EFFECT OF DIETARY FATS AND ANTIOXIDANTS ON THE FROZEN STORAGE LIFE OF COOKED PORK PRODUCTS

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By

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DEDICATIONS

I dedicate this thesis to my mother who always believed in me and who always generously supported me throughout my studies.

This thesis is also dedicated to my grandmother Elise and her sister, my great aunt Germaine, whose memories I will always cherish.

ABSTRACT

The objectives of this study were: 1) to investigate the oxidative stability of bacon manufactured from pork obtained from pigs fed a diet containing a combination of vitamin E (10 mg or 200 mg α-tocopheryl acetate per kilogram of feed) and fishmeal (0% or 5%) and processed with wood smoke only or with a combination of liquid smoke and wood smoke, 2) to investigate the oxidative stability of wiener sausages prepared from pork obtained from pigs fed a diet containing a combination of vitamin E (10 mg or 200 mg α-tocopheryl acetate per kilogram of feed) and fishmeal (0% or 5%) and manufactured with or without an antioxidant (0.03% rosemary extract or 2.5% whey powder).

Twelve (Large White x Landrace) gilts were randomly allotted to four dietary treatments containing two levels of vitamin E (10 or 200 mg/kg feed) and two levels of fishmeal (0 or 5%). Two products were manufactured from meat obtained from the animals after slaughter, bacon with or without liquid smoke and wiener sausages with or without rosemary extract and sweet whey powder. The oxidative stability of bacon was examined over 16 weeks of frozen storage and that of wieners during ten months frozen storage.

Lipid oxidation in the products was measured by means of thiobarbituric acid reactive substances (TBARS), fluorescence shift and sensory evaluation. The fluorescence shift method was unsatisfactory in the case of wieners, as it appeared that rosemary extract contained compounds that fluoresced and therefore interfered with the method.

The fatty acid composition of bacon fat showed that diet had no significant effect ($P > 0.05$) in the proportions of total saturated and total monounsaturated fatty acids. Bacon prepared from pigs fed the diet supplemented with vitamin E had higher levels ($P < 0.05$) of total polyunsaturated fatty acids than bacon manufactured from pigs fed the other experimental diets.

Analysis of vitamin E content in bacon fat and muscle showed that the diet supplemented with 200 mg vitamin E per kilogram of feed increased α-tocopherol levels by 3 fold in bacon fat and by 2 fold in bacon loin muscle compared to

bacon prepared from pigs fed a basal level of vitamin E (10 mg vitamin E per kilogram of feed).

Bacon processed with a combination of liquid and wood smoke proved to be significantly less ($P < 0.001$) susceptible to lipid oxidation compared to bacon processed with wood smoke only.

It was observed that dietary supplementation of fishmeal produced adverse effects on lipid oxidation in bacon, while dietary supplementation of vitamin E was shown to help reduce lipid oxidation in bacon whether fishmeal was present in the diet or not. Lipid oxidation in frozen bacon was most successfully reduced when bacon was manufactured from pigs fed a diet supplemented with 200 mg of α-tocopherol per kilogram of feed and processed with a combination of liquid and wood smoke. This trend was also observed in bacon manufactured from pigs fed fishmeal and vitamin E and processed with the combination of liquid and wood smoke. Sensory evaluation showed a slight increase in oxidation with time but no overall differences were observed between the dietary treatment groups.

No lipid oxidation as measured by TBARS, fluorescence shift and sensory analysis was observed in wieners stored at –20°C for ten months. The wieners were unaffected ($P > 0.05$) by dietary treatments or by the addition of antioxidants. Dietary vitamin E appeared to lower TBARS values, suggesting vitamin E helped retard lipid oxidation. The low TBARS, fluorescence shift levels and sensory scores obtained for wieners containing whey powder showed that slow lipid oxidation was taking place. A similar trend was observed with rosemary extract. The high oxidative stability of the wieners, even in the absence of antioxidants could be due to the presence of sodium erythorbate in the formulation as an additional antioxidant.

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Werribee, Australia

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STEPHANIE ANNE CORONADO

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LIST OF ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CAT	Catalase
CHD	Coronary heart disease
DHA	Docosahexaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FIS	Food Ingredients Specialities
FOFA	Fatty acids
GC	Gas chromatograph
GRAS	Generally regarded as safe
GSHPx	Glutathione peroxidase
HPLC	High performance liquid chromatography
HMW	High molecular weight
ID	Internal diameter
IQF	Individually quick freeze
LDPE	Polyethylene low density
LSD	Least significant differences of means
MA	Malonaldehyde
MAP	Modified atmosphere packaging
ME	Metabolizable energy
MetMb	Metmyoblobin
MUFA	Monounsaturated fatty acid
NACl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Ncotinamide adenine dinucleotide phosphate
OD	Outer diameter
PG	Propyl gallate
PAH	Polycyclic aromatic hydrocarbon

PUFA	Polyunsaturated fatty acid
PVC	Polyvinyl chloride
SAT	Saturated fatty acid
SED	Standard errors of differences of means
SEM	Standard error of the mean
SD	Standard deviation
SOD	Superoxide dismutase
Suppl.	Supplemented
STP	Sodium tripolyphosphate
TBA	2-Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-butylhydroquinone
TEP	1,1,3,3-Tetraethoxypropane

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INTRODUCTION

Consumers increasingly seek convenient products that can be readily prepared. This modern trend for convenience foods has resulted in increased production of precooked and restructured meat products and a range of frozen products containing meat toppings. Such foods are highly susceptible to lipid oxidation. Lipid oxidation is the primary cause of deterioration in the quality of meat and meat products. Oxidative deterioration can occur particularly during frozen storage and deterioration of meat lipids can directly affect colour, flavour, texture, nutritive value, and safety of food (Buckley et al., 1995; Maraschiello et al., 1998; Ruiz et al., 1999). Lipid oxidation products such as peroxides are believed to have adverse effects on the health of humans such as in coronary heart disease (CHD), atherosclerosis, cancer, and the aging process. Research studies found that oxidised lipids are more deleterious to arterial health than native lipids themselves (Jadhav et al., 1996; Addis and Park, 1989). A strong reaction between DNA and several primary peroxides produced from oxidation, indicated a possible role of the peroxides in carcinogenesis and it has been suggested that dietary lipid peroxides may function as promoters of carcinogenesis (Jadhav et al., 1996).

The rate of lipid oxidation in muscle foods depends on a number of factors; the most important of which is the level of polyunsaturated fatty acids (PUFAs) present in the muscle. The susceptibility of muscle tissues to lipid oxidation differs among species and also in muscles of the same species (Rhee, 1988). Pork contains higher levels of unsaturated fatty acids compared to meat from ruminants (Enser et al., 1996). Meat from pigs are therefore more prone to deterioration of lipids and myoglobin. Other factors that affect the rate of lipid oxidation include the presence of prooxidants such as heme and non-heme iron, antioxidants, and storage conditions. Processes that disrupt the integrity of the membranes include grinding, chopping, flaking of meat, exposure of the phospholipids to oxygen, enzymes, heme pigments and metal ions. These bring about the rapid development of rancidity even in fresh raw meat (Buckley et al., 1989; Ruiz et al., 1999).

There are growing interests in foods that contain high levels of unsaturated fatty acids. Several studies have focused on feeding diets supplemented with

unsaturated fats and oils to monogastric species since the body fat of these animals tends to reflect the fatty acid profile of the dietary fat (Ruiz et al., 1999). The dietary role of the long chain *n-3* (omega-3) PUFAs, especially eicosapentaenoic acid (EPA, C20:5 (*n-3*)) and docosahexaenoic acid (DHA, C22:6 (*n-3*)) has been widely studied. These *n-3*-PUFAs are found in abundance in fish oils but are scarce in animal fat (Irie and Sakimoto, 1992). There is an increasing interest in modifying the fatty acid composition of monogastric species with *n-3*-PUFAs as it is well recognised that these acids play a role in prevention of human disease including atherosclerosis and CHD (Irie and Sakimoto, 1992; Leskanich et al., 1997). However, unsaturated fatty acids in biomembranes are easily oxidised, which is a major cause of quality deterioration in meat during cooking and during refrigerated and frozen storage. This leads to production of off-flavours and odours, reduction of PUFAs and fat-soluble vitamin concentrations, and lower consumer acceptability (Ruiz et al., 1999).

Lipid oxidation can be controlled by antioxidants (Ruiz et al., 1999). The use of antioxidants in lipid-containing foods minimises rancidity, retards the formation of toxic oxidation products and allows maintenance of nutritional quality and increased shelf-life of a variety of lipid-containing foods (Jadhav et al., 1996). Both synthetic and natural antioxidants can be used to inhibit oxidation. However, in recent years, increasing consumer concerns about safety of synthetic antioxidants have favoured products containing natural antioxidants (Jadhav et al., 1996; Monahan and Troy, 1997). Natural antioxidants, such as tocopherol, herbs and spices (rosemary, sage, ginseng) have been shown to be effective in controlling oxidation in meat products. Many current studies are underway to determine the effectiveness of natural antioxidants in preventing lipid oxidation and in applying them in the manufacture of safe food products. Whey has shown some interesting antioxidant activity, however, the natural antioxidant activity of whey is yet to be determined in various food products (Browdy and Harris, 1997; Colbert and Decker, 1991). The prevention of lipid oxidation by addition of vitamin E, β-carotene, and selenium to the animal's diet is also currently investigated by researchers. Vitamin E (α-tocopherol) is an important fat-soluble antioxidant stored in the cell membranes of animals. Studies with diets

supplemented with vitamin E have shown that meat from animals fed supplemented vitamin E was less susceptible to lipid oxidation (De Winne and Dirinck, 1997).

Rancidity has been observed in some processed products including ham, bacon, and pepperoni (Trout et al., 1998). Rancidity was suspected to be due to animal feed supplemented with fishmeal or fish oils. Fish oils contain high levels of unsaturated fatty acids, especially C22:5 and C22:6. When fish oils are added to pig feed, the animals incorporate them into their fatty tissues. This leads to susceptibility of the meat to oxidation resulting in fishy off-odours (Trout et al., 1998). Development of rancidity in pork fat containing fish oil fatty acids (FOFA) is a major problem in cured products than their uncured counterpart. In the case of bacon, fishy odour developed after 3 to 4 months storage at –20°C, while in frozen sliced ham, rancid odour developed after 5 to 6 weeks of storage (Trout et al., 1998).

This study was divided into two parts. The first part of the study aimed at examining the combined effects of feeding fishmeal and vitamin E to pigs on the sensory characteristics and storage stability of frozen bacon manufactured with or without liquid smoke. The second part of the study investigated the combined effects of feeding fishmeal and vitamin E to pigs on the sensory characteristics and storage stability of frozen wiener sausages prepared with or without exogenous antioxidants (rosemary extract or sweet whey).

The specific objectives of the study were:

1. To investigate the oxidative stability of bacon manufactured with liquid smoke and wood smoke compared with wood smoke only.
2. To investigate the oxidative stability of bacon manufactured from pork obtained from pigs fed a diet containing a combination of vitamin E (10 mg or 200 mg α-tocopheryl acetate per kilogram of feed) and fishmeal (0% or 5%).
3. To investigate the oxidative stability of wiener sausages prepared from pork obtained from pigs fed a diet containing a combination of

vitamin E (10 mg or 200 mg α-tocopheryl acetate per kilogram of feed) and fishmeal (0% or 5%).

4. To investigate the oxidative stability of wiener sausages manufactured with 0.03% rosemary extract or 2.5% whey powder or without addition of antioxidant.

Chapter 1 contains Literature Review and Chapter 2 deals with Materials and Methods. Results and Discussion comprises four parts; Fat and Fatty Acids Composition is presented in Chapter 3.1 Vitamin E in Chapter 3.2, Bacon in Chapter 3.3 and Wieners Chapter 3.4. Conclusions for bacon appear in Chapter 4.1.1 and those for Wieners in Chapter 4.1.2. Implications to the Industry in Chapter 4.2 and Future Research in Chapter 4.3.

CHAPTER 1

LITERATURE REVIEW

1.1 LIPIDS

Lipids from animals and plant sources show differences in their fatty acid composition. Many foods of plant origin contain highly unsaturated lipids. Lipids of animal origin have low levels of unsaturated lipids, however, they contain certain amount of highly unsaturated fatty acids (Jadhav et al., 1996). Lipids occur in animals and plants either as storage lipid or as membrane lipid. Storage lipids are triglycerides, whereas membrane lipids include phospholipids, sterols, sphingolipids and glycolipids (Jadhav et al., 1996). In meat, lipids are found intermuscularly, intramuscularly, in adipose tissue, or neural tissue, and in blood (Dugan, 1987). Some of these lipids play important roles in metabolism, specifically the essential fatty acids, cholesterol, and phospholipids. The fatty acid esters are not as metabolically active, but are used as source of energy and as protection or cushioning for organs (Schweigert, 1987).

Lipids can be classified into two categories: (i) simple lipids that contain carbon, hydrogen and oxygen, and (ii) compound lipids that contain phosphorus, nitrogen and sulfur in addition to carbon, hydrogen and oxygen (Gray and Pearson, 1987).

Triglycerides and waxes are classified as simple lipids. Triglycerides, commonly called fats, are esters of glycerol containing three fatty acids. The fatty acids may be the same or may vary at the three positions on the glycerol molecule (Figure 1.1) (Dugan, 1987; Gray and Pearson, 1987; Nawar, 1985).

$CH_2OOC - R$
|
$CHOOC - R'$
|
$CH_2OOC - R''$

Figure 1.1 - General formula of a triglyceride, where R, R', R" represent three fatty acids esterified to a glycerol molecule.

Triglycerides vary in composition and susceptibility to oxidation depending on the length of fatty acids and degree of unsaturation (Gray and Pearson, 1987). Waxes are esters of higher fatty acids and fatty alcohols. Examples of waxes include sterol esters and vitamin A esters (Gray and Pearson, 1987).

