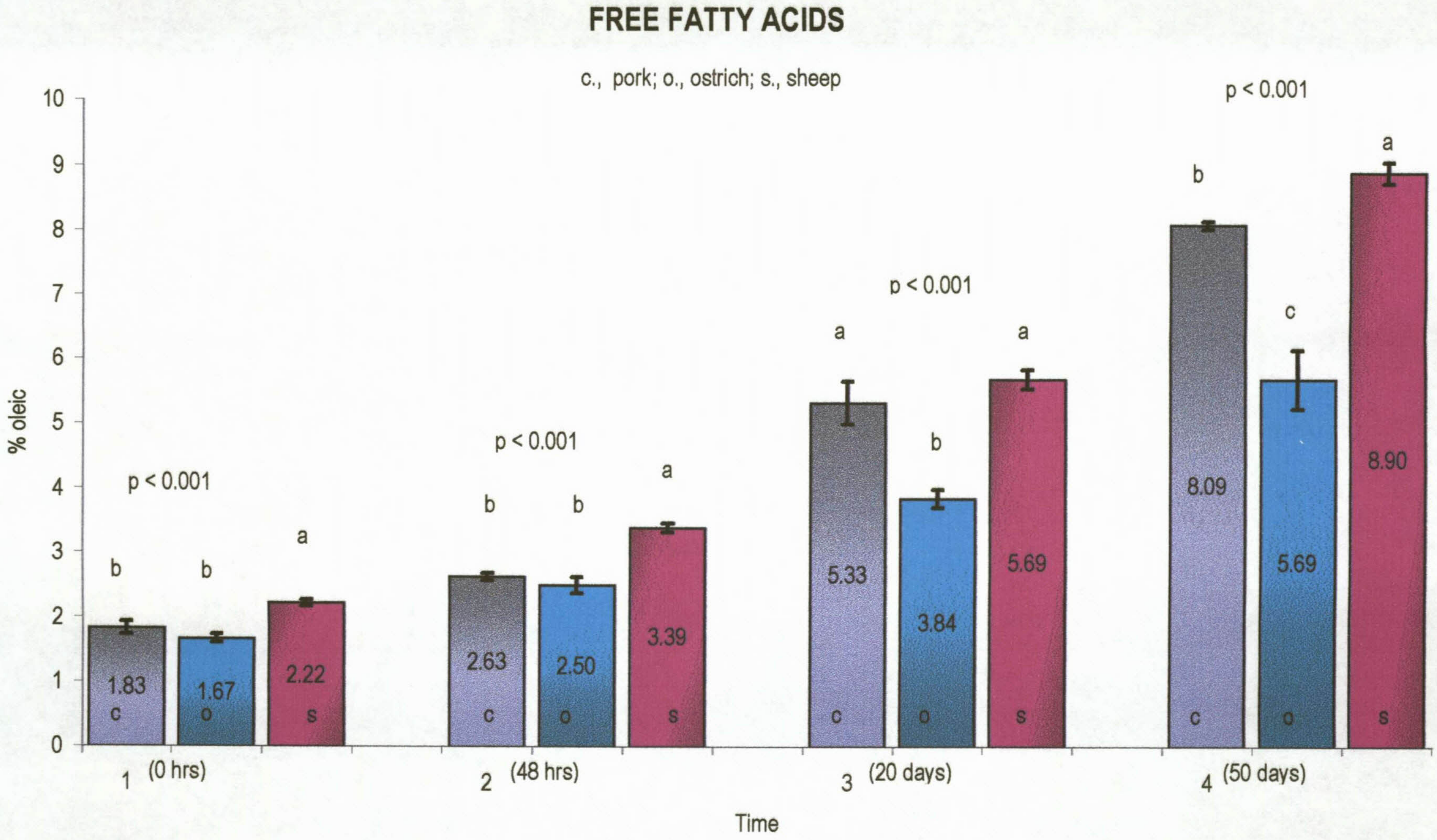
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 21. Total carbonyl values of salami during fermentation, ripening and storage.



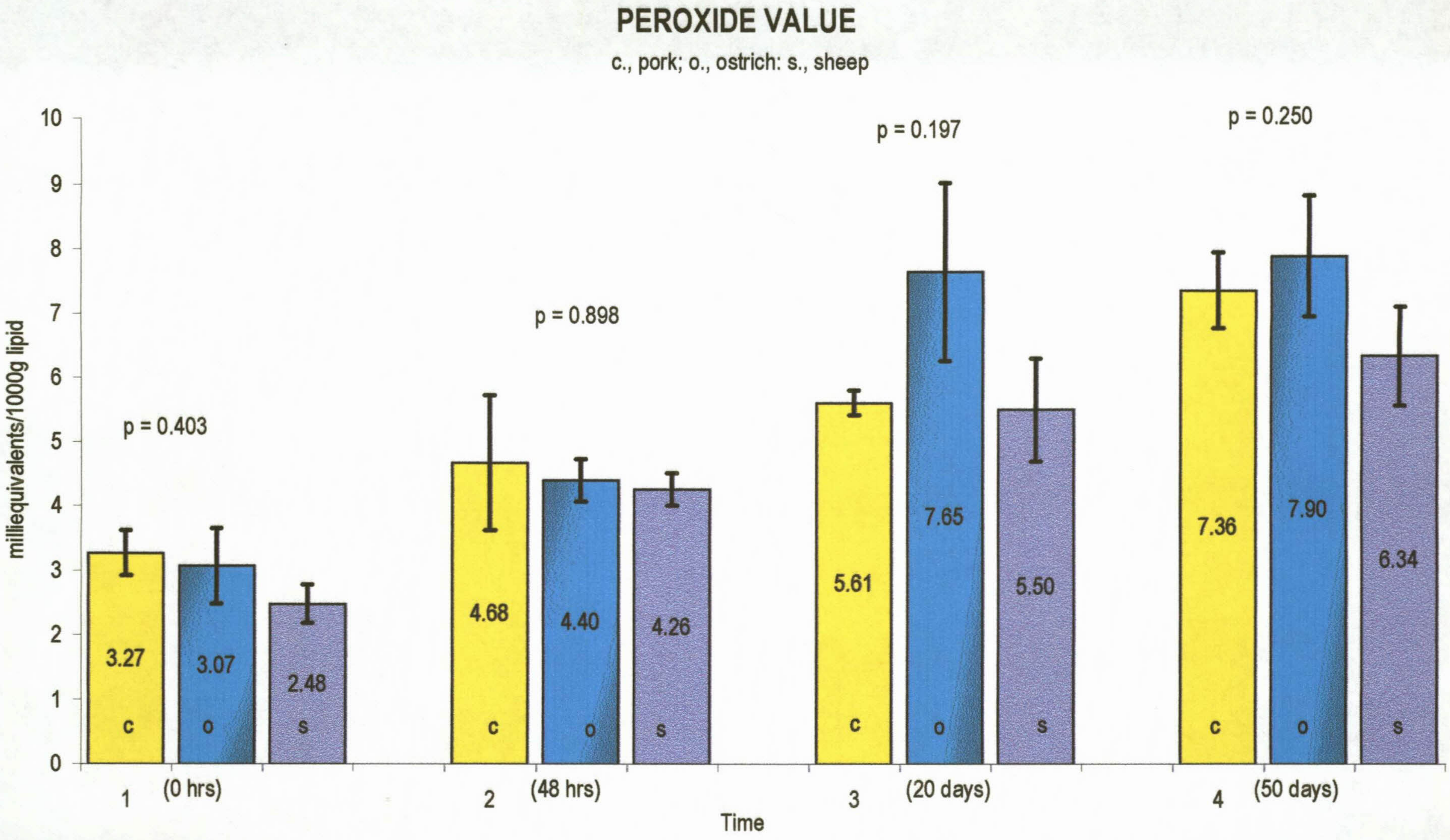
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 22. Changes in pH of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.