Compound lipids are formed by the linkage of lipids with carbohydrates, forming glycolipids, or with proteins, producing lipoproteins. Phospholipids are compound lipid. Phospholipids are similar to triglycerides except that one of the fatty acids attached to the glycerol molecule is replaced by phosphate and a nitrogen-containing compound. Example of phospholipids include lecithin, cephalin, phosphatidyl inositol, and phosphatidyl serine (Figure 1.2) (Ensminger et al., 1994).

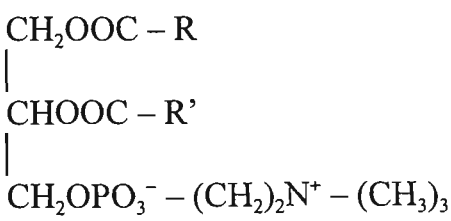

Figure 1.2 - α-Lecithin, where R, R' represent the fatty acids esterified at the respective positions to the glycerol molecule.

Phospholipids are mainly found in the membranes as components of mitochondria, the muscle fibre sarcolemma, the microsomes, and the sarcoplasmic reticulum (Gray and Pearson, 1987). Most of the phospholipids found in muscles are phosphoglycerides. Phospholipids are the major contributors to oxidative off-flavour in several animal muscles and their susceptibility to oxidation depends on the degree and the amounts of the unsaturated fatty acids (Dugan, 1987; Kanner, 1994).

1.1.1 Fatty acid composition

The lipid composition of the membranes depends on animal species and their diet. There are inter-species differences in the degree of unsaturation of the

fatty acids in triglycerides. The proportion of PUFAs is much higher in poultry meat and fish than in pork, lamb or beef (Allen and Hamilton, 1989; Gray and Pearson, 1987; Ranken, 1989). Some differences also exist between ruminants and non-ruminants in terms of incorporation of fatty acids in their body tissues (Gillett, 1987). In ruminants such as cattle, sheep and goat, the digestion involve microbial fermentation of the food prior to its exposure to their own digestive enzymes. Triacylglycerols present in foods consumed by ruminants are largely hydrogenated by bacteria, yielding first a monenoic acid and ultimately stearic acid (McDonald, 1995). The microorganisms also synthesis considerable quantities of lipids which are eventually incorporated in the milk and body fat of ruminants. Therefore, the majority of ingested unsaturated fatty acids are saturated by the rumen microorganisms (Gillett, 1987). Pigs are non-ruminants or monogastric animals and they lack the ability to saturate the fatty acids present in their diets. Monogastric animals cannot hydrogenate the unsaturated fatty acids, therefore, the degree of saturation of porcine fat will reflect the degree of saturation of their dietary fats (Gillett, 1987). Dietary supplement in animals feeds and the tendency of the different species to accumulate fatty acids in the membranous phospholipids affect the lipid composition of the membrane and their susceptibility to peroxidation (Kanner, 1994).

1.2 EFFECT OF DIETS ON MEAT QUALITY

Over the past 40 years, many researchers have investigated the effect of feeds on modification of fat, cholesterol content and fatty acid composition in animals. Jakobsen (1999) stated that the purpose of modifying fats is to produce high quality products, which meet the dietary recommendations for reduced intake of fat and cholesterol in the human diet and an optimal ratio between saturated, monounsaturated, and polyunsaturated fatty acids in order to minimise the risk for obesity, cancer, cardiovascular diseases and other life-style diseases. During the 1970s, people were encouraged to consume more plant oils such as sunflower oil, soy oil, maize oil and rapeseed oil, containing high proportion of linoleic acid (C18:2 n-6) (Jakobsen, 1999). However, during the 1980s, recommendations were towards higher intakes of fish fatty acids, particularly the *n-3* or ω3 PUFAs

(Jakobsen, 1999). Animal products are an important part of the human diet and they contribute significantly to the intake of fat, cholesterol and fatty acids. It is possible to produce a healthier product for humans by supplementing the animal feed with *n-3* and *n-6* fatty acids, which are absorbed unchanged by non-ruminants, and incorporated into their adipose tissue and cellular membranes. However, the inclusion of PUFAs in animal feed leads to increased susceptibility of lipid peroxidation, fishy odours or other off-flavours (Jakobsen, 1999, Sheard et al., 2000).

1.2.1 Plant oils

Feeding a high level of dietary corn oil (11.5%) produced more unsaturated fat in pigs than a low level (4.1%) (Gray and Pearson, 1987). Similar studies have been carried out with rapeseed oil (Hertzman et al., 1988), soya oil (Morgan et al., 1992), high oleic acid sunflower oil (Bosi et al., 2000), and α-linolenic acid (Ahn and Sim, 1996). Rapeseed and rapeseed meal are found to give slightly higher sensory score after freeze-storage of pork loins than pigs fed no rapeseed oil, while a combination of rapeseed and fishmeal in the diet produced pork that was highly sensitive to oxidation (Hertzman et al., 1988). Pigs fed a supplement of soya oil, which is high in linoleic acid (18:2), resulted in high levels of this fatty acid in semitendinosus muscle in pigs than those that were not fed the supplement (Morgan et al., 1992).

High oleic acid sunflower oil can be used to increase the oleic acid content in meats and subcutaneous fats in pigs without any adverse effects on fresh meat quality (Bosi et al., 2000). A study by Ahn and Sim (1996) found that dietary α-linolenic acid increased the proportion of *n-3* fatty acids significantly and the degree of unsaturation in the neutral lipids and phospholipids. Diets enriched in α-linolenic acid were also shown to increase oxidation and had a detrimental effect on the acceptability of cooked pork loins held for two days in loose packaging (Ahn and Sim, 1996). Maximising saturated fatty acid composition in the diet by adding tallow to pig feed has been reported. This results in muscles oxidatively more stable; however, this is undesirable from nutritional standpoint and texture of the product (Decker and Xu, 1998b; Morgan et al., 1992). The

effect of fishmeal/fish oils fed to pigs has been well studied, some researchers are now looking at the effect of feeding a combination of fish oils and vitamin E.

1.2.2 Fish oils and fishmeal

Incorporation of fish oils and fishmeal to animal feed can affect meat quality and increases its susceptibility to oxidation. Meat quality is affected when pigs are fed feed rich in fish meal or fish oils as it leads to fishy odours and off-flavours in meat (Trout et al., 1998). Fish oils and fishmeal are added directly to pig feed. They have growth promoting effects, are a cheap source of energy and contain significant amounts of vitamin A and D (Karrick, 1990). The high nutritional value of fishmeal, provides high quality proteins, amino acids, B vitamins, calcium and zinc and is often added to the diets of young animals (Karrick, 1990; McDonald et al., 1995). The energy of fishmeal is present entirely in the form of fat and protein and is largely a reflection of the oil content (Karrick, 1990). Fish oils are high in unsaturated fatty acids, especially C22:5 and C22:6 fatty acids, and when added to pig feed, the animals incorporate them into their own fatty tissues. The animal fat is therefore more prone to oxidise and produce fishy odours (Trout et al., 1998). Twenty percent fishmeal was found to give a definite fishy odour in bacon and ham and the pork quality was poor (Karrick, 1967). Recommendations regarding the incorporation of fishmeal and fish oils in pork feed in order to avoid fishy taint were made over 60 years ago. These recommendations were: pig feed should not contain more than 5% fishmeal or 0.5% of fish oil and fish products should be removed from the feed at least two weeks before slaughter (Coxon et al., 1986). It was also recommended that pigs should not be fed more than 10 grams of fish oil per day to avoid development of fishy odour during storage (Karrick, 1967).

The addition of vitamin E to feed containing fishmeal/fish oil improves the quality and shelf life of meat. Feeding animals with more unsaturated fatty acids improves the polyunsaturated to saturated ratio, while feeding *n-3*-PUFAs as linseed or fish oil lowers the *n-6*:*n-3* ratio and increases susceptibility of the meat to oxidation. When fishmeal or fish oil is added to the feed, it is necessary to add higher dietary levels of vitamin E to prevent flavour deterioration due to lipid

oxidation (Wood and Enser, 1997). Irie and Sakimoto (1992) studied the effect of adding different levels (2, 4 or 6%) of fish oils in pig feed. All pigs were also supplemented with 1000 ppm dl-α-tocopherol. *n-3*-PUFAs increased linearly with the addition of fish oil. As levels of fish oil increased in the diet, the content of myristic, palmitoleic, linolenic, arachidonic + erucic acids, EPA, docosapentaenoic acid (C22:5), and DHA in pig fat increased, but there was a significant decrease in oleic acid. The study by Irie and Sakimoto (1992) showed that the EPA and DHA, which are present in high concentrations in fish oil, can be elevated in porcine fat by feeding pigs diets containing fish oil for 2 to 4 weeks before slaughter. Leskanich and co-workers (1996) found that pigs fed 10 g fish oil/kg diet at 52-95 kg live weight required 250 mg vitamin E/kg in the feed to maintain acceptable chemical and organoleptic levels.

1.2.3 Effect of antioxidants in diets

Inclusion of antioxidant into animal feed can be an effective method of increasing the oxidative stability of muscle foods especially in products where addition of exogenous antioxidant is difficult (Decker and Xu, 1998b). Many studies have involved supplementation of the animal feed with selenium, β-carotene and vitamin E.

1.2.3.1 Selenium

Selenium is an essential micronutrient showing antioxidant properties (Madhavi et al., 1996). Selenium acts as antioxidant at the cellular level and is necessary for the synthesis and activity of glutathione peroxidase (GSHPx), a primary cellular antioxidant enzyme (Madhavi et al., 1996). In animals nearly 90% of dietary selenium is absorbed from the gastrointestinal tract. In tissues, selenate is reduced to selenide, which is incorporated into selenocysteine, as present at the active site of GSHPx (Madhavi et al., 1996). Studies with selenium dietary supplementation have shown to decrease the thiobarbituric acid reactive substances (TBARS) formation during the storage of minced muscle suggesting that dietary selenium could increase oxidative stability of muscle foods (Decker

and Xu, 1998b). It was also observed that selenium and vitamin E could work synergistically in reducing lipid peroxide formation (Madhavi et al., 1996).

1.2.3.2 β-Carotene

β-Carotene has antioxidant properties, which are enhanced in the presence of vitamin E. β-Carotene has been reported to be a singlet oxygen quencher, and therefore, is an antioxidant despite the system of conjugated double bonds in the molecule that imparts a prooxidant character (Ruiz et al., 1999). Ruiz and co-workers (1999) studied the effect of β-carotene and vitamin E on the oxidative stability of broiler leg meat fed different supplemental diets. They observed that β-carotene and α-tocopherol could act synergistically in tissues. However, β-carotene showed antioxidant properties only if vitamin E in tissues reached a certain level. According to Ruiz and co-workers (1999), β-carotene acts as an antioxidant in meat, and it must be supplemented in feed at certain levels together with sufficient amount of vitamin E. They found that if the proportion of β-carotene to vitamin E ratio was too high, deposition of vitamin E may be impaired, resulting in higher lipid oxidation.

1.2.3.3 Vitamin E

Vitamin E functions as an antioxidant that protects tissue lipids from free-radical damage. It has been demonstrated that dietary Vitamin E supplementation can increase colour stability, reduce drip loss, extend display and storage life and can reduce development of oxidative rancidity in fresh pork (Channon et al., 1997).

Vitamin E consists of a mixture of four different isomers α-, β-, δ-, and γ- tocopherol, and it has been demonstrated that α-tocopherol has a much higher antioxidant properties than the other isomers (Buckley et al., 1989; Channon et al., 1997; De Winne and Dirinck, 1997). Tocopherols occur naturally as lipid soluble antioxidant in the highly unsaturated bilayer of phospholipids of the cell membranes. They act as free radical scavenger by breaking the chain of lipid peroxidation in cell membranes and thus prevent the formation of lipid hydroperoxides (Walsh et al., 1998).

Vitamin E is added to feed as dietary α-tocopheryl acetate but it does not function as an antioxidant until de-esterified in the gastrointestinal tract (Buckley et al., 1995). Several studies have observed that dietary α-tocopheryl acetate supplementation in the diet of pigs increases muscle α-tocopherol concentrations and stabilises PUFAs and cholesterol in muscles against oxidative deterioration (Asghar et al., 1991; Buckley et al., 1995; Monahan et al., 1992a,b). The requirement for Vitamin E is known to increase as the dietary concentration of PUFAs increases. For lean pigs, vitamin E requirements may be greater than for fat pigs as the levels of PUFAs are higher in pigs with higher lean:fat ratio (Channon et al., 1997). The rate and extent of lipid oxidation in meats are dependent on the α-tocopherol concentration in tissues. Monahan and co-workers (1990) showed that dietary supplementation of up to 200 mg of α-tocopheryl acetate per kilogram of feed significantly improved the oxidative stability of both raw and cooked pork muscles during 8 days storage at 4°C. In addition, α-tocopherol stabilised the membrane-bound lipid against metmyoglobin/H_2O_2 initiated oxidation (Buckley et al., 1995). Other studies showed that to achieve a maximum stability in pig lipids a concentration of 7-10 μg/g of α-tocopherol in fresh meat is required; this can be achieved by adding 100-200 mg of Vitamin E/kg of feed (Channon et al., 1997).

Buckley and co-workers (1989) showed that pork patties prepared from a short-term (4 weeks) and a long-term (10 weeks) α-tocopherol supplementation were not significantly different in terms of oxidative stability when the patties were prepared without salt and stored at 4°C under light and dark conditions. However, when patties contained salt, the long-term diet showed more effectiveness in improving oxidation stability than the short-term feeding trial.