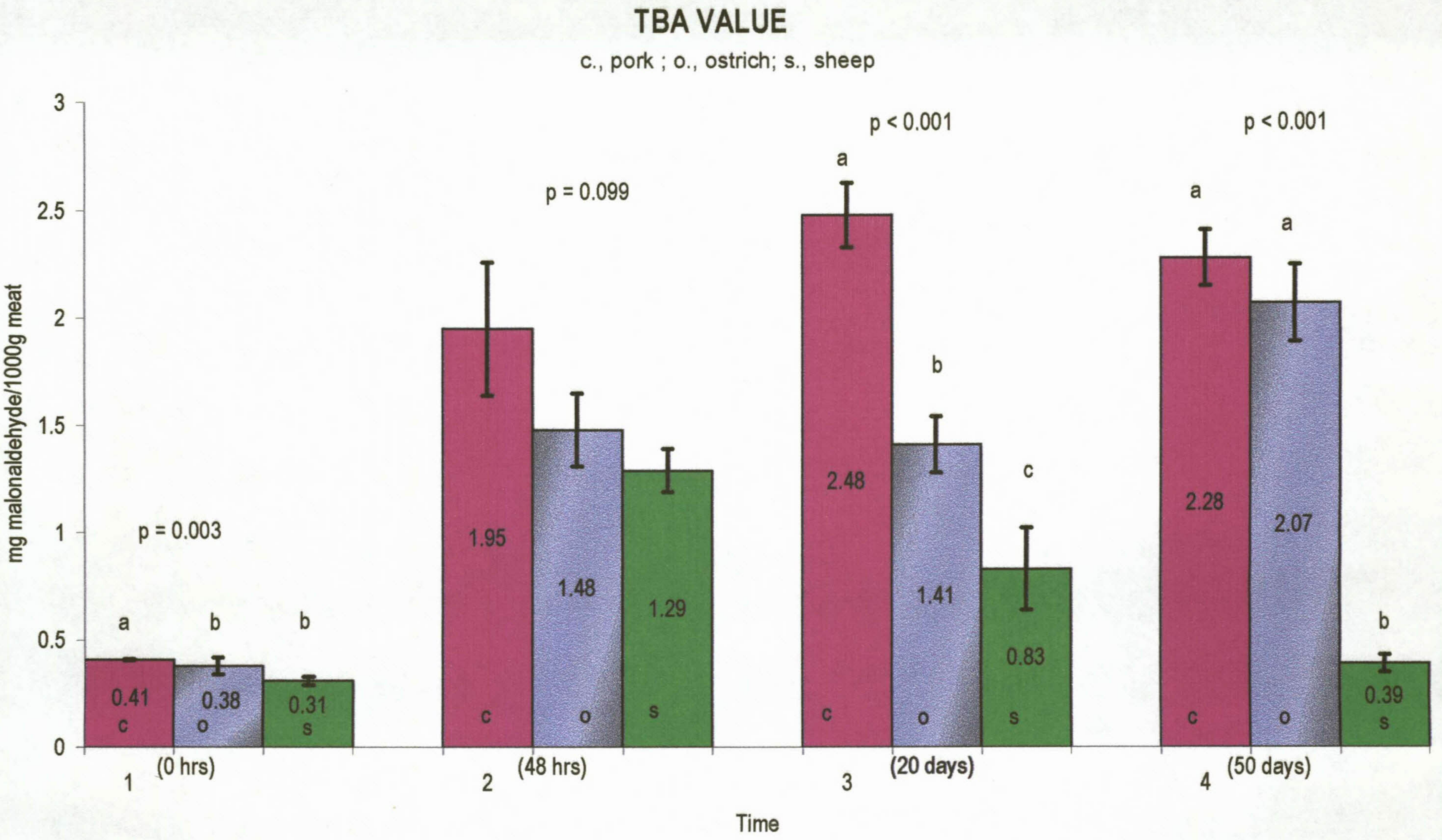
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 23. Free fatty acid contents of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.



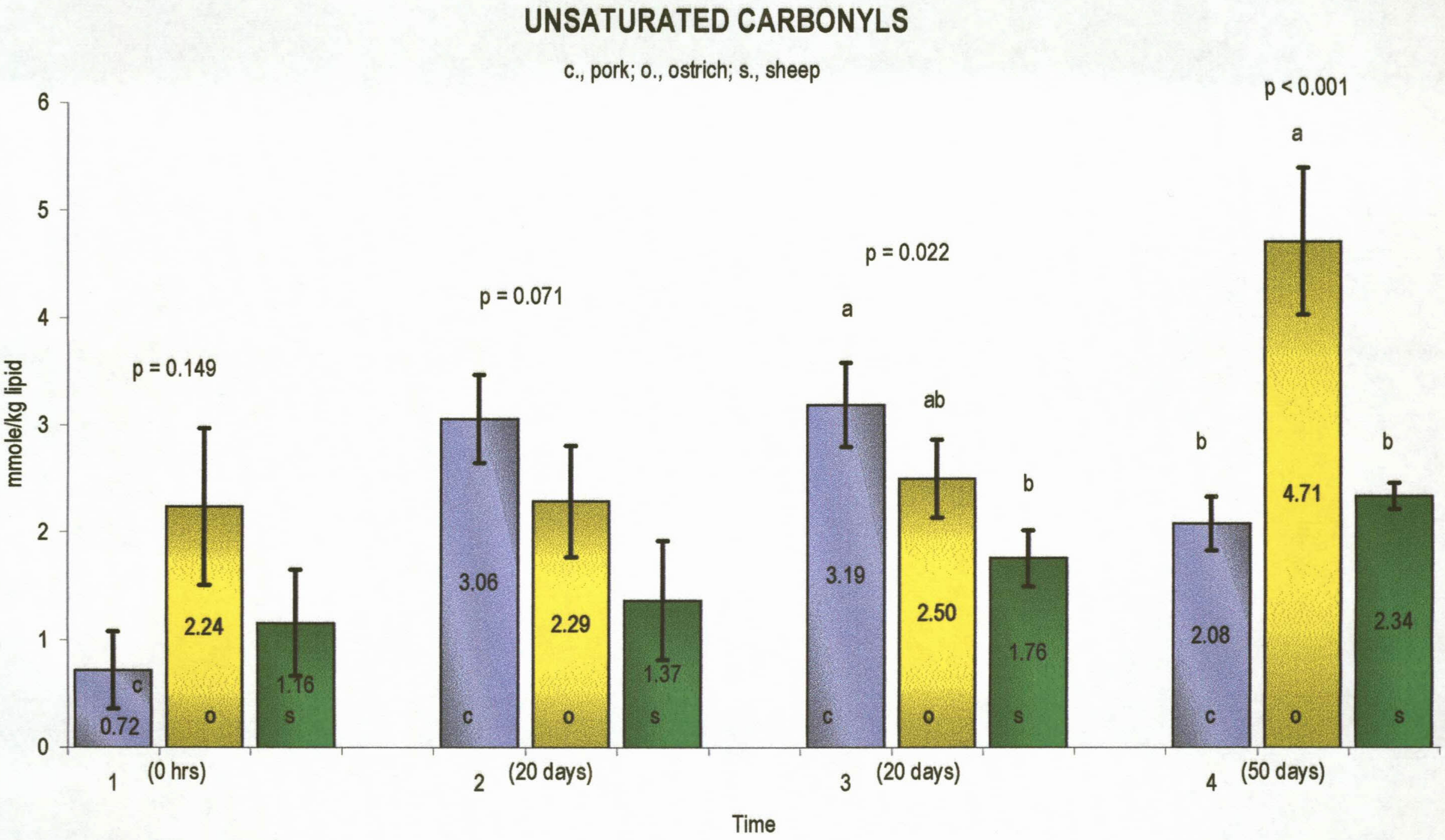
(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 24. Peroxide values of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.



(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 25. TBA values of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.



(within a group (1,2,3,4), means with the same superscript letter do not differ significantly).