Isabel and co-workers (1999a) showed that dietary α-tocopheryl acetate supplementation increased the concentration of α-tocopherol in muscles and in processed hams prepared from muscles. In this study, pork from pigs fed a level of 200 mg α-tocopheryl acetate per kilogram of feed were less susceptible to oxidation than those fed a lower level (10 mg/kg feed). De Winne and Dirinck (1997) also found that ham samples from animals supplemented with vitamin E were more stable and less susceptible to lipid oxidation.

Vitamin E added to processed meat products could be a more cost effective way of reducing rancidity but research shows that this is not effective. Studies have shown that vitamin E added to post-mortem muscle does not become an integral part of the cellular membranes and is not as effective an antioxidant as when vitamin E is incorporated into the membranes by dietary means (Channon et al., 1997).

1.3 LIPID OXIDATION / RANCIDITY IN MUSCLE FOODS

The extent of lipid oxidation varies between animal species and within muscles of the same species. It is well known that the more unsaturated the fat, the more prone it is to oxidation. Rancidity therefore develops faster in the relatively unsaturated pork fats than in the harder beef or sheep fats. The oxidation process is also faster in soft chicken fat and fastest in fish oils (Allen and Hamilton, 1989; Gray and Pearson, 1987; Kirk and Sawyer, 1991; Ranken, 1989).

Kanner (1994) reported that when cells are injured, such as in muscle after slaughtering, oxidative processes are favoured. These oxidative processes affect lipids, pigments, proteins, carbohydrates, vitamins, and the overall quality of foods. Many factors seem to affect lipid peroxidation in animal tissues including species, anatomical location, diet, environmental temperature, sex, age, and phospholipid composition and its content. Also during processing, handling and storage of food products, the rate of lipid peroxidation can be influenced by factors, such as composition and freshness of raw meat, cooking or heating, chopping, flaking, emulsification, deboning and adding exogenous compounds including salt, nitrite, spices and antioxidants (Kanner, 1994).

1.3.1 Mechanisms of rancidity

Two of the typical reactions of lipids are oxidation and hydrolysis. The development of rancidity or off-flavour compounds, polymerisation, reversion and a number of other reactions causing reduction in the shelf life and nutritive value of the food product can be due to changes that occur from reactions with atmospheric oxygen (oxidative rancidity) (Hamilton, 1989; Jadhav et al., 1996). Hydrolytic rancidity may be due to direct chemical reaction or due to reaction

which is catalysed by enzymes, however, the reaction is only significant in meat and meat products in limited circumstances (Hamilton, 1989; Ranken, 1989).

1.3.1.1 Mechanisms of oxidative rancidity

Oxidative rancidity is referred to as autoxidation. The autocatalytic peroxidation process probably begins immediately after slaughter. The path depends on the production of free radicals ($R^{\cdot}$) from lipid molecules (RH) and by their interaction with oxygen in the presence of a catalyst. The initiation can occur by the action of external energy sources such as heat, light or high-energy radiation, by moisture or by the presence of various pro-oxidant catalysts such as traces of transition metals (eg. copper, nickel, iron) or metalloproteins such as heme. Lowering the temperature of storage does not stop oxidative rancidity (Buckley et al., 1995; Hamilton, 1989; Jadhav et al., 1996; Kirk and Sawyer, 1991; Morrissey et al., 1998).

The initiation step is not fully understood. The free radical ($R^{\cdot}$) produced in the initiation step can then react to form a lipid peroxy radical ($ROO^{\cdot}$), which can react further to give the hydroperoxide (ROOH). The propagation steps also provide a further free radical ($R^{\cdot}$), making it a self-propagating chain process. The self-propagating chain reaction can be stopped by termination step, where two radicals combine to give products (Hamilton, 1989; Jadhav et al., 1996; Kirk and Sawyer, 1991; Morrissey et al., 1998).

1. Initiation:	(a)	$RH + O_2 \rightarrow R^{\cdot} + {}^{\cdot}OOH$
2. Propagation:	(b)	$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$
	(c)	$RH + ROO^{\cdot} \rightarrow ROOH + R^{\cdot}$
	(d)	$ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH$
3. Termination:	(e)	$R^{\cdot} + R^{\cdot} \rightarrow R - R$
	(f)	$R^{\cdot} + ROO^{\cdot} \rightarrow ROOR$
	(g)	$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$

The induction period is the initial phase during which fat undergoes oxidation slowly and at a relatively uniform rate. At the end of the induction period, when the amount of peroxide formation reaches a certain level, the reaction enters a second phase, which has a rapid and accelerating rate of oxidation. At this point or soon after, the fat begins to smell or taste rancid (Hamilton, 1989; Kirk and Sawyer, 1991).

Ferryl ions were found to catalyse peroxidation. Myoglobin and haemoglobin in the ferrous or ferric states are activated by H_2O_2, producing a short-lived intermediate of ferryl (Fe^{4+} or oxo-ferryl), which can initiate membrane and non-membrane lipid peroxidation (Kanner, 1994). Free metal ions play an important role as catalysts in muscle foods. Transition metals such as iron and copper have labile d-electron system, which makes them well suited to catalyse redox reactions (Kanner, 1994). Iron is an important catalyst in biological systems, about two-thirds of body iron is found in haemoglobin and smaller amounts in myoglobin. A small amount of iron is included in various iron-containing enzymes, and in the transport protein transferrin. The remainder is present in intracellular storage proteins, ferritin and hemosiderine (Kanner, 1994). All the iron compounds are capable of decomposing H_2O_2 or ROOH to form free radicals. The main source of free iron in cells seems to be ferritin (Kanner, 1994). Ferritins are the main proteins that store iron in cells. Iron can be released from ferritin and utilised by mitochondria for the synthesis of haemoproteins. During storage of muscle foods, ferritin loses iron at a significant rate, and this amount was found to initiate membrane lipid peroxidation. The amount of free copper in muscle foods appears to be very low and mostly chelated to histidine dipeptides, such as carnosine. This prevents copper from catalysing lipid peroxidation (Kanner, 1994).

Ferrous ions in aerobic aqueous solution produce superoxide, hydrogen peroxide and hydroxyl radical by the following reactions (Kanner, 1994; Morrissey et al., 1998):

$$Fe^{2+} + O_2^{\cdot -} \rightarrow Fe^{3+} + O_2$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow HO^{\cdot} + HO^- + Fe^{3+}$$

Ferrous ions can stimulate lipid peroxidation by generating the $^{\cdot}OH$ from H_2O_2 but also by the breakdown of preformed lipid peroxides ROOH to form the alkoxyl ($RO^{\cdot}$) radical as follows:

$$RH + HO^{\cdot} \rightarrow R^{\cdot} + H_2O$$

$$R^{\cdot} + O_2 \xrightarrow{LH} ROOH + R^{\cdot}$$

$$ROOH + Fe^{2+} \rightarrow RO^{\cdot} + HO^{-} + Fe^{3+}$$

$$RH + RO^{\cdot} \rightarrow R^{\cdot} + ROH$$

In iron redox cycle-dependent membranes, lipid peroxidation needs to attack unsaturated fatty acids only by a few $^{\cdot}OH$ radicals. Once the first hydroperoxides are formed, propagation of lipid peroxidation will be catalysed by the breakdown of ROOH to free radicals and these reactions are not affected by superoxide dismutase (SOD), catalase (CAT) or $^{\cdot}OH$ scavengers (Kanner, 1994).

1.3.1.2 Photo-oxidation

Photo-oxidation is another mechanism for free radical formation. Different peroxides are formed when light and certain photosensitiser molecules are present. A photosensitiser is able to convert a triplet oxygen to a singlet oxygen. The photosensitiser (S) is converted to its electronically excited state due to absorption of light in the visible or near-UV region. It can then transfer its excess energy to an oxygen molecule, giving rise to a singlet oxygen, which in turn can react with a lipid molecule to yield a hydroperoxide:

$$S \xrightarrow{h\nu} {}^1S \rightarrow {}^3S^*$$

$${}^3S^* + {}^3O_2 \rightarrow {}^1O_2 + {}^1S^*$$

$${}^1O_2^* + RH \rightarrow ROOH$$

where, ^1S is the singlet-state sensitiser, $^1S^*$ is the excited singlet-state sensitiser, $^3S^*$ is the excited triplet-state sensitiser, $^3O_2$ is the normal triplet oxygen, $^1O_2^*$ is the excited singlet-state oxygen and hν is ultraviolet light energy in photons (Jadhav et al., 1996).

1.3.1.3 Mechanisms of hydrolytic rancidity

Hydrolytic rancidity of lipids is caused by hydrolysis of the ester linkage resulting in the formation of free fatty acids and glycerol. The reaction is catalysed by high temperature, acids, lipolytic enzymes and high moisture content (Ranken, 1989). Hydrolytic changes initiated by enzymes may occur in meat or meat fats where there is microbiological growth, especially mould growth but sometimes caused by yeasts or bacteria (Ranken, 1989). The presence of fatty acids is an indication of lipase activity or other hydrolytic action. Hydrolytic changes occur during storage resulting in the production of an unpleasant taste and odour. Such fat is referred to as rancid. The unpleasant organoleptic characteristics are in part caused by the presence of free fatty acids but the major development of rancidity is brought about by atmospheric oxidation or autoxidation (Kirk and Sawyer, 1991). Antioxidants can effectively retard the process of oxidation, but they cannot reverse it. Further, they are not effective in suppressing hydrolytic rancidity (Jadhav et al., 1996).

1.3.1.4 Enzymic catalysis

Hultin (1980) demonstrated the presence of enzymic systems in microsomal fractions from chicken and fish skeletal muscles responsible for catalysing the oxidation of microsomal lipids in the presence of cofactors. Lipid peroxidation in isolated microsomes requires nicotinamide adenine dinucleotide phosphate (NADPH) or nicotinamide adenine dinucleotide (NADH), cytochrome reductase, molecular oxygen and is stimulated by ADP-Fe^{3+} (Kanner, 1994). The presence of a similar lipid oxidation system associated with microsomes of beef and pork muscle was also found. In addition to microsomal lipid peroxidation systems, mitochondrial enzymic lipid peroxidation was demonstrated by Lou and Hultin (1986). The latter was found to be dependent on the same cofactors as the microsomes. A similar enzymic lipid peroxidation system still needs to be demonstrated in beef chicken and pork muscle mitochondria (Kanner, 1994; Rhee, 1988). It is important to note that microsomal and mitochondrial lipid peroxidation is not a direct reaction of an enzyme towards unsaturated fatty acids,

but only a generator of O_2^-, and lipid oxidation is merely a non-enzymic process (Rhee, 1988).

Currently available research data indicate that enzymes or enzyme systems possibly activate metmyoglobin (MetMb) and are responsible for initiation of lipid oxidation in raw muscle tissues (Rhee, 1988). Further, it was found that metal catalysts such as non-heme iron in red meat also promote lipid oxidation in raw tissues, possibly through their role in propagation of free radicals in the oxidation process (Rhee, 1988).

Lipoxygenase enzymes are present in spices, wheat flour and vegetables. The enzyme lipoxygenase induces oxidation in a similar way as autoxidation; however, lipoxygenase is very specific about the substrate and the mode of oxidation of the substrate. Lipoxygenase specifically oxidises polyethenoid acids containing methylene-interrupted double bonds that are in the *cis* geometrical configuration such as those in linoleic, linolenic and arachidonic acids but not in oleic acid. During lipoxygenase catalysis, free-radicals intermediates are formed, which in turn will oxidise compounds such as carotenoids and polyphenols (Jadhav et al., 1996).

1.3.2 Lipid oxidation in uncooked meat products

For oxidation to occur, an oxidising agent must be present and must gain access to the fat. The most common oxidising agent is oxygen from the air. Measures, which exclude oxygen, such as vacuum packaging, or those which reduce its concentration, as in controlled atmosphere packaging can prevent or reduce oxidative rancidity (Rhee, 1988). Oxidative changes in lipids can become a serious problem for uncooked meat when it is subjected to size reduction such as grinding, chopping, flaking, emulsification, freeze-thawing, temperature abuse in handling and distribution and/or prolonged storage (Rhee, 1988). Comminution of the meat disrupts membranes and introduces air or oxygen into the tissues, therefore, exposing the lipids. This increases their susceptibility to oxidation and hasten development of oxidative rancidity and warm-over-flavour (Gray and Pearson, 1987; Ranken, 1989). Phospholipids present in subcellular membranes (mitochondria or microsomes) are high in PUFAs. When the cells are damaged,

because of their close proximity to a range of prooxidants, they are very susceptible to oxidation, as they are the first to be exposed to oxidation. Other phospholipids are more finely dispersed in the lean meat or musculature where similar considerations apply (Allen and Hamilton, 1989; Buckley et al., 1995; Ranken, 1989). Membrane-bound lipid associated with the muscle cell wall, mitochondria, microsomes and the sarcoplasmic reticulum are especially vulnerable to oxidative deterioration (Gray and Pearson, 1987).

The meat pigment, myoglobin is shown to play an important role in the catalysis of lipid oxidation in uncooked red meat. Harel and Kanner (1985) suggested that raw meat is relatively more stable compared to cooked meat, in regard to lipid oxidation because the catalase enzyme present in raw meat might partially prevent the activation of MetMb. They also suggested that there might be a direct involvement of haemoproteins (MetMb and methaemoglobin) in lipid oxidation in muscle tissues. They reported that the interaction of H_2O_2 with MetMb generated activated MetMb capable of initiating lipid oxidation. Little or no lipid oxidation occurred in the sarcosomal fraction from turkey dark muscle in the presence of H_2O_2 or MetMb alone, but oxidation occurred readily in the presence of MetMb plus H_2O_2 (Rhee, 1988). Some studies also suggest that the autoxidation of oxymyoglobin or oxyhaemoglobin leads to the formation of MetMb or methaemoglobin and the superoxide radical O_2^-, which dismutates to H_2O_2. It has also been suggested that in muscle tissues, a large part of H_2O_2 is generated by a non-enzymic reaction, presumably due to oxidation of heme pigments. Since the oxidation of oxymyoglobin to MetMb is common in postmortem red meat, especially during storage, it was assumed that H_2O_2 could be produced from the oxidation of pigments in sufficient quantities for the MetMb - H_2O_2 mediated initiation of lipid oxidation in red meat (Rhee, 1988).

Harel and Kanner (1985) suggested that lipid peroxidation in microsomes is initiated by a hydrogen peroxide-activated MetMb complex. Rhee and co-workers (1987) suggested that the heme pigment system plays a major role in catalysing lipid oxidation in raw and cooked meat. They suggested that the MetMb -hydrogen peroxide complex was the primary initiator of lipid oxidation

in raw meat and that non-heme iron was the major catalysts of lipid oxidation in cooked meat (Buckley et al., 1989).

1.3.3 Lipid oxidation in cooked meat products

Cooking of meat accelerates oxidation, resulting in serious oxidative deterioration within a matter of hours in contrast to the slower development of rancidity during refrigeration or freeze-storage (Gray and Pearson, 1987). The term warm-over-flavour (WOF) was first introduced by Tims and Watts in 1958 to describe the rapid onset of rancidity in cooked meat during refrigerated storage. It was demonstrated that the major phospholipid involved in lipid peroxidation and, therefore, WOF in cooked meat was phosphatidylethanolamine (Ahn and Sim, 1996; Kanner, 1994; Mei et al., 1994).

Heating can affect many factors involved in lipid peroxidation (Kanner, 1994). Heat coagulates and denatures meat proteins, hence disrupts muscle cells structure, inactivates indigenous proteolytic enzymes, destroys considerable numbers of microorganisms and releases oxygen from oxymyoglobin (Kanner, 1994; Pearson and Gillett, 1996). The release of oxygen from oxymyoglobin produces H_2O_2 and this reaction is increased at 60°C (Harel and Kanner, 1985). The level of free iron greatly increases during cooking, however, it was found to be released more at low temperatures and during slow heating than at high temperatures (Rhee et al., 1987). These results were assumed to be connected with the production of H_2O_2, which activates and destroys the porphyrin structure, releasing free iron. At high temperature, it seems that more O_2 escapes from the meat tissue without oxidising the pigment, thus rapidly producing an environment very low in oxygen (Kanner, 1994).

Several hypotheses have been proposed to explain how cooking causes chemical and physical alterations in meat, resulting in the acceleration of oxidative reactions. Mei and co-workers (1994) stated that these hypotheses include increases in prooxidant iron concentrations caused by heat-induced release of protein bound iron, increased generation of hydrogen peroxide and MetMb leading to formation of the prooxidant, hydrogen peroxide-activated MetMb, and

heat-induces disruption of muscle membrane systems resulting in mixing of unsaturated fatty acids with endogenous lipid oxidation catalysts

1.3.4 Lipid oxidation in cured meat products

Products such as bacon and ham can be cured using two different techniques: i) dry-cure, and ii) pickle-cure. Dry-curing consists of rubbing the curing mixture onto the surface of the pieces of meat. Pickle-curing involves soaking the meat pieces in brine or injecting the brine directly into the meat muscle using a multiple-stitch needle machine or through an artery-injection needle into the arterial system from where it diffuses into the muscle tissue (Pearson and Gillett, 1996). Very often, cured meats are subsequently heated and smoked, which kills most microorganisms on or in the product (Niven, 1987) and contribute to their stability (Urbain and Campbell, 1987).

A typical curing pickle consists of salt, sugar, sodium nitrite and phosphate. Nitrite and phosphate have antioxidant properties but the main purpose of using nitrite is to develop the typical colour of cured meat products while phosphate is used to increase water binding capacity (Pearson and Gillett, 1996). Sugar is added primarily for flavour. Salt is a bacteriostatic agent, but it also affects flavour, increases water solubility as well as water-holding capacity. It may also cause undesirable effects by acting as pro-oxidant and accelerate oxidation of pigments and fats (Hernández et al., 1999). Sodium chloride is known to accelerate lipid oxidation when there are low free moisture conditions such as in the frozen state. Therefore, cured meats are especially subject to oxidative changes when stored in a freezer (Zipser et al., 1964). A consequence of oxidation is that fat peroxides destroy cured meat pigments producing some discolouration in frozen cured meats (Zipser et al., 1964). In addition to rancidity, changes in flavour and texture occur in frozen cured meats (Urbain and Campbell, 1987).

1.4 PREVENTION OF OXIDATION

Living cells have several mechanisms of protection against oxidative processes, including two categories of antioxidants - the preventive antioxidants and the chain-breaking antioxidants. The first class is composed of the antioxidant

enzymes such as catalase (CAT), superoxide dismutase (SOD) and the glutathione peroxidase (GSHPx), which are considered to be the major peroxide-removing enzymes. Their function involves reducing the lipid hydroperoxides into their respective alcohols (Maraschiello et al., 1999). α-Tocopherol (vitamin E), ascorbic acid and β-carotene form the second group of protective agents. They are capable of scavenging free radicals and by this together with the action of the preventive antioxidants, prevent or delay the onset of the lipid peroxidation process (Maraschiello et al., 1999).

The balance between antioxidant and prooxidants is destroyed in postmortem conditions. However, it has been found that dietary antioxidant supplementation such as vitamin E, is an efficient means for increasing the oxidative stability of raw and cooked meat. The cytosolic antioxidant enzymes are another limiting factor of the oxidative processes affecting the meat. Enzymes such as CAT, SOD and GSHPx have also found to delay the onset of rancidity in stored meats (Maraschiello et al., 1999). However, these enzymes are only active in raw meat since their activity is lost when meat is cooked (Maraschiello et al., 1999).

1.4.1 Addition of antioxidants

Lipid oxidation can be inhibited by nitrite, metal-chelating agents and synthetic or natural antioxidants. In recent years, consumers increasing concern about safety of synthetic antioxidants has favoured products containing natural antioxidants. This has led to increased interest in the antioxidant properties of naturally occurring substances, including vitamin E, ascorbic acid, β-carotene, glutathione, carnosine, homocarnosine and anserine as well as herbs and spices and whey proteins (Buckley et al., 1995; Jadhav et al., 1996; Monahan and Troy, 1997).

The level of antioxidants needed to inhibit oxidation effectively depends on the degree of oxidative stress in the product. The degree of unsaturated lipids in the product, the presence of heme iron and the extent of comminution all affect susceptibility to lipid oxidation (Jadhav et al., 1996; Monahan and Troy, 1997).

1.4.1.1 Natural antioxidants

Several chemicals that are found in animal or plant tissues are also available as synthetic molecules and used in food applications. Vitamin E, vitamin C and uric acid are synthetic products of natural origin capable of participating in the *in vivo* radical defence mechanism.

1.4.1.1.1 Plant extract, herbs and spices

Plant extracts contain a variety of natural products, including some with potential antioxidant activity. Tea antioxidants could be a source of natural antioxidant if they are used in large quantity (Zandi and Gordon, 1999). Herbs and spices including rosemary, sage, pepper, garlic, mustard, and ginseng, can be used directly for their antioxidant characteristics. Rosemary is used commercially as an antioxidant. Both crude and refined extracts of *Rosmarinus officinalis* L. (rosemary) have shown excellent antioxidant properties (Abd El-Alim et al., 1999; Barbut et al., 1985; Liu et al., 1992; Lopez-Bote et al., 1998). The crude extract, however, imparts objectionable colour, odour and taste. By purifying the crude extract, objectionable characteristics can be avoided. It has been observed that about 90% of the antioxidant activity of rosemary can be attributed to carnosol, a phenolic diterpene. Other effective components include, carnosic acid, rosmanol, rosemaridiphenol and rosmariquinone (Jadhav et al., 1996; Madhavi et al., 1996). The recommended levels for rosemary in food products are 200-1000 mg/kg food (Madhavi et al., 1996).

1.4.1.1.2 Whey

Whey is a by-product of the cheese industry and its incorporation in other products would partially solve whey disposal problems (Colbert and Decker, 1991). Whey and whey ultrafiltration permeate have been found to have some antioxidant activity and have been proposed to be used as a natural antioxidant in foods (Browdy and Harris, 1997; Colbert and Decker, 1991). However, few studies have looked at incorporating whey in meat products. Colbert and Decker (1991) reported that acid whey and acid whey permeate were both capable of inhibiting several different lipid oxidation catalysts including iron, lipoxidase,

photoactivated riboflavin and hydrogen peroxide-activated MetMb. It was suggested that whey antioxidant activity was not only due to chelation but also due to inhibition of lipid oxidation by hydrogen donation. Tong and co-workers (2000) investigated the high molecular weight (HMW) fraction of whey from pasteurised milk and found that the antioxidant activity of this HMW fraction increased with concentration. The HMW fraction was able to scavenge peroxyl radicals and chelate iron. Studies are currently underway to determine the mechanisms by which whey proteins inhibit lipid oxidation.

1.4.1.1.3 Carnosine

Carnosine is a naturally occurring skeletal muscle dipeptide, which consists of β-alanine and histidine. The antioxidant effect of carnosine has been studied by several researchers (Chastain et al., 1982; Decker and Faraji, 1990, Decker and Crum, 1991; Lee et al., 1998; O'Neill et al., 1999; O'Neill et al., 1998; Zhou and Decker, 1999). Carnosine has been shown to inhibit iron-catalysed oxidation of phosphatidylcholine liposomes over the pH (5.5-6.8) and temperature (4-37°C) range common to muscle foods (Decker and Crum, 1991). Carnosine is also capable of inhibiting other lipid oxidation catalysts such as hydrogen peroxide-activated haemoglobin, photoactivated riboflavin and lipoxygenase (Decker and Faraji, 1990). The antioxidant mechanism of carnosine is probably due to inactivation of water-soluble lipid oxidation catalysts and/or active oxygen species (Decker and Crum, 1991).

Carnosine has been shown to inhibit lipid oxidation *in vitro*, but the antioxidant activity of carnosine in muscle foods has not yet been reported. Studies on the frozen storage (–15°C) of salted ground pork showed that concentrations as low as 0.5% carnosine inhibited lipid oxidation for up to 6 months. Inhibition of lipid oxidation and colour changes by 1.5% carnosine was better than other antioxidants commonly used in foods including sodium tripolyphosphate (STP), butylated hydroxytoluene (BHT) and α-tocopherol as determined by both chemical and sensory analysis (Decker and Crum, 1991).

1.4.1.2 Synthetic antioxidants

Typical synthetic antioxidants used in the food industry are BHT, butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) (Jadhav et al., 1996). These synthetic antioxidants are primarily aromatic amines and hindered phenols and act as chain-breaking hydrogen (electron) donors. Synthetic antioxidants have been thoroughly tested for their toxicological behaviour and the levels at which they can be added to various foods are strictly regulated (Deshpande et al., 1996).

1.5 EFFECT OF PROCESSING

1.5.1 Salt

Sodium chloride (NaCl) is an important additive in the meat industry and has been reported to act as a prooxidant or as an antioxidant (Kanner, 1994). The action by which sodium chloride accelerates muscle lipid peroxidation is not well understood and more studies are required to clarify the effect of NaCl on the catalysis of lipid peroxidation (Kanner, 1994). Sodium chloride can decrease enzyme activity by causing enzyme denaturation or by reducing catalytic activity. It was also found that NaCl enhances the activity of ionic iron. The prooxidant effect of NaCl was inhibited by ethylenediaminetetraacetic acid (EDTA) and ceruloplasmin (Kanner, 1994). It has been assumed that NaCl can stop interactions between iron ions and protein macromolecules, allowing more free iron to interact with the lipid reaction and catalyse lipid oxidation. Rhee and co-workers (1983) found that salt inhibited lipid oxidation in ground pork when added at concentrations greater than 2% but accelerated lipid oxidation when added at lower concentrations.

1.5.2 Phosphates

Phosphates are added to the cure to increase the water-binding capacity and, therefore, the yield of the product. Phosphates improve water retention by raising the pH and by causing an unfolding of the muscle proteins, making more sites available for water binding. Phosphates also chelate trace metal ions and

retard developments of rancidity in meat products (Pearson and Gillett, 1996). More complex polyphosphates may be used in the curing mixture in order to chelate metal ions, especially calcium and iron. The binding of calcium ions aids in restoring the meat to pre-rigor state by breaking the bonds between actin and myosin. Chelation of iron aids to prevention of oxidation, since iron is a prooxidant (Pearson and Gillett, 1996).

In hams and bacon, the curing brine consists of a mixture of tripolyphosphate and sodium hexametaphosphate. They are dissolved in water and injected into hams and bacons where they are slowly hydrolysed to diphosphates and become active slowly (Pearson and Gillett, 1996). In sausages, phosphates improve emulsification and protein extraction. They also reduce the viscosity of the batter and make the stuffing more uniform. For emulsion products, pyrophosphates or diphosphates are best, however, a combination works even better (Pearson and Gillett, 1996).

1.5.3 Nitrite

Sodium nitrite is used in the preparation of cured meats. Its functions are to stabilise the colour of the lean tissues, to contribute to the characteristic flavour of cured meat, to inhibit the growth of a number of food poisoning and spoilage microorganisms and to retard development of rancidity (Pearson and Gillett, 1996). However, there are concerns regarding the levels of nitrite used in meat curing, as it has been shown to produce nitrosamine, which is an acknowledged carcinogen (Walsh et al., 1998).