Fig. 26. Unsaturated carbonyl values of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.

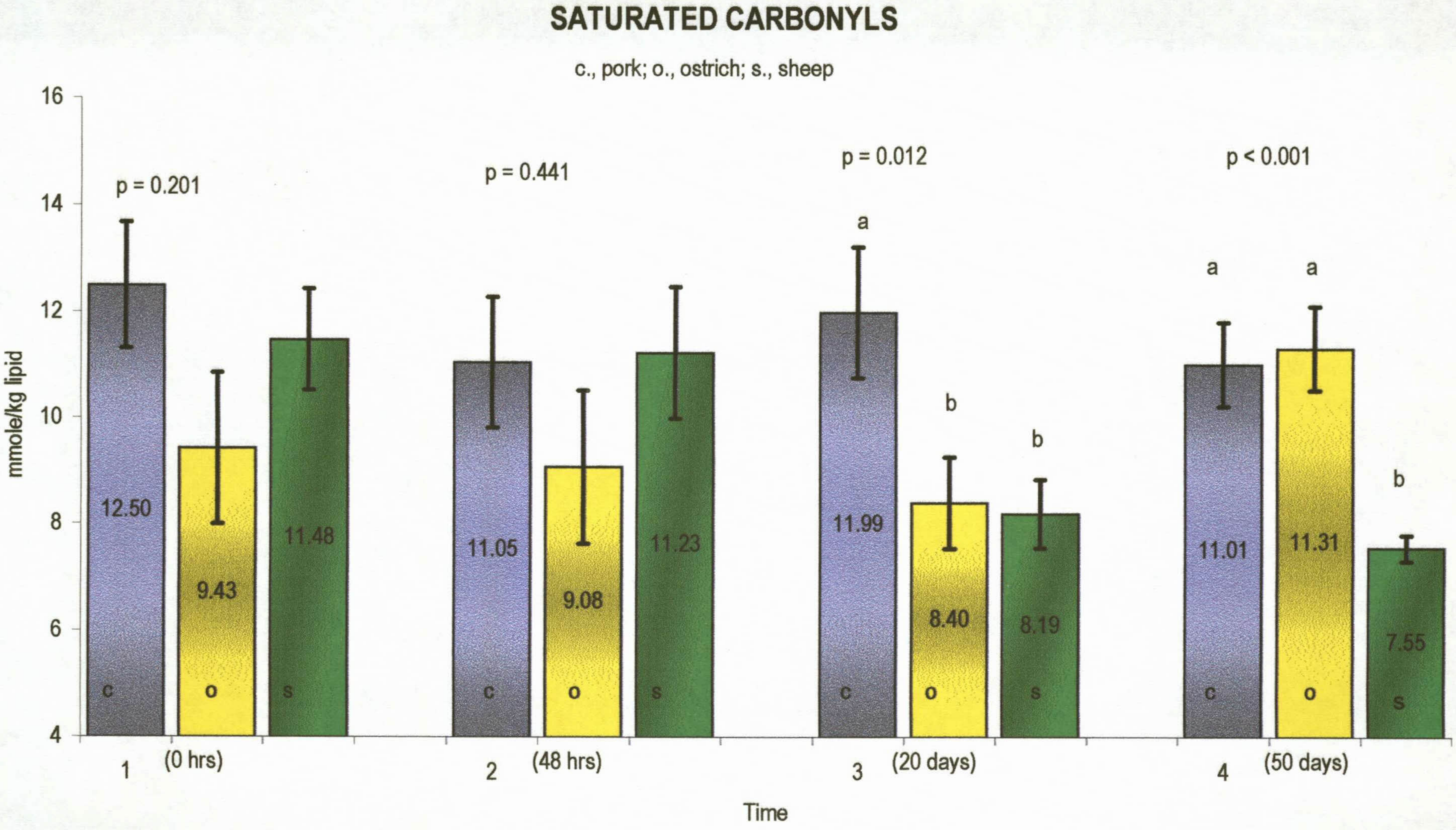


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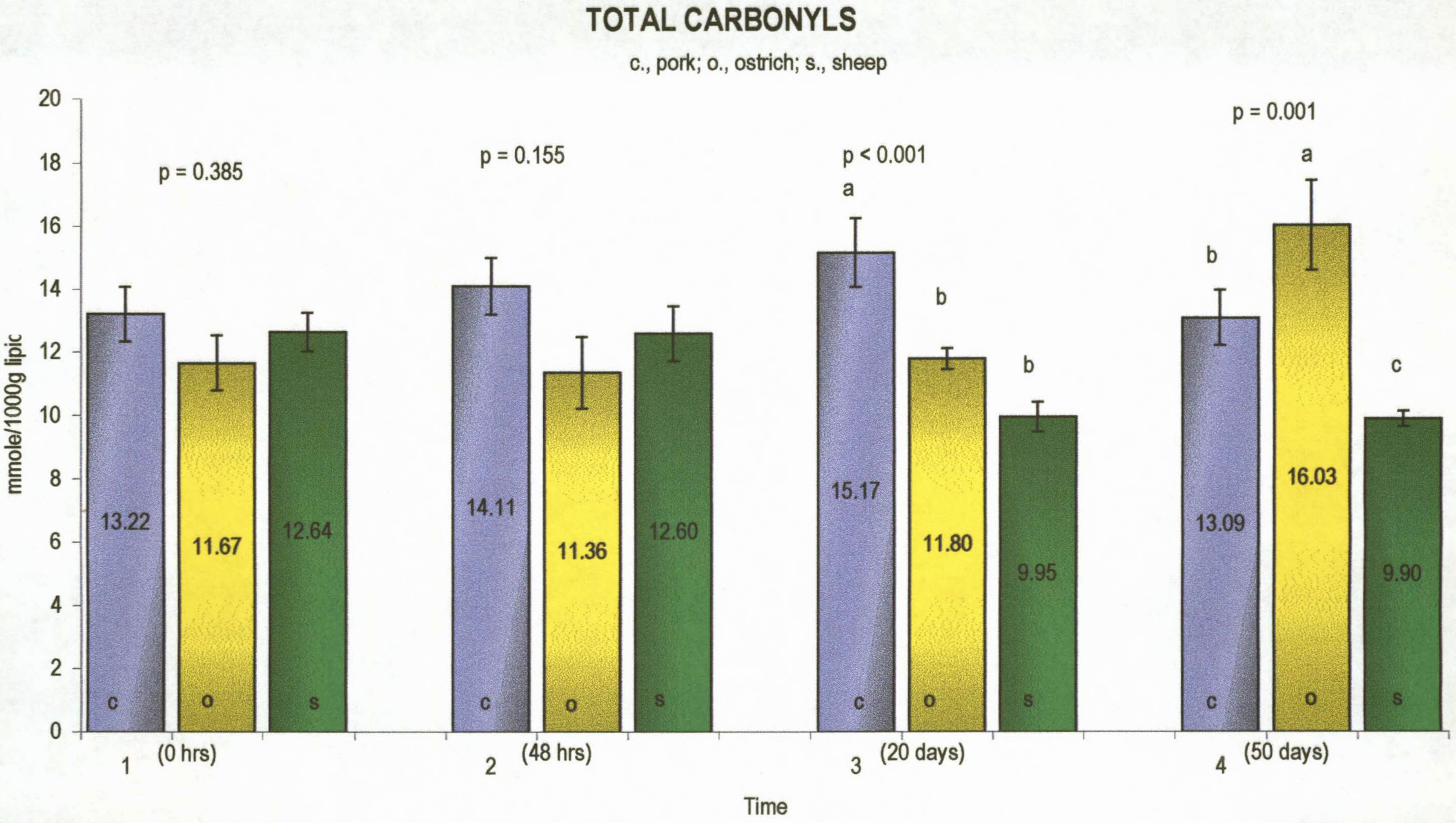
Fig. 27. Saturated carbonyl values of pork backfat, ostrich and sheeptail salami during fermentation, ripening and storage.



(within a group (1,2,3,4), means with the same superscript letter do not differ significantly)

Fig. 28. Total carbonyl values of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.

Table 6: Changes in fatty acid composition (% total lipids) of salami during processing

	Time (days)		
Fatty acids (%)	0	2	27
C14:0	1.55 ± 0.16^{b}	1.58 ± 0.19^{b}	1.92 ± 0.10^{a}
C16:0	22.06 ± 0.91^{a}	22.29 ± 1.01^{a}	22.58 ± 1.53^{a}
C16:1c9	2.65 ± 0.14^{a}	2.70 ± 0.12^{a}	2.61 ± 0.13^{a}
C17:0	0.40 ± 0.09^{b}	0.40 ± 0.08^{b}	0.50 ± 0.05^{a}
C17:1c10	0.32 ± 0.04^{a}	0.34 ± 0.03^{a}	0.29 ± 0.03^{b}
C18:0	11.97 ± 0.08^{b}	11.85 ± 0.63^{b}	12.58 ± 0.52^{a}
C18:1t9	0.42 ± 0.19^{a}	0.34 ± 0.12^{ab}	0.20 ± 0.22^{b}
C18:1c9	43.22 ± 1.02^{a}	43.55 ± 0.87^{a}	41.46 ± 0.68^{b}
C18:1c7	2.23 ± 0.37^{b}	2.16 ± 0.30^{b}	2.80 ± 0.09^{a}
C18:2c9, 12	9.46 ± 0.72^{b}	9.52 ± 0.68^{b}	10.57 ± 0.18^{a}
C20:0	0.52 ± 0.15^{a}	0.50 ± 0.15^{a}	0.23 ± 0.08^{b}
C20:1c11	1.45 ± 0.10^{a}	1.38 ± 0.11^{ab}	1.33 ± 0.14^{b}
C18:3c9,12, 15	0.77 ± 0.10^{b}	0.77 ± 0.11^{b}	0.91 ± 0.09^{a}
C21:0	0.29 ± 0.19^{a}	0.29 ± 0.18^{a}	0.08 ± 0.03^{b}
C20:2c11, 14	0.72 ± 0.12	0.69 ± 0.09	0.69 ± 0.08
C20:3c11, 14, 17	0.18 ± 0.09^{a}	0.15 ± 0.10^{a}	0.07 ± 0.08^{b}
C20:3c8,11, 14	0.20 ± 0.10^{a}	0.16 ± 0.11^{a}	0.08 ± 0.03^{b}
C20:4c5,8,11, 14	0.38 ± 0.05	0.37 ± 0.06	0.36 ± 0.05
SFA	36.71 ± 0.68^{b}	36.91 ± 0.67^{b}	37.81 ± 1.03^{a}
MUFA	50.29 ± 0.93^{a}	50.48 ± 0.66^{a}	48.68 ± 0.81^{b}
PUFA	11.71 ± 0.78^{b}	11.60 ± 0.79^{b}	12.61 ± 0.35^{a}

Results are means ± standard deviations ($n = 12$).

^{a,b} In the same row, means with the same superscript letters do not differ significantly ($p < 0.05$).

SFA: saturated fatty acids.

MUFA: monounsaturated fatty acids.

PUFA: polyunsaturated fatty acids.

Table 7: Quality parameters related to lipid stability of salami during storage at 4, 12 and 25 ^{0}C

	Time (days)						
	0	15			30		
Parameter		4 ^{0}C	12 ^{0}C	25 ^{0}C	4 ^{0}C	12 ^{0}C	25 ^{0}C
pH	4.84 ± 0.14^{abc}	4.89 ± 0.08^{ab}	4.79 ± 0.13^{bc}	4.47 ± 0.09^{d}	4.93 ± 0.05^{ab}	4.78 ± 0.14^{bc}	4.44 ± 0.06^{d}
Moisture (%)	44.94 ± 1.21^{b}	48.84 ± 2.26^{a}	49.63 ± 1.84^{a}	49.67 ± 2.27^{a}	49.11 ± 1.38^{a}	49.74 ± 1.29^{a}	49.87 ± 1.90^{a}
Fat (%)	23.92 ± 1.01^{ab}	22.07 ± 1.39^{bcd}	23.39 ± 1.50^{abc}	21.71 ± 1.50^{cd}	23.97 ± 1.17^{ab}	24.22 ± 2.04^{ab}	22.07 ± 1.83^{bcd}
FFDM (%)	31.14 ± 1.11^{a}	28.46 ± 0.72^{bc}	26.82 ± 0.90^{cdef}	28.83 ± 1.77^{bc}	26.91 ± 1.12^{def}	26.04 ± 1.23^{def}	27.96 ± 1.23^{bcd}
Neutral (%)	96.24 ± 1.44	94.62 ± 1.38	95.02 ± 1.30	95.57 ± 1.35	95.37 ± 0.66	95.97 ± 2.83	94.55 ± 1.27
Glyco (%)	2.69 ± 0.43^{de}	2.68 ± 0.30^{de}	3.21 ± 0.32^{bcd}	3.85 ± 0.62^{a}	3.00 ± 0.44^{cde}	3.23 ± 0.37^{bcd}	3.47 ± 0.35^{bc}
Phospho (%)	1.03 ± 0.42^{ab}	1.03 ± 0.42^{ab}	0.98 ± 0.49^{ab}	1.29 ± 0.53^{a}	0.91 ± 0.45^{ab}	0.63 ± 0.17^{b}	1.38 ± 0.62^{a}
FFA (% Oleic)	4.30 ± 0.79^{c}	8.34 ± 1.67^{b}	8.36 ± 1.39^{b}	7.17 ± 1.62^{b}	13.46 ± 3.08^{a}	7.17 ± 3.61^{b}	11.69 ± 3.42^{a}
TBA	0.39 ± 0.09^{de}	0.57 ± 0.18^{cde}	0.75 ± 0.14^{cd}	1.55 ± 0.37^{b}	0.77 ± 0.26^{cd}	1.61 ± 0.60^{b}	3.82 ± 0.32^{a}
PV	10.28 ± 3.41^{a}	8.09 ± 3.31^{a}	5.94 ± 2.66^{b}	4.40 ± 1.90^{b}	3.63 ± 1.05^{b}	5.56 ± 1.53^{b}	4.90 ± 2.36^{b}
IV	55.20 ± 4.28^{def}	58.60 ± 1.27^{bcde}	62.31 ± 1.59^{ab}	57.78 ± 2.93^{bcdef}	56.33 ± 5.50^{cdef}	62.36 ± 1.70^{ab}	60.71 ± 0.91^{abcd}
UC	10.64 ± 2.98^{b}	ND	ND	ND	6.50 ± 4.55^{c}	11.00 ± 5.27^{b}	25.83 ± 3.40^{a}
SC	22.62 ± 10.38^{bc}	ND	ND	ND	14.90 ± 4.91^{cd}	20.95 ± 6.69^{bcd}	44.14 ± 7.02^{a}
TC	33.26 ± 8.97^{b}	ND	ND	ND	20.57 ± 5.89^{c}	31.95 ± 11.43^{b}	69.82 ± 5.38^{a}

Results are means ± standard deviations (n = 12)
0., zero days of storage.
^{a,b,c,d,e,f} In the same row, means with the same superscript letter do not differ significantly
PV: peroxide value (milliequivalent/1000 g lipid)
IV: iodine value (g iodine/100 g lipid)
UC: unsaturated carbonyls (mmole/kg lipid)
SC: saturated carbonyls (mmole/kg lipid)
TC: total carbonyls (mmole/kg lipid)
TBA: mg malonaldehyde/1000 g meat.
ND: not determined

Table 8: Changes in fatty acid composition (% total lipids) of salami during storage at 4, 12 and 25 ^{0}C