The antioxidative effects of nitric oxide seem to derive from its capability to ligand to ferrous ion and to work as an electron donor and a free radical scavenger (Kanner, 1994). According to Kanner (1994), the formation of nitric oxide from nitrite during the curing process induces an antioxidative effect by: (1) interacting with iron non-heme and iron heme proteins to prevent metal catalysis; (2) acting as free radical scavengers; (3) complexing with heme proteins to prevent iron release from the porphyrin during an attack of H_2O_2 or hydroperoxides; (4) stabilising the unsaturated lipid within the membrane. Sato and Hegarty (1971) suggested that nitrite may interact with the lipid component of

meat cell membranes or may bind to the natural prooxidants such as iron present in meat. The antioxidant mechanism of nitrite may also be due to its ability to stabilise heme-containing proteins, chelate free iron, stabilise lipid molecules and form nitrosated heme-compounds which possess antioxidant activity (Channon et al., 1997; Kanner, 1994).

Nitrosylhaemochrome is a stable complex formed on heating following the addition of nitrite to a meat system. It prevents the release of heme iron as non-heme iron, which can act as a lipid oxidation catalyst (Channon et al., 1997). A few studies showed that nitric oxide myoglobin, nitric oxide ferrous iron complexes and S-nitrosocysteine act as antioxidants (Kanner, 1994). Nitric oxide was recently found to prevent the release of iron from nitric oxide myoglobin by H_2O_2 through a mechanism related to its antioxidative effect. In addition to these effects, during the curing process nitrite and by-products from nitrite seem to also interact with lipids, thus changing their susceptibility to oxidation (Kanner, 1994).

1.5.4 Sodium erythorbate

Sodium erythorbate is often used in the meat industry in cured cuts and cured, pulverised products to accelerate colour fixing in curing (Winter, 1989). It is also used as oxidising/reducing agent, preservative, antioxidant and antimicrobial agent (Burdoch, 1997).

1.5.5 Smoking

1.5.5.1 Wood smoke

The preservation effect of smoke on meat has been known for thousands of years. The main purpose is to develop aroma and flavour, to preserve, to develop colour, to create new products, to form a protective skin in emulsion-type sausages and to protect the products from oxidation (Pearson and Gillett, 1996; Schwanke et al., 1996).

Smoke is produced by using wood or wood sawdust. First the outer surface of meats is oxidised and the inner surface is gradually dehydrated before they can be oxidised. Once the internal moisture level of the sawdust reaches zero, the

temperature rises rapidly to 300-400°C, which allows thermal decomposition to occur thus smoke is generated (Pearson and Gillett, 1996).

The amount of smoke on meat depends on the rate of smoke deposition and is influenced by the smoke density, the smokehouse velocity, the smokehouse relative humidity, the type of casing used and the surface of the product being smoked (Pearson and Gillett, 1996).

Smoking and cooking are usually carried out together and are involved in the development of colour of cured meat. Browning or Maillard reaction is responsible for the development of the characteristic brown colour on the surface of smoked products (Pearson and Gillett, 1996).

Smoking of bacon has been shown to effectively reduce the number of surface bacteria and to extend its storage life. This is due to the bactericidal and bacteriostatic properties of smoke. These properties are attributable to some smoke components such as phenols and acids. Smoking also removes moisture from the surface of the meat, which helps to retard or reduce microbial growth (Pearson and Gillett, 1996).

Smoke also has an influence on the development of rancidity due to its antioxidant activity. Wood smoke is composed of hundreds of different compounds. The most common chemical components found in wood smoke include phenols, organic acids, alcohols, carbonyls, hydrocarbons, and some gaseous components, such as carbon dioxide, carbon monoxide, oxygen, nitrogen, and nitrous oxide (Pearson and Gillett, 1996). About 20 phenols from wood smoke have been isolated and identified. They act as antioxidants, contribute to the colour and flavour of smoked products and have bacteriostatic effect, which contributes to preservation (Pearson and Gillett, 1996).

1.5.5.2 Liquid smoke

The first attempts to produce liquid smoke were made in 1811. Ernest Wright from Kansas City produced liquid smoke using hickory that is still the basis for the production of liquid smoke. Liquid smoke as we know it today begun 35 to 40 years ago (Potthast, 1993). Its use is increasing among processors due to several advantages it has over traditional smoking (Pszczola, 1995). Liquid smoke

can be easily applied to products and the concentration can be easily controlled (Suñen, 1998). Smoke flavourings are available in various physical states, colours and odours (Guillén and Ibargotia, 1998) and are considered as GRAS (Generally Regarded As Safe) (Schwanke et al., 1996).

Liquid smoke is made by placing hardwood sawdust in sealed retorts, where pyrolysis takes place. Intense heat makes the wood smoulder, releasing the gases seen in ordinary smoke. These gases are quickly chilled in condensers, which liquefies the smoke. It is then forced through several refining vats and a multistage filtration to remove impurities. Finally, it is filled into containers (Hermey and Patzelt, 1994). If properly processed, liquid smoke is practically free from all polycyclic aromatic hydrocarbons (PAH).

Liquid smoke has antioxidative and antimicrobial properties. It contains phenol syringol, guaiacol, catechol and eugenol, which were generated during the pyrolysis of the lignin fraction of wood as well as acetic, propionic and other organic acids which lower pH and destroy the walls of bacterial cells (Pszczola, 1995). A concentration of 0.2 to 5% liquid smoke in product formulation was found to have effective antioxidant properties (Schwanke et al., 1996, Maga, 1988). However, high levels (10%) of liquid smoke have shown to have a prooxidant effect (Chomiak and Goryn, 1976; Maga, 1988).

1.5.5.3 Polycyclic aromatic hydrocarbons in products processed with natural wood smoke or liquid smoke

Presence of polycyclic aromatic hydrocarbons (PAHs) in smoked meats is of major concern as some of the PAHs are considered carcinogenic. The main carcinogenic PAHs in food as well as in air include benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, dibenzo[*a,h*]anthracene and indeno [1,2,3-*c,d*]pyrene (Gomaa, 1993).

Surveys of several commercially available products showed differences in PAHs concentrations between smoked products (Gomaa et al., 1993). Those PAHs are formed by incomplete combustion or pyrolysis of organic material (Gomaa et al., 1993). Their occurrence in food may result from the contamination of the environment or from the food preparation. PAHs have been detected and

quantified in many foods and are often found in low concentrations (Yabiku et al., 1993). Studies involving various commercial products showed that processed products with natural wood smoke had higher total carcinogenic PAHs content than those processed with liquid smoke flavouring (Gomaa et al., 1993; Yabiku et al., 1993). In a study by Yabiku and co-workers (1993), benzo[*a*]pyrene, usually used as a reference indicator for carcinogenic PAHs, appeared to be present in more than 50% of the smoked food samples tested; however, their levels were generally under 1.0 μg/kg. The maximum level recommended by FAO/WHO for benz[*a*]pyrene is 10μg/kg.

1.6 MEAT PRESERVATION

Fresh meat is not normally marketed immediately after slaughter. *Rigor mortis* must have passed before it is suitable for consumption. The most common preservation methods for fresh meat are cooling or freezing together with packaging. The shelf life of processed meat can be extended by various means such as by smoking, drying, salting, fermenting, pickling and chilling or freezing combined with packaging. Vacuum packaging and modified atmosphere packaging (MAP) in combination with chilling or freezing are largely used for extending the shelf life of meats (Ooraikul and Stiles, 1991; Hanlin and Evancho, 1992).

1.6.1 Chilling

Chilling involves a reduction in temperature below the minimum necessary for microbial growth. Chilling extends the generation time of microorganisms and prevents or retards their reproduction (Fellows, 1988), particularly the growth of thermophilic and mesophilic microorganisms. A number of psychrophilic microorganisms cause food spoilage but there are no psychrophilic pathogens (Fellows, 1988).

Chilling to temperatures below 5-7°C retards microbial spoilage and prevents the growth of pathogens. At 0°C, the storage life of carcass meat is 3-6 weeks, during which microbial growth and lipid oxidation are retarded (Ooraikul and Stiles, 1991). The rate of biochemical changes by either

microorganisms or naturally occurring enzymes changes logarithmically with temperature. Chilling therefore reduces the rate of enzymic and microbiological change and retards respiration of fresh foods (Fellows, 1988).

The shelf life of processed chilled foods is determined by the following factors: (1) the type of food; (2) the degree of microbial destruction or enzyme inactivation achieved by the process; (3) control of hygiene during processing and packaging; (4) the barrier properties of the package; and (5) the temperature during distribution and storage (Fellows, 1988). At chill temperature, meat can spoil rapidly due to the development of aerobic microflora, usually dominated by species of *Pseudomonas* (Devine et al., 1996). Strict aerobes bacteria can be effectively controlled by manipulating the oxygen concentration in the atmosphere surrounding the meat. An anaerobic gaseous environment around the meat will cause the microflora developing at chill temperature to become dominated by lactic acid bacteria of the genera *Lactobacillus*, *Carnobacterium* and related cocci (Devine et al., 1996).

The spoilage pattern for cooked cured meat products is very similar to fresh meat; however, the presence of salt and nitrite can effectively control the microflora of the product (Nielsen, 1985). The most common method to produce anaerobic conditions is vacuum packaging (vacuum packaging is further described in section 1.6.3.2). Another packaging method that involves the modification of the atmosphere surrounding the product is MAP (MAP is further described in section 1.6.3.3).

1.6.2 Freezing

All frozen products are initially chilled. In addition to microbial changes during the chilling process, undesirable chemical changes such as rancidity are likely to occur. If rancidity is initiated during chilled storage, this chemical reaction will continue to take place at an accelerated rate after freezing (Devine et al., 1996).

Freezing involves cooling food from a refrigerated temperature (~4-5°C) to a temperature below the freezing point (at least –15°C), at which temperature the product can be stored for prolonged periods without significant deterioration in its

quality attributes (Mandigo and Osborn, 1996). Freezing and storage of meat at –18°C to -20°C and 90% relative humidity will extend its storage life to 9-15 months (Ooraikul and Stiles, 1991).

Freezing involves crystallisation of the water in foods. The quality of frozen foods depends on the speed of freezing. Slow freezing (0.1-0.2 cm/h) produces very large ice crystals within the cells and causes serious damage to the cell walls allowing greater weight loss during freezing, thawing and cooking, resulting in lower water-binding capacity, decreased protein solubility and tougher cooked meat (Mandigo and Osborn, 1996). Quick freezing (5.0 cm/h) produces fine ice crystals, therefore causing little damage to the frozen tissue (Mandigo and Osborn, 1996). Freezing rapidly is a major requirement for producing quality frozen products. The quicker a product is frozen, the more effectively it can be kept in its original state and its quality is maintained, even after thawing. Furthermore, microbial growth can be hindered by lowering the temperature quickly (Mandigo and Osborn, 1996).

Frozen storage of foods is an effective means of preservation, however, during long-term storage, deterioration may still occur at a reduced rate (Stuchell and Krochta, 1997). Food quality can be greatly affected by reactions such as oxidation, insolubilisation of proteins, and glycolysis, which frequently accelerate during freezing. Uncooked meats are susceptible to degradation under frozen conditions through moisture loss, lipid oxidation, protein alteration leading to changes in water binding capacity and in texture, flavour, colour and aroma (Stuchell and Krochta, 1997). The quality of cured and processed meat products is directly related to the quality of the raw materials used in their manufacture. Additionally, cured and processed meats products contain ingredients such as salt, spices, sodium nitrite, phosphate, smoke, added singly or in combination, that affect quality, shelf life and overall acceptability of the products and the natural chemical reactions occurring within these products during frozen storage (Mandigo and Osborn, 1996).

Fat oxidation is one of the few chemical reactions that is accelerated at temperatures below the freezing point of water, with a maximum rate around –10°C (Ranken, 1989). The acceleration in rancidity is due to the water in the fatty

tissue which, when frozen increases the concentration of the reactants. Freezing and the presence of salt such as in processed meat greatly accelerates peroxidation (Ranken, 1989).

1.6.3 Packaging

Packaging is used to provide a protective barrier between the food and the environment in order to extend the shelf life of the product. It controls light transmission, transfer of heat, moisture and gases, and movement of microorganisms or insects (Fellows, 1988). It also advertises foods at the point of sale and protects food from mechanical damage. The most currently used packaging methods for meat are vacuum packaging and MAP. Both methods involve the removal of the source of oxygen together with low temperature storage, which help delay the onset of lipid oxidation (Madhavi et al., 1996).

1.6.3.1 Vacuum packaging

Vacuum packaging involves the removal of all the air within the package without deliberately replacing it with another gas (Brody, 1989). Vacuum shrink packaging has been derived from vacuum packaging and is widely used for primal and subprimal cuts of red meat. Meat is placed in a flexible film, barrier-pouch, and put inside a vacuum-packaging chamber where oxygen is evacuated, then closed and heat-shrunk around the meat. This creates a skintight package wall and protects against the entry or escape of gases such as air, CO_2 or water vapour. It assures inhibition of microbial growth and inhibits water loss and freezer burn (Brody, 1989). The minimum concentration of CO_2 for an inhibitory effect to take place is between 20 and 30 % (Stiles, 1991).

Enzymatic and microbial activities in meat tissue produce carbon dioxide, thus increasing the carbon dioxide level inside the package. This, together with chilled temperature (0 to 5°C) account for prolonged shelf life of vacuum packaged fresh meats (Brody, 1989). The increase in CO_2 levels inside the package retards the growth of gram-negative, aerobic, psychrotrophic bacteria but allows the development of facultative anaerobic bacteria such as *Lactobacillus*, *Leuconostoc* and *Streptococcus* species. *Pseudomonas* species are inhibited with

CO_2 levels as low as 10% (Brody, 1989). With uncooked cured meats, the main advantage of vacuum packaging is microbiological, however, its contribution is small regarding rancidity and colour (Ranken, 1989). With cooked cured meats, microbiological spoilage is slower and vacuum packaging gives a significant protection against colour loss and rancid off-flavour (Ranken, 1989).