	Time (days)						
	0	15			30		
Fatty acid (%)		4 ^{0}C	12 ^{0}C	25 ^{0}C	4 ^{0}C	12 ^{0}C	25 ^{0}C
C14:0	1.92 ± 0.10^{ab}	1.82 ± 0.10^{abc}	1.81 ± 0.15^{abc}	1.82 ± 0.13^{abc}	1.74 ± 0.10^{bcd}	1.65 ± 0.18^{cde}	1.60 ± 0.21^{de}
C16:0	22.38 ± 1.53^{a}	20.85 ± 0.80^{b}	21.96 ± 1.28^{ab}	21.35 ± 1.07^{ab}	20.92 ± 1.12^{b}	22.00 ± 0.80^{ab}	22.05 ± 0.86^{ab}
C16:1c9	2.61 ± 0.13^{a}	2.45 ± 0.10^{bc}	2.45 ± 0.16^{bc}	2.47 ± 0.15^{bc}	2.40 ± 0.11^{bcd}	2.32 ± 0.18^{bcd}	2.26 ± 0.22^{cd}
C17:0	0.50 ± 0.05^{a}	0.52 ± 0.04^{a}	0.48 ± 0.08^{a}	0.51 ± 0.07^{a}	0.50 ± 0.06^{a}	0.39 ± 0.09^{b}	0.36 ± 0.09^{b}
C17:1c10	0.29 ± 0.03^{a}	0.28 ± 0.03^{a}	0.27 ± 0.03^{a}	0.28 ± 0.02^{a}	0.28 ± 0.02^{a}	0.25 ± 0.04^{ab}	0.24 ± 0.02^{b}
C18:0	12.58 ± 0.52^{b}	13.02 ± 0.48^{a}	12.84 ± 0.30^{ab}	13.20 ± 0.33^{a}	13.10 ± 0.30^{a}	12.93 ± 0.27^{ab}	13.24 ± 0.33^{a}
C18:1t9	0.20 ± 0.22	0.34 ± 0.25	0.32 ± 0.25	0.38 ± 0.23	0.40 ± 0.20	0.26 ± 0.20	0.34 ± 0.25
C18:1c9	41.46 ± 0.68	42.50 ± 0.37	41.00 ± 3.26	42.24 ± 0.51	42.27 ± 0.64	42.12 ± 0.67	42.08 ± 0.62
C18:1c7	2.80 ± 0.09^{ab}	2.60 ± 0.06^{abc}	2.38 ± 0.30^{bcd}	2.59 ± 0.20^{abc}	2.67 ± 0.31^{abc}	2.20 ± 0.45^{cd}	2.15 ± 0.49^{cd}
C18:2c9,12	10.57 ± 0.18^{a}	10.69 ± 0.17^{a}	10.64 ± 0.14^{a}	10.55 ± 0.33^{a}	10.71 ± 0.13^{a}	10.56 ± 0.19^{a}	10.33 ± 0.25^{b}
C20:0	0.23 ± 0.08^{b}	0.29 ± 0.05^{b}	0.32 ± 0.18^{b}	0.30 ± 0.13^{b}	0.31 ± 0.11^{b}	0.56 ± 0.26^{a}	0.58 ± 0.16^{a}
C20:1c11	1.33 ± 0.14^{b}	1.50 ± 0.14^{a}	1.40 ± 0.20^{ab}	1.46 ± 0.12^{ab}	1.52 ± 0.15^{a}	1.53 ± 0.17^{a}	1.56 ± 0.10^{a}
C18:3c9, 12, 15	0.91 ± 0.09^{ab}	0.93 ± 0.06^{ab}	0.85 ± 0.09^{abc}	0.89 ± 0.07^{ab}	0.89 ± 0.07^{ab}	0.81 ± 0.07^{bcd}	0.76 ± 0.10^{cd}
C20:2c11, 14	0.69 ± 0.08	0.80 ± 0.06	0.77 ± 0.09	0.76 ± 0.08	0.76 ± 0.07	0.93 ± 0.47	0.95 ± 0.39
C20:4c5, 8, 11, 14	0.36 ± 0.05	0.35 ± 0.05	0.36 ± 0.07	0.35 ± 0.06	0.36 ± 0.05	0.35 ± 0.05	0.36 ± 0.06
SFA	37.81 ± 1.03^{a}	36.50 ± 0.54^{b}	37.41 ± 1.09^{ab}	37.18 ± 0.90^{ab}	36.56 ± 0.93^{b}	37.58 ± 0.60^{a}	37.92 ± 0.89^{a}
MUFA	48.68 ± 0.81^{ab}	49.67 ± 0.39^{a}	47.83 ± 3.55^{b}	49.40 ± 0.51^{ab}	49.54 ± 0.78^{ab}	48.67 ± 0.72^{ab}	48.63 ± 0.43^{ab}
PUFA	12.61 ± 0.35	12.95 ± 0.22	12.77 ± 0.34	12.64 ± 0.56	12.83 ± 0.21	12.96 ± 0.51	12.71 ± 0.48

Results are means ± standard deviation (n = 12). ^{a,b,c,d} In the same row, means with the same superscript letter do not differ significantly.
0: zero time of storage; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 9: Fatty acid composition (% of total lipids) of the feed

	Treatment	
Fatty acid (%)	Control	Sunflower oil supplemented
C16 0	13.30 ± 0.39^a	10.70 ± 0.29^b
C18 0	3.20 ± 0.15^b	3.81 ± 0.11^a
C18 1c9	27.28 ± 0.48^a	24.86 ± 0.44^b
C18 2c9, 12	50.73 ± 0.62^b	55.93 ± 0.64^a
C18 3c9, 12, 15	2.89 ± 0.13^a	1.95 ± 0.11^b
SFA	17.51 ± 0.42^a	15.51 ± 0.43^b
MUFA	28.80 ± 0.60^a	26.27 ± 0.38^b
PUFA	53.62 ± 0.61^b	57.88 ± 0.65^a

Results are means ± standard deviations (n = 12).

[a,b] In the same row, means with the same superscript letter do not differ significantly.

SFA., saturated fatty acids.

MUFA., monounsaturated fatty acids.

PUFA., polyunsaturated fatty acids.

Table 10: Fatty acid composition (% of total lipids) and quality characteristics of the backfat

	Treatment	
Parameter	Control	Sunflower oil supplemented
C10:0 (%)	1.79 ± 0.18	1.69 ± 0.23
C16:0 (%)	21.24 ± 1.71^{a}	19.55 ± 2.02^{b}
C16:1c9 (%)	2.46 ± 0.38^{a}	2.04 ± 0.41^{b}
C18:0 (%)	11.10 ± 0.91	10.17 ± 1.29
C18:1c9 (%)	36.47 ± 1.00^{a}	32.35 ± 1.47^{b}
C18:1c7 (%)	3.00 ± 0.36^{a}	2.28 ± 0.40^{b}
C18:2c9, 12 (%)	18.65 ± 2.69^{b}	27.42 ± 4.15^{a}
C18:3c9, 12, 15 (%)	1.33 ± 0.16^{a}	1.19 ± 0.18^{b}
C20:2c11, 14 (%)	0.82 ± 0.13^{b}	1.12 ± 0.17^{a}
SFA (%)	34.81 ± 2.22^{a}	31.94 ± 3.08^{b}
MUFA (%)	43.08 ± 1.53^{a}	37.46 ± 2.15^{b}
PUFA (%)	21.00 ± 3.01^{b}	30.03 ± 4.53^{a}
IV (g iodine/100g lipid)	71.91 ± 5.06^{b}	82.00 ± 5.26^{a}
DBI	92.77 ± 5.94^{b}	105.30 ± 7.72^{a}

Results are means ± standard deviations (n = 12).

[a,b] In the same row, means with the same superscript letter do not differ significantly.

SFA., saturated fatty acids.

MUFA., monounsaturated fatty acids.

PUFA., polyunsaturated fatty acids.

IV., iodine value.

DBI., double bond index.

Table 11: Fatty acid composition (% of total lipids) of the muscle

	Treatment	
Fatty acid (%)	Control	Sunflower oil supplemented
C14:0	1.95 ± 0.21	1.93 ± 0.20
C16:0	21.98 ± 0.78	21.68 ± 1.90
C16:1c9	3.58 ± 0.51	3.12 ± 0.62
C18:0	11.17 ± 0.63	11.39 ± 0.66
C18:1c9	36.42 ± 2.14	35.16 ± 4.14
C18:1c7	5.05 ± 0.71	4.26 ± 0.81
C18:2c9, 12	12.74 ± 2.03	15.34 ± 4.27
C20:4c5, 8, 11, 14	2.06 ± 0.48	2.29 ± 1.49
SFA	35.64 ± 0.63	35.58 ± 2.31
MUFA	45.77 ± 2.91	43.38 ± 5.09
PUFA	16.15 ± 2.49	19.00 ± 5.90

Results are means ± standard deviations (n = 12).
SFA., saturated fatty acids.
MUFA., monounsaturated fatty acids.
PUFA., polyunsaturated fatty acids.

Table 12: Proximate values of salami during fermentation, ripening and storage

	Dietary treatment							
	Control				Sunflower oil supplememted			
Parameter	0 hrs	48 hrs	21 days	51 days	0 hrs	48 hrs	21 days	51 days
Moisture (%)	57.50 ± 1.44	58.07 ± 1.21	51.73 ± 1.38	50.67 ± 1.55	58.35 ± 1.70	58.17 ± 1.76	52.48 ± 1.52	51.25 ± 1.62
Fat (%)	19.32 ± 1.35	18.90 ± 1.54	22.49 ± 1.89	22.67 ± 1.94	18.38 ± 1.54	18.70 ± 1.76	21.61 ± 1.69	21.50 ± 1.75
FFDM (%)	23.18 ± 1.02	23.03 ± 0.74	25.78 ± 0.88	26.83 ± 0.98	23.27 ± 0.54	23.13 ± 1.03	25.91 ± 0.73	27.25 ± 0.81
Protein (%)	17.81	18.09	22.71	22.71	16.06	16.37	20.60	20.60
Nitrite (ppm $NaNO_2$)	90	91	115	115	90	92	115	115
Salt (% NaCl)	2.69	2.74	3.44	3.44	2.89	2.94	3.71	3.71
Weight loss (%)	0	1.54	21.56	ND	0	1.89	22.04	ND

Results are means ± standard deviations (n = 12, except for protein nitrite, salt and weight loss, where n = 3).
FFDM., fat free dry matter.
Statistical analysis was not done on protein, nitrite, salt and weight loss because of the small number of replicates used.
ND., not done.

Table 13: Changes in the fatty acid composition (% of total lipids) of salami during fermentation, ripening and storage

	Dietary treatment									
	Control					Sunflower oil supplemented				
Fatty acid (%)	0 hrs	48 hrs	21 days	51 days	Δ%	0 hrs	48 hrs	21 days	51 days	Δ%
C14:0	1.77 ± 0.12	1.68 ± 0.07	1.75 ± 0.07	1.74 ± 0.09	− 0.03	1.59 ± 0.51	1.77 ± 0.11	1.78 ± 0.06	1.75 ± 0.09	+ 0.16
C16:0	22.87 ± 0.90^{a}	22.57 ± 0.79^{a}	23.00 ± 0.57^{a}	22.70 ± 0.70^{a}	− 0.17	21.67 ± 0.78^{b}	21.76 ± 0.89^{b}	21.90 ± 0.59^{b}	21.73 ± 0.92^{b}	+ 0.06
C16:1c9	2.30 ± 0.16^{a}	2.21 ± 0.14^{a}	2.31 ± 0.09^{a}	2.31 ± 0.12^{a}	+ 0.01	1.98 ± 0.10^{b}	2.00 ± 0.14^{b}	2.02 ± 0.17^{b}	1.95 ± 0.17^{b}	− 0.03
C18:0	12.34 ± 0.43^{bcd}	12.93 ± 0.46^{a}	12.63 ± 0.35^{abc}	12.60 ± 0.40^{abc}	+ 0.26	11.99 ± 0.70^{cd}	11.89 ± 0.50^{cd}	11.86 ± 0.36^{cd}	12.12 ± 0.35^{cd}	+ 0.13
C18:1c9	35.95 ± 1.74^{a}	36.46 ± 2.00^{a}	36.34 ± 0.74^{a}	35.85 ± 1.53^{a}	− 0.10	31.85 ± 1.56^{b}	31.76 ± 1.44^{b}	31.66 ± 1.44^{b}	31.63 ± 2.03^{a}	− 0.22
C18:1c9	2.38 ± 0.21^{a}	2.45 ± 0.16^{a}	2.44 ± 0.10^{a}	2.38 ± 0.36^{a}	0.00	1.95 ± 0.10^{b}	1.93 ± 0.09^{b}	1.94 ± 0.04^{b}	1.96 ± 0.11^{b}	+ 0.01
C18:2c9, 12	16.13 ± 2.86^{b}	15.82 ± 1.05^{b}	15.42 ± 0.79^{b}	16.26 ± 1.02^{b}	+ 0.13	22.32 ± 1.83^{a}	22.37 ± 1.42^{a}	21.44 ± 2.04^{a}	21.52 ± 2.04^{a}	− 0.80
C18:3c9,12,15	1.03 ± 0.05^{a}	1.02 ± 0.09^{a}	1.02 ± 0.06^{a}	1.03 ± 0.08^{a}	0.00	0.94 ± 0.05^{c}	0.95 ± 0.07^{bc}	0.94 ± 0.10^{c}	0.93 ± 0.11^{c}	− 0.01
C20:2c11, 14	0.68 ± 0.06^{b}	0.77 ± 0 23^{b}	0.77 ± 0.44^{b}	1.00 ± 0.92^{b}	+ 0.32	1.83 ± 1.34^{a}	1.38 ± 1.15^{ab}	0.96 ± 0.17^{ab}	1.57 ± 1.34^{ab}	− 0.26
SFA	38.64 ± 1.31^{a}	38.04 ± 0.55^{a}	38.76 ± 0.58^{a}	38.16 ± 0.73^{a}	− 0.48	36.58 ± 0.75^{b}	36.55 ± 0.92^{b}	36.90 ± 0.59^{b}	36.72 ± 0.89^{b}	+ 0.14
MUFA	41.84 ± 2.16^{a}	42.35 ± 2.29^{a}	42.33 ± 0.81^{a}	41.69 ± 1.57^{a}	− 0.15	36.88 ± 1.37^{b}	36.73 ± 1.40^{b}	36.76 ± 1.34^{b}	36.62 ± 2.08^{b}	− 0.26
PUFA	18.29 ± 3.05^{b}	18.09 ± 1.06^{b}	17.63 ± 0.80^{b}	18.83 ± 0.90^{b}	+ 0.54	25.85 ± 1.91^{a}	25.41 ± 0.98^{a}	24.11 ± 1.96^{a}	24.71 ± 1.40^{a}	− 1.14

Results are means ± standard deviations (n = 12).
^{a,b,c,d} In the same row, means with the same superscript letter do not differ significantly.
SFA., saturated fatty acids.
MUFA., mounsaturated fatty acids.
PUFA., polyunsaturated fatty acids.
Δ%., fatty acid percentage change.