1.6.3.2 Modified atmosphere packaging (MAP)

MAP is the initial alteration of the gaseous environment in the immediate vicinity of the product, allowing the packaged product interactions to naturally vary their immediate gaseous environment (Brody, 1989). This is carried out by removing the air by vacuum and back flushing with carbon dioxide, nitrogen or a combination of the two and heat sealing (Brody, 1989; Fellows, 1988). Mixtures of carbon dioxide with nitrogen or oxygen give very satisfactory protection against colour and rancidity problems (Decker and Xu, 1998b; Madhavi et al., 1996; Ranken, 1989). This process is performed in conjunction with a flexible oxygen barrier film. The packaging material itself transmits oxygen, carbon dioxide and water vapour allowing further changes in the gaseous environment surrounding the product (Brody, 1989). The changes in gas composition during storage depend on: (1) the respiration rate of fresh food and the temperature of storage; (2) the permeability of the packaging material to water vapour and gases; (3) the external relative humidity which may affect the permeability of some films; and (4) the surface area of the package in relation to the amount of food it contains (Fellows, 1988). The modification of gas environment allows protection from spoilage, oxidation, dehydration, weight loss and freezer burn and extends the shelf life (Vaclavik and Christian, 1998).

Gas mixtures used for fresh meat packaging include O_2/CO_2, 20/80 and $O_2/CO_2/N_2$, 20/69/11 (Inns, 1987). The oxygen concentration in MAP is sufficient to inhibit anaerobic bacteria and to retain the red colour of oxymyoglobin of fresh red meats (Inns, 1987). The gas atmosphere creates a selective pressure on the microflora of meats; however, it is important to maintain low microbial loads through high standard of hygiene during meat preparation. A high initial microbial load will counteract the benefits of MAP (Stiles, 1991). In MAP, lactic acid

bacteria are not controlled, therefore if a H_2S producer is present in fresh meat or if strains of *Lactobacillus* or *Leuconostoc* are present in processed meats with added carbohydrate, then excess acidity or slime can develop and cause product spoilage (Stiles, 1991).

Storing processed meats with MAP differ from fresh meat, principally due to the lack of CO_2 production, the absence of pseudomonads, the negative O_2 effects, and the need for CO_2 to inhibit mould (Gill and Molin, 1991). The cells in processed meat tissues are dead, therefore, their CO_2 metabolism is ended. In a package of processed meat, only the microflora still present will continue to metabolise but the production of CO_2 and the consumption of O_2 take place at a much slower rate than in a package of fresh meat (Gill and Molin, 1991). Spoilage microorganisms such as *Pseudomonas* and Enterobacteriaceae normally do not grow on processed meat due to the addition of salt, nitrite or other additives with antibacterial action or due to the decrease in water activity. The presence of O_2 due to a leak in the package can initiate oxidation reactions as well as can allow the growth of moulds and yeasts. Ideal atmosphere conditions for processed meat do not contain O_2 but gas mixtures of N_2 supplemented with 10-30% CO_2 (Gill and Molin, 1991).

Unlike vacuum packaging, MAP uses films that remain loose fitting. This avoids the crushing effect of skintight vacuum packaging. When used in combination with aseptic packaging, which reduces the microbial load, and reduced temperature, MAP becomes a very effective packaging method (Vaclavik and Christian, 1998).

1.6.3.3 Retail Packaging

Meat for the retail market needs to be prepacked to prevent dehydration, to maintain appearance and to protect from discolouration (Tohma, 1990). This is carried out by means of a combination of stretchable polyvinyl chloride (PVC) wrap film and clear polystyrene or foamed polystyrene trays (Tomioka, 1990). The film has oxygen permeability above 9 L/m^2 to allow the meat to bloom to its bright red colour. Due to its high oxygen transmission, this type of packaging does not control aerobic growth and the retail display is very short (1 to 3 days) (Brody,

1989). Vacuum packaging and MAP are also used for the retail of fresh meat. For processed meat, a wide range of packaging is available. Packaging methods include vacuum packaging, MAP, canning, double sterilisation packaging, boil- and steam-cooking packaging, retort sterilised packaging, oxygen-absorbing agent packaging, and aseptic packaging (Tomioka, 1990).

CHAPTER 2

MATERIALS AND METHODS

2.1 ANIMALS AND FEEDING

2.1.1 Experimental design

Twelve (Large White x Landrace) 10 week old gilts with an average initial weight of 27.0 kg (± 2.5 kg) were stratified on liveweight into 4 blocks of 3 pigs and then within each block randomly allotted to four dietary treatment groups. The dietary treatments were assigned in a 2 x 2 factorial design with the respective factors being fishmeal (0 or 5%) and vitamin E (10 or 200 mg per kilogram of feed). The vitamin E was supplemented in the form of α-tocopheryl acetate.

2.1.2 Experimental treatments

The composition of the control and experimental diets is shown in Table 2.1. All diets were formulated to meet the nutritional requirements of growing pigs (Standing Committee on Agriculture, 1987) and to be isocaloric and have the same essential amino acids content. The nutrient contents of the experimental diets are shown in Table 2.2. The fat content, anisidine value and thiobarbituric acid reactive substances (TBARS) of fishmeal and the experimental diets are given in Table 2.3.

Pigs were housed at the Pig Research and Training Centre, Victorian Institute of Animal Science, Werribee, Victoria, Australia. The pigs were fed their respective diets *ad libitum* for 10 weeks (average live weight 88.9kg ± 7.4kg), after which time they were slaughtered at a commercial abattoir. At this point, the animals had reached an average live weight of 88.9 kg. Animals were CO_2 stunned, slaughtered, dressed and chilled at a local commercial abattoir. The carcasses were stored for 5 days at 4°C before being processed.

Two products were manufactured from meats obtained from the animals after slaughter: (1) bacon with two treatments - with or without liquid smoke;

(2) wiener sausages with three treatments - rosemary extract, sweet whey powder, control.

Table 2.1 : Ingredients (% w/w) of the control and experimental diets.

Ingredients	Control Diet	Vitamin E Supplemented Diet	Fishmeal Supplemented Diet	Fishmeal + Vitamin E Supplemented Diet
DL methionine	0.078	0.078	0.033	0.033
Lysine	0.100	0.100	0.100	0.100
Threonine	0.062	0.062	0.076	0.076
Wheat	66.903	66.903	74.754	74.754
Tallow	2.000	2.000	2.000	2.000
Peas	15.000	15.000	10.229	10.229
Dicalcium phosphate	0.402	0.402	–	–
Lime	1.242	1.242	1.381	1.381
Salt	0.200	0.200	0.200	0.200
Vita-Grow	0.200	0.200	0.200	0.200
Blood meal	3.000	3.000	3.000	3.000
M&B ML 50	4.178	4.178	3.028	3.028
Soy 48	6.635	6.635	–	–
Fishmeal	–	–	5.000	5.000
α-tocopheryl acetate [mg/kg diet]	10	200	10	200

Table 2.2 : Nutrient composition (% w/w) of the control and experimental diets.

Nutrients	Control Diet	Vitamin E Supplemented Diet	Fishmeal Supplemented Diet	Fishmeal + Vitamin E Supplemented Diet
M.E. pigs, [MJ/kg]	14.22	14.22	14.22	14.22
Crude protein	20.00	20.00	19.46	19.46
Lysine (available)	0.995	0.995	0.995	0.995
Isoleucine	0.655	0.655	0.622	0.622
Leucine	1.534	1.534	1.475	1.475
Methionine	0.321	0.321	0.320	0.320
Methionine + cysteine	0.621	0.621	0.608	0.608
Phenylalanine + tyrosine	1.550	1.550	1.470	1.470
Threonine	0.729	0.729	0.725	0.725
Tryptophan	0.221	0.221	0.210	0.210
Calcium	1.100	1.100	1.100	1.100
Phosphorus	0.400	0.400	0.400	0.400
Crude fibre	3.238	3.238	2.773	2.773

Table 2.3 : Fat content, anisidine value and TBARS values for fishmeal and the experimental diets.

	Fat content [%]	Anisidine value	TBARS [mg malonaldehyde/ kg sample]
Fishmeal	6.3	3.8	3.2
Control Diet	4.3	0.0	0.8
Vitamin E Supplemented Diet	4.6	0.0	0.8
Fishmeal Supplemented Diet	4.6	0.2	0.9
Fishmeal + Vitamin E Supplemented Diet	4.8	0.3	0.8

Table 2.4: Fatty acid composition (% wt/wt of total fatty acids) of fishmeal

Fatty acid	Fishmeal	SEM [a]
14:0	6.33	0.02
16:0	27.42	0.08
16:1 (*n*-7)	4.42	0.01
18:0	7.52	0.01
18:1 (*n*-9)	20.59	0.02
18:1 (*n*-7)	4.88	0.00
18:2 (*n*-6)	1.65	0.00
18:3 (*n*-3)	0.88	0.01
20:0	1.50	0.01
18:4 (*n*-3)	0.30	0.01
20:1 (*n*-9)	6.67	0.02
20:3 (*n*-6)	0.11	0.03
20:4 (*n*-6)	0.70	0.01
20:5 (*n*-3)	1.03	0.00
22:1 (*n*-9)	6.57	0.02
21:5 (*n*-3)	0.16	0.01
22:4 (*n*-6)	0.17	0.00
22:5 (*n*-3)	2.03	0.00
22:6 (*n*-3)	6.47	0.01

[a] SEM, standard error of the mean

2.2 PRODUCT MANUFACTURE

Bacon and wiener sausages were produced at a commercial meat processing plant, in the month of February 2000.

2.2.1 Bacon manufacture

Two types of bacon were manufactured from the middles. The left middle from each pig was cured using a curing mixture containing salt (2.2 to 2.5%), nitrite (residual nitrite 40 to 80ppm), phosphates as sodium tripolyphosphate and sodium hexametaphosphate (level of 0.5 to 0.6% in finished product). The right middle from each pig was cured using a curing mixture containing salt (2.2 to 2.5%), nitrite (residual nitrite 40 to 80ppm), phosphates as sodium tripolyphosphate and sodium hexametaphosphate (level of 0.5 to 0.6% in finished product), and liquid smoke 0.2% (Royal S, Grayson Trading, Bayswater, Victoria, Australia). The details of the manufacturing process are shown in the flow diagram (Figure 2.1).

The pork middles were injected with the curing mixture, using a multi-needle Fomaco injector (Model FGM 88DW; Koge, Denmark). The injected middles were immersed in brine for 3 days at 4°C and allowed to equilibrate. They were then drained and cooked in a smokehouse from Vermag (Model Micromat S; Verden, Germany) to an internal temperature of 59°C. The bacon was then chilled to 4°C, sliced (thickness 3mm), packaged in cardboard boxes, blast frozen to –20°C and stored at -20°C for 4 months.

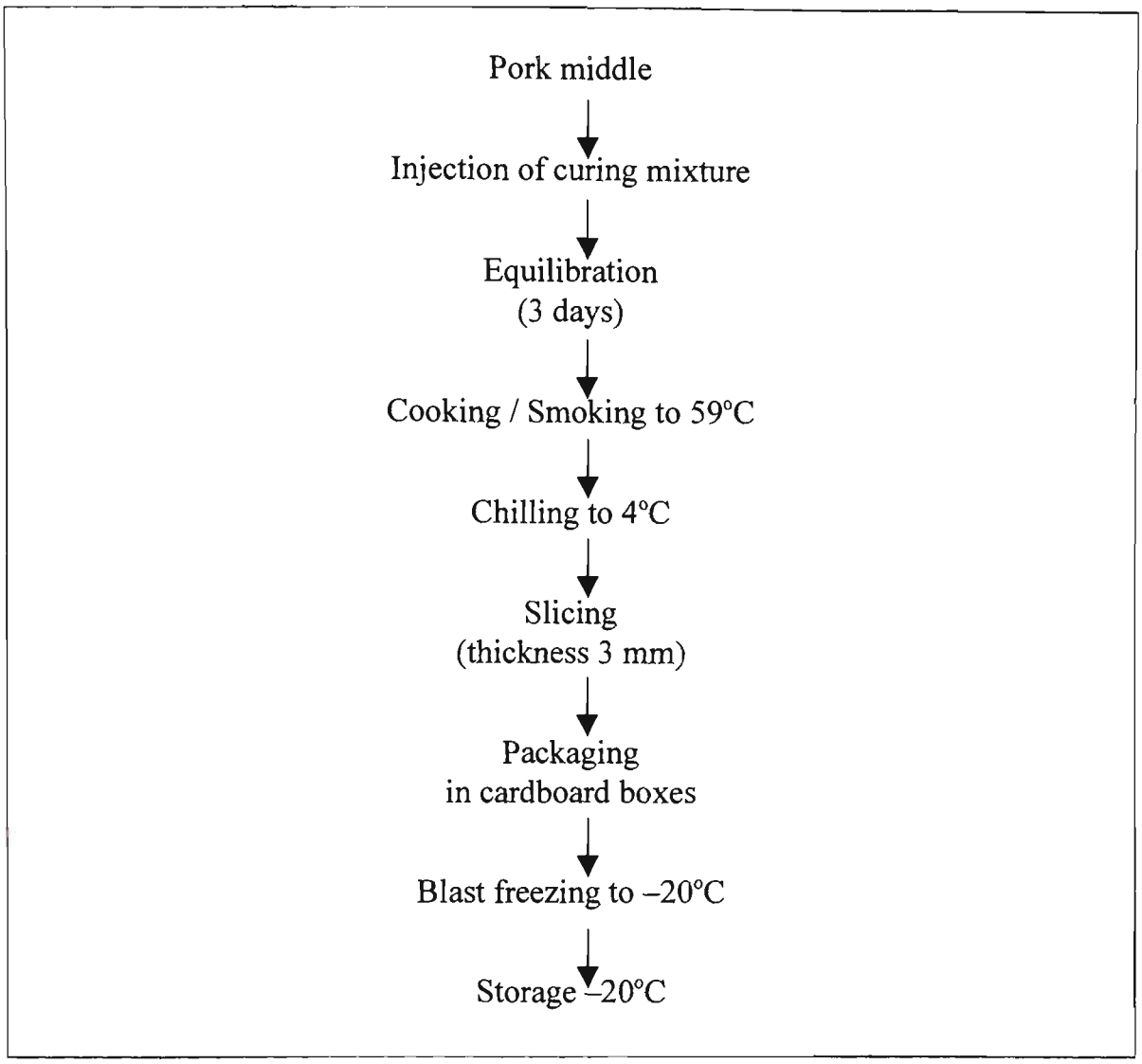

Figure 2.1 A process flow diagram for the manufacture of bacon.