Table 14: Quality characteristics of salami fat

	Type of fat source		
Parameter	Pork backfat	Ostrich	Sheeptail
Moisture (%)	10.78 ± 0.45	7.73 ± 2.95	6.69 ± 1.61
Fat (%)	78.64 ± 0.55	83.56 ± 4.00	84.41 ± 1.03
FFDM (%)	10.58 ± 0.46	8.71 ± 1.17	8.89 ± 0.61
Protein (%)	2.13 ± 0.44	1.08 ± 0.18	0.76 ± 0.17
L-hydroxyproline (%)	0.14 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
FFA (% oleic)	1.22 ± 0.04	1.20 ± 0.06	1.52 ± 0.06
PV (milliequivalent/kg lipid)	10.63 ± 0.61	7.68 ± 1.86	14.54 ± 1.14
IV (g Iodine/100 g lipid)	62.35 ± 1.86	67.43 ± 2.21	48.27 ± 1.64
UC (mmole/1000 g lipid)	1.09 ± 2.26	0.50 ± 0.75	2.90 ± 2.90
SC (mmole/1000 g lipid)	8.61 ± 4.95	5.18 ± 1.39	3.82 ± 5.21
TC (mmole/1000 g lipid)	9.70 ± 2.73	5.68 ± 0.64	6.72 ± 3.28
C10:0 (%)	ND	ND	0.25 ± 0.02
C12:0 (%)	ND	ND	0.50 ± 0.03
C14:0 (%)	1.44 ± 0.04	0.83 ± 0.03	6.42 ± 0.02
C14:1c9 (%)	ND	0.08 ± 0.07	0.39 ± 0.04
C15:0 (%)	ND	0.22 ± 0.01	0.88 ± 0.02
C16:0 (%)	25.70 ± 035	29.81 ± 0.31	25.36 ± 0.06
C16:1c9 (%)	2.61 ± 0.09	8.20 ± 0.24	3.49 ± 0.10
C17:0 (%)	0.37 ± 0.01	0.14 ± 0.06	1.80 ± 0.14
C17:1c10 (%)	0.33 ± 0.03	0.04 ± 0.06	1.16 ± 0.10
C18:0 (%)	14.05 ± 0.26	6.37 ± 0.02	12.74 ± 0.77
C18:1t9 (%)	0.17 ± 0.09	0.31 ± 0.09	1.30 ± 0.18
C18:1c9 (%)	39.88 ± 0.39	34.46 ± 0.41	36.20 ± 1.92
C18:1c7 (%)	1.67 ± 0.08	1.03 ± 0.14	0.32 ± 0.03
C18:2t9, 12 (%)	ND	0.08 ± 0.07	0.34 ± 0.03
C18:2c9, 12 (%)	9.49 ± 0.43	15.05 ± 0.22	1.78 ± 0.01
C20:0 (%)	0.25 ± 0.01	0.04 ± 0.04	0.39 ± 0.30
C18:3c6, 9, 12 (%)	ND	0.03 ± 0.05	ND
C20:1c11 (%)	0.82 ± 0.04	0.18 ± 0.02	0.11 ± 0.04
C18:3c9, 12, 15 (%)	0.60 ± 0.02	1.78 ± 0.04	0.69 ± 0.11
C21:0 (%)	0.01 ± 0.01	0.01 ± 0.01	0.25 ± 0.25
C20:2c11, 14 (%)	0.55 ± 0.14	0.41 ± 0.50	2.71 ± 2.46
C20:3c11, 14, 17 (%)	0.01 ± 0.01	0.02 ± 0.04	ND
C20:3c8, 11, 14 (%)	0.08 ± 0.01	ND	ND
C20:4c5, 8, 11, 14 (%)	0.13 ± 0.02	0.23 ± 0.03	ND
C22:2c13, 16 (%)	0.03 ± 0.02	ND	ND
C24:0 (%)	0.36 ± 0.02	ND	ND
C22:6c4, 7, 10, 13, 16, 19 (%)	0.54 ± 0.01	ND	ND
SFA (%)	42.18 ± 0.52	37.40 ± 0.33	47.57 ± 0.88
MUFA (%)	42.87 ± 0.43	36.10 ± 0.36	39.47 ± 2.17
PUFA (%)	11.52 ± 0.34	17.60 ± 0.40	5.53 ± 2.32
Double Bond Index (DBI)	71.71 ± 0.73	81.78 ± 0.38	54.70 ± 2.29

Results are means ± standard deviation (n =12).

FDM: fat free dry matter; IV: iodine value; PV: peroxide value; UC: unsaturated carbonyls; SC: saturated carbonyls; TC: total carbonyls; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ND: not detected

Table 15: Proximate values for pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.

	Type of fat source											
	Pork backfat				Ostrich fat				Sheeptail fat			
Parameter	0 hrs	48 hrs	20 days	50 days	0 hrs	48 hrs	20 days	50 days	0 hrs	48 hrs	20 days	50 days
Moisture (%)	58.63 ± 2.59	58.77 ± 2.77	48.70 ± 1.53	47.42 ± 1.05^{ab}	58.20 ± 0.79	58.19 ± 0.79	48.29 ± 1.62	46.91 ± 0.41^{b}	58.26 ± 1.45	58.69 ± 1.67	48.55 ± 1.57	48.06 ± 0.96^{a}
Fat (%)	16.43 ± 2.34	18.94 ± 0.99	22.55 ± 1.67	23.63 ± 1.36	17.61 ± 1.28	18.95 ± 1.31	22.85 ± 1.23	24.00 ± 1.09	17.78 ± 1.81	18.32 ± 1.61	22.03 ± 1.20	23.57 ± 1.17
FFDM (%)	24.94 ± 1.23	22.29 ± 0.36	28.75 ± 1.29	28.95 ± 1.33	24.19 ± 1.04	22.86 ± 1.01	28.85 ± 1.27	28.92 ± 1.05	23.96 ± 1.03	22.99 ± 0.67	29.42 ± 1.44	28.61 ± 1.00
Protein (%)	15.06	15.44	18.95	18.95	15.17	15.44	18.38	18.38	14.72	14.97	18.14	18.14
Salt	3.02	3.10	4.30	4.30	2.99	3.05	4.35	4.35	2.99	3.04	4.27	4.27
Nitrites	90	90	120	120	90	90	120	120	90	90	120	120
Weight loss	0	2.34	28.04	ND	0	2.04	26.34	ND	0	1.87	26.55	ND

Results are means ± standard deviations (n = 12, except for protein, nitrite, salt and weight loss where n = 3).
^{a,b} In the same row, means with the same superscript letter do not differ significantly.
Statistical analysis was not done on the protein, salt and nitrite because of the small number of replicates used.
FFDM., fat free dry matter.
Salt., % NaCl.
Nitrites., ppm $NaNO_2$.
Weight loss ., % of original salami weight.
ND., not done.

Table 16: Changes in fatty acid composition (% total lipids) of pork backfat, ostrich and sheeptail fat salami during fermentation, ripening and storage.

	Type of fat source											
	Pork backfat				Ostrich				Sheeptail			
Fatty acid (%)	0 hrs	48 hrs	20 days	50 days	0 hrs	48 hrs	20 days	50 days8	0 hrs	4 8 hrs	0 days	50 days
C14: 0	1.49 ± 0.08^{d}	1.49 ± 0.05^{d}	1.51 ± 0.09^{d}	1.50 ± 0.04^{d}	1.04 ± 0.04^{e}	1.04 ± 0.00^{e}	1.01 ± 0.03^{e}	1.03 ± 0.03^{e}	5.57 ± 0.20^{ab}	5.49 ± 0.00^{abc}	5.41 ± 0.17^{bc}	5.36 ± 0.16^{bc}
C15: 0	ND	0.03 ± 0.06^{c}	0.10 ± 0.04^{c}	0.09 ± 0.03^{c}	0.20 ± 0.10	0.21 ± 0.07^{b}	0.22 ± 0.07^{b}	0.21 ± 0.07^{b}	0.80 ± 0.03^{a}	0.78 ± 0.04^{a}	0.78 ± 0.03^{a}	0.76 ± 0.02^{a}
C16: 0	25.28 ± 0.68^{b}	25.49 ± 0.68^{b}	25.43 ± 0.54^{b}	25.24 ± 0.35^{b}	28.85 ± 0.35^{a}	28.98 ± 0.38^{a}	28.87± 0.29^{a}	28.98 ± 0.33^{a}	24.47 ± 0.54^{c}	24.05 ± 0.30^{d}	22.87 ± 0.42^{d}	23.92 ± 0.31^{d}
C16: 1c9	2.66 ± 0.08^{e}	2.56 ± 0.24^{e}	2.49 ± 0.26^{e}	2.52 ± 0.26^{e}	7.03 ± 0.61^{c}	7.37 ± 0.17^{a}	7.25 ± 0.14^{b}	7.15 ± 0.55^{b}	3.71 ± 0.24^{d}	3.58 ± 0.32^{d}	3.66 ± 0.18^{d}	3.49 ± 0.28^{d}
C17: 0	0.35 ± 0.04^{b}	0.36 ± 0.04^{b}	0.35 ± 0.03^{b}	0.36 ± 0.02^{b}	0.19 ± 0.03^{c}	0.17 ± 0.02^{c}	0.18 ± 0.03^{c}	0.19 ± 0.03^{c}	1.66 ± 0.04^{a}	1.67 ± 0.04^{a}	1.68 ± 0.05^{a}	1.69 ± 0.04^{a}
C17: 1c10	0.33 ± 0.03^{c}	0.31 ± 0.03^{c}	0.30 ± 0.05^{c}	0.32 ± 0.04^{c}	0.07 ± 0.08^{d}	0.08 ± 0.07^{d}	0.13 ± 0.02^{d}	0.11 ± 0.05^{d}	1.22 ± 0.11^{a}	1.22 ± 0.07^{a}	1.23 ± 0.08^{a}	1.15 ± 0.09^{b}
C18: 0	13.78 ± 0.20^{bc}	13.98 ± 0.30^{bc}	14.15 ± 0.22^{ab}	14.06 ± 0.18^{ab}	7.83 ± 0.17^{g}	7.74 ± 0.14^{g}	7.88 ± 0.16^{g}	7.77 ± 0.12^{g}	12.71 ± 0.44^{e}f	12.97 ± 0.21^{cd}	12.86 ± 0.44^{cdef}	13.00 ± 0.36^{cd}
C18: 1t9	0.08 ± 0.16^{cd}	0.30 ± 0.49^{bcd}	0.27 ± 0.35^{bcd}	0.26 ± 0.32^{bcd}	0.40 ± 0.38^{bcd}	0.28 ± 0.04^{bcd}	0.29 ± 0.06^{bcd}	0.29 ± 0.09^{bcd}	0.79 ± 0.46^{ab}	0.77 ± 0.48^{ab}	0.62 ± 0.42^{abc}	0.82 ± 0.54^{ab}
C18: 1c9	39.88 ± 0.40^{a}	39.07 ± 2.67^{a}	38.02 ± 3.30^{a}	38.47 ± 2.90^{a}	34.81 ± 2.60^{b}	35.37 ± 0.70^{b}	35.67 ± 0.36^{b}	35.53 ± 0.45^{b}	38.34 ± 2.26^{a}	38.85 ± 1.29^{a}	38.73 ± 1.35^{a}	37.58 ± 2.36^{a}
C18: 1c7	1.89 ± 0.04^{a}	1.78 ± 0.10^{a}	1.64 ± 0.15^{b}	1.69 ± 0.15^{b}	1.15 ± 0.09^{c}	1.19 ± 0.03^{b}	1.11 ± 0.03^{c}	1.07 ± 0.07^{d}	0.58 ± 0.04^{e}	0.59 ± 0.07^{e}	0.52 ± 0.04^{e}	0.51 ± 0.04^{e}
C18: 2c9, 12	10.34 ± 0.29^{b}	10.21 ± 0.20^{b}	9.85 ± 0.65^{c}	9.81 ± 0.68^{c}	13.96 ± 0.55^{a}	14.21 ± 0.12^{a}	13.90 ± 0.41^{a}	14.07 ± 0.12^{a}	3.05 ± 0.23^{d}	3.28 ± 0.30^{d}	3.45 ± 0.36^{d}	3.18 ± 0.21^{d}
C20: 0	0.23 ± 0.02^{a}	0.24 ± 0.02^{a}	0.24 ± 0.02^{a}	0.22 ± 0.03^{a}	0.03 ± 0.03^{cd}	0.05 ± 0.03^{bcd}	0.06 ± 0.02^{bcd}	0.05 ± 0.02^{bcd}	0.07 ± 0.03^{bc}	0.07 ± 0.03^{bc}	0.08 ± 0.03^{bc}	0.08 ± 0.02^{bc}
C20: 1c11	0.77 ± 0.04^{a}	0.78 ± 0.12^{a}	0.75 ± 0.08^{a}	0.78 ± 0.07^{a}	0.26 ± 0.02^{b}	0.26 ± 0.03^{b}	0.26 ± 0.02^{b}	0.24 ± 0.02^{b}	0.13 ± 0.03^{c}	0.13 ± 0.05^{c}	0.13 ± 0.03^{c}	0.15 ± 0.05^{c}
C18: 3c9, 12, 15	0.59 ± 0.04^{c}	0.61 ± 0.05^{c}	0.56 ± 0.05^{c}	0.57 ± 0.06^{c}	1.61 ± 0.14^{a}	1.63 ± 0.06^{a}	1.60 ± 0.10^{a}	1.63 ± 0.04^{a}	0.77 ± 0.08^{b}	0.78 ± 0.05^{b}	0.79 ± 0.05^{b}	0.72 ± 0.07^{b}
C20: 2c 11, 14	0.59 ± 0.26	0.65 ± 0.35	1.39 ± 1.52	1.83 ± 2.53	0.96 ± 1.82	0.29 ± 0.34	0.33 ± 0.33	0.34 ± 0.54	0.51 ± 1.03	1.12 ± 2.50	0.59 ± 1.11	1.65± 2.04
SFA	42.38 ± 0.75^{d}	41.68 ± 0.62^{d}	41.92 ± 0.20^{d}	41.62 ± 0.35^{d}	38.14 ± 0.40^{e}	38.19 ± 0.28^{e}	39.23 ± 0.41^{e}	38.24 ± 0.36^{e}	46.15 ± 0.78^{ab}	45.84 ± 0.29^{abc}	45.55 ± 0.63^{bc}	45.48 ± 0.64^{abc}
MUFA	45.61 ± 0.75	44.80 ± 2.40	43.47 ± 3.63	44.04 ± 3.22	43.77 ± 2.98	44.50 ±0.72	44.84 ± 0.36	44.47 ± 0.83	45.17 ± 2.52	45.51 ± 1.54	45.30 ± 1.51	44.07 ± 2.68
PUFA	12.23 ± 0.50^{b}	12.19 ± 0.41^{b}	12.63 ± 1.37^{b}	13.12 ± 1.86^{b}	16.96 ± 1.45^{a}	16.54 ± 0.40^{a}	16.24 ± 0.44^{a}	16.44 ± 0.56^{a}	4.76 ± 0.96^{de}	5.72 ± 2.32^{cde}	5.43 ± 1.14^{cde}	6.12 ± 1.84^{cd}

Results are means ± standard deviations (n = 12).

^{,a,b,c,d}, In the same row, means with the same superscript letter do not differ significantly.

SFA., saturated fatty acids; MUFA., monounsaturated fatty acids., PUFA., polyunsaturated fatty acids; ND., not detected.

Table 17: Physical measurements of pork backfat, ostrich and sheeptail fat salami after storage.

	Type of fat source		
Parameter	Pork backfat	Ostrich	Sheeptail
L*	50.74 ± 4.40	47.47 ± 3.79	47.90 ± 2.18
a*	12.76 ± 1.89^{b}	14.89 ± 2.14^{a}	13.83 ± 1.42^{ab}
b*	9.35 ± 1.09^{b}	11.27 ± 1.29^{a}	9.33 ± 0.40^{b}

Results are means ± standard deviations (n =12).

^{a,b} In the same row, means with the same superscript letter do not differ significantly.

L*., lightness

a*., redness

b*., yellowness