2.2.2 Wiener sausages manufacture

Twelve batches of wiener sausages were manufactured with or without antioxidants (rosemary extract or whey powder) added to the formulation. Three types of sausages were produced with different formulations using trims and fat from all three pigs of the same dietary treatment. The basic formulation contained pork trims, fat (20%), modified corn starch, dextrose monohydrate, salt (1.2%), wiener spice mix, sucrose, monosodium glutamate, Heglutal M (a flavour enhancer), sodium erythorbate (0.08%), ice/water.

The three treatments of wieners were produced: (1) Control (basic formulation); (2) Basic formulation with the addition of 0.03% rosemary extract,

Herbor (FIS, Food Ingredients Specialities, St. Albans, Victoria, Australia); and (3) Basic formulation with the modified corn starch replaced by 2.5% sweet whey powder (FIS).

Wiener sausages were made in 20 kg batches. Trim and fat from all the three pigs of the same dietary treatment were weighed separately for the three batches. The trim and fat were then combined to give a pre-determined fat level (35%) and minced using a mincer with a 5 mm plate (Model 7; Butchers Service Engineering Pty. Ltd. Melbourne, Australia). The mince was mixed evenly by hand before weighing the quantities required for three batches. One batch of mince and the dry ingredients were placed in a custom manufactured double-armed paddle mixer (commercial meat processing plant). Ingredients were allowed to mix through the mince before adding a mixture of ice/water. The batch was mixed for 30 minutes, the sausage mix was kept refrigerated at 4°C overnight, followed by filling into fresh sheep casing using a sausage filler (Model KF 650; Hoegger Alpina, Gossau, Switzerland). Sausages were subsequently hung on trolleys and cooked to an internal temperature of 72°C in a smokehouse (Model Micromat S; Vermag, Verden, Germany) and chilled overnight to 4°C. The sausages were then individually quick frozen (IQF) to –20°C, placed into polyethylene low density (LDPE) bags, 450 x 580 mm; thickness, 75 μm, permeability to O_2=2.6 L m^{-2} 24 h^{-1}; at 25°C (Mike's Plastic Bags Pty. Ltd., Moorabbin, Australia), boxed in cardboard boxes and stored at –20°C for 40 weeks. The details of the manufacturing process for wiener sausages are shown in Figure 2.2.

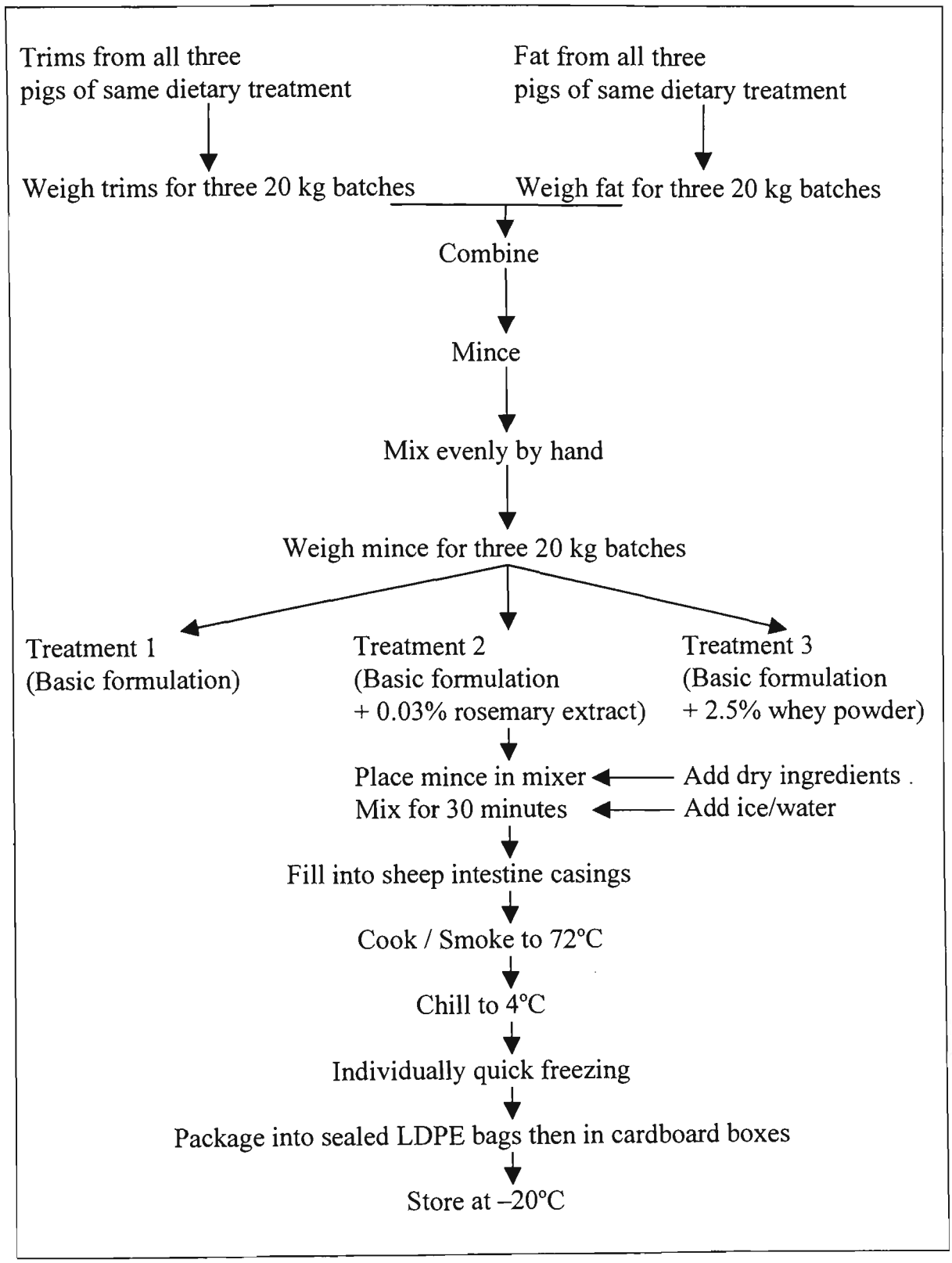

Figure 2.2 A process flow diagram for the manufacture of wiener sausages

2.3 CHEMICALS

All chemicals were of "Analar" or of higher purity and were obtained from Merck (Merck Pty. Ltd., Kilsyth, Victoria, Australia) and Sigma (Sigma Chemical Company Ltd., Castle Hill, New South Wales, Australia).

2.4 FATTY ACID COMPOSITION OF PORCINE BODY FAT

Saturated and unsaturated fatty acids from bacon fat and pig feed were determined qualitatively using the method described by Bannon et al (1982). The fatty acids were first esterified with sodium methoxide, acidified with HCl and finally the fatty acids were extracted with hexane. The extracted fatty acids were then separated by gas chromatography (GC) using a FID detector. All samples were analysed in triplicate.

2.4.1 Method

A 8 g sample of fishmeal/pig feed or a 0.5000 g of fat tissue taken from a slice of thawed bacon middle under the rind and finely chopped was accurately weighed in a 50 mL screw top tube in case of fishmeal/pig feed or in a 15 mL glass screw top tube in case of bacon. Three millilitres of 0.5 M sodium methoxide was added, and the sample was vortexed. The tube was placed in a 60°C water bath for 15 minutes, followed by mixing with a vortex for 2 minutes and cooled to room temperature (~20°C). One millilitre of 2.0 M HCl containing methyl orange indicator was added and the solution thoroughly mixed (pink colour of solution indicates acidic conditions), then 5.0 mL of 5%NaCl and 3.0 mL of 95% hexane were added, and mixed thoroughly. The layers were allowed to separate for approximately 10 minutes followed by removing about 1 mL of the hexane layer into an amber GC vial for analysis. Gas chromatography was carried out with a Varian Star 3400 CX gas chromatograph, using a BPX 70 column (25 m x 0.33 mm O.D. x 0.22 mm I.D., 0.25μm, film thickness) obtained from SGE (Austin, Texas, USA). The chromatographic conditions consisted of the following: initial GC injector temperature of 240°C, initial GC injector hold time of 35 minutes, initial column temperature of 150°C, final temperature of 210°C,

rate of 2.0°C/minute, hold time of 5 minutes, FID detector temperature of 280°C, detector attenuation of 16, detector range of 12, split of 40 mL/min., injection volume of 1.0 µl, helium as carrier gas, and gas pressure of 15 psi.

2.5 DETERMINATION OF THE VITAMIN E CONTENT IN FAT AND MUSCLE TISSUES

Vitamin E in fat and muscle were quantitatively determined using the method described by Liu et al. (1996). Vitamin E was first saponified, then extracted with diethyl ether for fat and hexane for muscle. Samples were evaporated to dryness and redissolved in ethanol before being quantified by High Performance Liquid Chromatography (HPLC). All samples were analysed in duplicate.

2.5.1 Method

2.5.1.1 Determination of vitamin E in fat

A 0.5 g sample of finely diced bacon fat taken from a slice of thawed bacon middle under the rind was accurately weighed into a 20 x 150 mm culture tube, and 0.25 g of ascorbic acid and 2.0 mL 20% KOH in methanol were added followed by vortexing. The tube was flushed with nitrogen, capped and heated in the dark in a 65°C water bath and mixed at every five minutes using a vortex until saponification was completed (30 minutes). Six millilitre of distilled water was then added and the tube was thoroughly vortexed and allowed to cool before extracting twice with 8.0 mL of diethyl ether. The extracts were pooled into another tube, and washed twice subsequently with an equal volume of distilled water. A few grams (~2g) of anhydrous Na_2SO_4 were added after the final wash to remove any last traces of water. The solution was then transferred to a clean scintillation vial and evaporated to dryness under nitrogen. Finally the residue was redissolved (if the solution was cloudy, or if there was a precipitate present, the sample was warmed until completely dissolved prior to analysis using HPLC) in 2000 µl of ethanol and transferred to an insert in an amber bottle ready for HPLC. The analysis was carried out using a Varian 9010 solvent delivery system,

Varian 9070 fluorescence detector and a column symmetry C_8, 5μm particle size, 150 mm x 3.0 mm O.D. (Waters; Milford, Massachusetts, USA). The chromatographic conditions consisted of methanol:water (97:3), injection volume of 50 μl, fluorescence detection at 296 nm excitation and 325 nm emission, run time of 6 minutes, and wash time of 6 minutes.

2.5.1.2 Determination of vitamin E in muscle

One gram of bacon muscle from the loin was previously thawed, finely diced and accurately weighed into a 20 x 150mm culture tube. Ascorbic acid (0.25 g) followed by 7.3 mL of KOH (11 % in water:methanol (45:55)) were added and vortexed. The tube was then flushed with nitrogen, capped and heated in dark in a 75°C water bath and mixed every five minutes on a vortex until saponification was completed (30 minutes). The tube was allowed to cool in an ice-bath before extracting twice with 4.0 mL of hexane. The extracts were pooled in a separate tube and washed twice with an equal volume of distilled water. A few grams of anhydrous Na_2SO_4 were added after the final wash to remove any last traces of water. The solution was then transferred to a clean scintillation vial and evaporated to dryness under nitrogen. Finally, the residue was redissolved in 500 μl of ethanol transferred to an Eppendorf tube and centrifuged for 10 minutes at 14,000 rpm on a Eppendorf centrifuge (Model 5415 C; Eppendorf, Germany). The clear supernatant was transferred to an amber bottle ready for HPLC. The analysis was carried out with the same instrument and conditions as for the determination of vitamin E in fat (section 2.5.1.1).

2.5.1.3 Preparation of standards

A standard stock solution was prepared by weighing 0.01g of α-tocopherol and diluting to 100 mL with ethanol. Working solutions were prepared by diluting the stock solution with ethanol. The E-value of the working solutions were checked prior to their use, using a spectrophotometer (Model 4054; UV/visible spectrophotometer; LKB Biochrom Ultrospec Plus, Amersham Pharmacia Biotech, Uppsala, Sweden). $E^{1\%}_{1cm}$ value for α-tocopherol = 75.8 at 295 nm. For calculation of concentration (μg/mL) = absorbance x 131.93.

2.6 FAT (CRUDE) DETERMINATION IN FISHMEAL

The fat content of fishmeal and pig feed was determined using the acetone extraction method (AOAC, 1990). All the samples were analysed in duplicate.

2.6.1 Method

A 5 g sample of fishmeal or a 10 g sample of pig feed was weighed to the nearest 0.001 g into a paper extraction thimble, covered with a thin layer of cotton and extracted with acetone in a continuous extractor for 16 hours. The acetone was distilled off until the volume in the flask was 10-15 mL. The residue was transferred to a 100 mL tared beaker, and the flask was washed free of all oils with fresh acetone then evaporated over a steam bath. When no water was left, the beaker was placed in an oven at 80°C overnight, then transferred to a desiccator, cooled and weighed. The extracted sample was transferred from the thimble to a 150 mL beaker and any remaining solvent was removed by heating in an oven at 80°C. Sixty millilitres of 4N HCl was added and the sample was digested for one hour at boiling point on a hot plate, occasionally stirring with a glass rod and adding distilled water as needed to maintain the volume in the beaker. The mixture was then filtered through fluted filtered paper and the residue was washed until acid-free. The filter paper with the sample was placed in a 150 mL beaker and dried overnight in an oven at 80°C. The filter content and sample was transferred to a paper extraction thimble and extracted with acetone in a continuous extractor for 16 hours. The acetone was distilled off until the volume in the flask was 10-15 mL. The residue was transferred to a 100 mL tared beaker, and the flask was washed free of all oils with fresh acetone then evaporated over a steam bath. When no water was left, the beaker was placed in an oven at 80°C overnight, then transferred into a desiccator, cooled and weighed. The sum of the weights from the extractions gave the total fat.

2.7 P-ANISIDINE VALUE

The extent of oxidation in oils and fats was determined by measuring the formation of carbonyl compounds according to Kirk and Sawyer (1991). All the samples were analysed in duplicate.

2.7.1 Method

A 0.4000g of dry sample (*M*) was accurately weighed into a 25 mL volumetric flask then diluted to the mark with iso-octane. The solution was filtered prior to measuring the absorbance (*A1*) of the solution at 350 nm in a 10 mm cell against a blank of iso-octane using a spectrophotometer (Model Novaspec®II; Amersham Pharmacia Biotech, Uppsala, Sweden). Five millilitres of the sample solution was pipetted into a 10 mL screw-capped test tube and 1 mL of *p*-anisidine solution (2.5 g *p*-anisidine/L in glacial acetic acid) was added to it. Similarly, a reagent blank determination was carried out. After exactly 10 minutes, the absorbance (*A2*) was measured as before against the reagent blank. The *p*-anisidine value was expressed as $25 \times (1.2\,A2 - A1) / M$.

2.8 ASSESSMENT OF LIPID OXIDATION

Three methods were used for assessment of lipid oxidation: (i) determination of thiobarbituric acid reactive substances, (ii) determination of fluorescence shift and (iii) sensory analyses. All samples were analysed in triplicate.

2.8.1 Determination of thiobarbituric acid reactive substances (TBARS)

Both products were analysed at different time intervals to measure the concentration of TBARS using the distillation method of Ke et al. (1984). TBARS was used to determine the degree of oxidation in the product and the value was expressed in milligrams malonaldehyde per kilogram of sample. TBARS method is known to have some limitations with products that have been stored for a long time. This is due to instability of malonaldehyde and other short chain carbon products of lipid oxidation. These products oxidise and yield organic alcohols and acids, which are not determined by the TBARS test.

2.8.1.1 Preparation of reagents

2.8.1.1.1 TBA reagent

A 1.44 g of 2-thiobarbituric acid (TBA) and 50 mL of deionised water were mixed in a 500 mL volumetric flask. Approximately 300 mL of glacial acetic acid was added and the mixture was stirred until the TBA was completely dissolved. The flask was then filled to the mark with glacial acetic acid.

2.8.1.1.2 TEP standard solution

A 2.268 g of 1,1,3,3 – tetraethoxypropane (TEP) was accurately weighed into a 1000 mL volumetric flask and the flask filled to the mark with distilled water to produce a 1 x 10^{-3} M solution. A 10 mL aliquot was taken and diluted to 1000 mL to produce a 1 x 10^{-4} M stock solution. The flask containing the solution was covered with a foil and kept refrigerated until used.

2.8.1.2 Preparation of standard curve

A 1 x 10^{-5} M working solution of TEP was prepared by diluting 10 mL of the stock solution to 100 mL. Aliquots of 0, 0.4, 0.8, 1.2, 1.6 and 2.0 mL of working TEP standard solution were accurately pipetted into screw-cap test tubes and distilled water was added to obtain a total volume of 5.0 mL. Five millilitres of TBA reagent was added and the tubes were tightly closed. Standards were placed in a boiling water bath at the same time as the samples and heated at 100°C for 45 minutes. The tubes were then cooled under tap water for 3 min and the absorbance was read at 538 nm using a spectrophotometer (Model Novaspec®II; Pharmacia LKB Biotech). The blank (0.0 mL TEP) was set to zero. The TEP concentrations in μM were then plotted against the absorbance at 538 nm.

2.8.1.3 Method

Ten grams of slightly thawed meat (or 1/3 of fat and 2/3 of muscle meat in case of bacon) was finely chopped and weighed into a 100 mL Schott bottle and 0.1 g of propyl gallate, 0.1 g of EDTA and 50 mL of deionised water were added. The bottle was placed in ice bath and the mixture blended for 20 seconds at

20,500 rpm with an Ultraturrax homogeniser (Model T25, dispersing element S25 N-18G; Janke & Kunkel, IKA Werke, Staufen, Germany). The homogenate was then transferred to a 800 mL Kjeldahl flask and the Schott bottle was rinsed with 45 mL of deionised water and by blending for a further 10 seconds. The content of bottle was then added to the homogenate in the Kjeldahl flask. Ninety five millilitres of 4N HCl was subsequently added and the distillation started immediately. Forty five millilitres of distillate was collected in a 50 mL graduated cylinder within 35 minutes or less, using a Kjeldahl distillation unit (Kjeltec system, 1002 distilling unit, Höganäs, Sweden). The distillate was transferred into a 50 mL volumetric flask and the volume made up to the mark with distilled water. Five millilitres of distillate and 5.0 mL of TBA reagent were pipetted into a screw-cap test tube and capped tightly. Samples, standards and blank were placed in a boiling water–bath and heated at 100°C for 45 minutes The tubes were then cooled under tap water for 3 min and the absorbance was read at 538 nm using a spectrophotometer (Model Novaspec®II; Amersham Pharmacia Biotech, Uppsala, Sweden). TBARS value was calculated from the standard and was expressed as milligram malonaldehyde per kilogram of sample.

2.8.2 Determination of fluorescence shift

Both products were analysed in triplicate at different time intervals to measure the shift in fluorescence as means to assess lipid oxidation using the method of Aubourg et al. (1997).

2.8.2.1 Method

A 0.2 g solution of propyl gallate (PG) per litre of methanol was first prepared. Five grams of slightly thawed meat (or 1/3 of fat and 2/3 of muscle meat in case of bacon) was finely ground and weighed in a 50 mL centrifuge tube and 5 mL chloroform was added. Ten millilitre of PG-methanol solution and 4 mL of deionised water was subsequently added to provide a 1:2:1.8 chloroform:methanol:water ratio. The tube was placed in ice/water and the mixture was homogenised for 20 seconds at 13,500 rpm using an Ultraturrax homogeniser (Model T25; dispersing element S25 N-18G, Janke & Kunkel, IKA

Werke, Staufen, Germany). Chloroform (5 mL) and water (5 mL) were then added to provide a 2:2:1.8 chloroform:methanol:water ratio. The tubes were capped and centrifuged at 4000 rpm for 25 min at 15°C using a Sorvall® Du Pont centrifuge (Model RT7, rotor RTH-750; Sorvall Inc., Newtown, Connecticut, USA). The aqueous layer was removed by aspiration with a Pasteur pipette then the organic layer was carefully transferred to another 50 mL centrifuge tube. The remaining organic phase was washed by adding 5 mL of 0.5% sodium chloride. The washing step was repeated with 5 mL of distilled water. Centrifugation at 4000 rpm for 10 min at 15°C was used between wash. The sample was then evaporated to dryness under nitrogen. Finally the lipid extract was made up to 5 mL using chloroform, which was used for fluorescence measurements. A standard quinine sulfate (1 μg/mL quinine sulfate in 0.05M sulfuric acid) was prepared and the fluorescence shift was measured at room temperature with a Perkin Elmer Luminescence spectrometer (Model LS 50B; Norwalk, Connecticut, USA) using a 10 mm path-length quartz cuvette at 393 nm / 463 nm and 327 nm / 415 nm wavelengthes. The fluorescence shift was expressed as $\delta F = (F3/F3st) / (F1/F1st)$, where, F3 and F1 were the fluorescence intensities of the sample at 393/463 and 327/415 nm, respectively, and F3st and F1st were the fluorescence intensities of a quinine sulfate solution (1 μg/mL quinine sulfate in 0.05M sulfuric acid) at the corresponding wavelengths.

2.9 SENSORY EVALUATION

The product was assessed by an untrained panel consisting of 10 (5 females and 5 males, age between 22 and 35 years old) postgraduate students and staff of the School of Life Science and Technology, Werribee campus of Victoria University, using a 9 point scale questionnaire to rate the rancid odour (1 - no rancid odour, 9 - extremely strong rancid odour). The sensory analysis was conducted on the same week as the lipid oxidation measurements for bacon and wiener sausages. Panellists were asked to assess each product for off-odour (rancid odour). A fresh sample and a rancid sample were presented as controls. Samples for sensory analysis were prepared by chopping 18 g of wiener sausages (or 1/3 of fat and 2/3 of muscle meat in case of bacon to keep the fat and muscle in

a constant proportion). Samples were identified by 3-digit random numbers and placed in a 100 mL Schott bottle, capped and placed in a 60°C water-bath for 30 minutes before the panellists were asked to assess the odour while the product was still warm.

2.10 STATISTICAL ANALYSIS

Data were analysed by analysis of variance using Gensat 5 program (Payne et al., 1987) to determine significant differences between dietary treatments. Data for the wieners were analysed assuming a three factor interaction can be used as error (Cochran and Cox, 1957). Significance of differences between means was determined by the method of Least Significance Differences (LDS) and Student's t-test (small sample inferences concerning the difference between two means, Mendenhall and Beaver, 1994).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 FAT AND FATTY ACID COMPOSITION

3.1.1 Fatty acid composition of pig feeds

The fatty acid composition of the pig feed is shown in Table 3.1. Statistical analyses are shown in Appendix 6.1, Tables 6.1 to 6.69. No significant differences ($P > 0.05$) were found between the control and the vitamin E supplemented diet. Similarly, the fatty acid composition of the fishmeal and fishmeal plus vitamin E supplemented feed showed no significant differences ($P > 0.05$). These results were expected as the control and the vitamin E supplemented feed were prepared with the same formulation. Diets containing fishmeal differed from those without the fishmeal by the absence of soy and dicalcium phosphate in the feed (section 2.1.2, Table 2.1). All components in the diets were balanced in order to reach the same levels of metabolisable energy and essential amino acids per kilogram of the feed (section 2.1.2, Table 2.1 and 2.2).

The fishmeal diets showed lower levels of C18:0 fatty acid and higher levels of C14:0, C16:1 *(n-7)*, C20:0, C18:4 *(n-3)*, C20:4 *(n-6)*, C22:1 *(n-9)*, C22:4 *(n-6)*, C22:5 *(n-3)* and C22:6 *(n-3)* fatty acids than the control (Table 3.1). Feeds supplemented with 5% fishmeal (equivalent to 0.3% fish oil) contained 0.5% of 22:6 (*n*-3) fatty acid. This fatty acid was absent in diets without fishmeal. The level of 22:6 (*n*-3) fatty acid in the fishmeal diet was almost one-sixth of that reported by Hertzman and co-workers (1988) for a similar fish oil level.

Feeds enriched with fishmeal have been shown to alter the fatty acid composition in pigs, especially the level of long chain *n-3* (omega -3) polyunsaturated fatty acids oxidation (Hertzman et al., 1988; Irie and Sakimoto, 1992; Morgan et al., 1992; Taugbøl, 1993; Howe et al., 1996; Leskanich et al., 1997). In the present study, diets supplemented with fishmeal showed the

presence of higher levels of total *n*-3 fatty acids than those without fishmeal. Those fatty acids included 18:4 (*n*-3), 22:5 (*n*-3) and 22:6 (*n*-3). These results are in agreement with those of Leskanich et al. (1997).

No significant differences ($P > 0.05$) were found between diets in the proportions of saturated (SAT), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Table 3.1). The ratio of MUFA plus PUFA to SAT was similar for all dietary treatments.

Table 3.1 : Fatty acid composition (% wt/wt of total fatty acids) of pig feed (n =2).

Fatty acid	**Control Diet**	**Vitamin E Supplemented Diet**	**Fishmeal Supplemented Diet**	**Fishmeal + Vitamin E Supplemented Diet**
14:0 [%]	2.05^{e}	2.06^{e}	2.29^{ef}	2.43^{f}
16:0 [%]	23.34	23.32	23.63	23.82
16:1 (*n*-7) [%]	1.87^{ef}	1.86^{e}	2.01^{f}	2.05^{ef}
18:0 [%]	13.14^{e}	12.97^{ef}	12.29^{f}	12.39^{f}
18:1 (*n*-9) [%]	31.07	30.85	29.67	29.57
18:1 (*n*-7) [%]	1.15	1.16	1.67	1.67
18:2 (*n*-6) [%]	23.08^{ef}	23.44^{e}	22.91^{ef}	22.19^{f}
18:3 (*n*-3) [%]	2.22^{ef}	2.26^{e}	2.05^{f}	2.01^{f}
20:0 [%]	0.25^{e}	0.25^{e}	0.30^{ef}	0.32^{f}
18:4 (*n*-3) [%]	0.48^{e}	0.48^{e}	0.93^{ef}	1.00^{f}
20:1 (*n*-9) [%]	0.18	0.19	0.19	0.18
20:4 (*n*-6) [%]	0.11^{e}	0.11^{e}	0.16^{f}	0.15^{f}
20:5 (*n*-3) [%]	0.13	0.14	0.14	0.12
22:1 (*n*-9) [%]	0.11^{e}	0.12^{e}	0.39^{ef}	0.62^{f}
21:5 (*n*-3) [%]	0.10	0.10	0.11	0.10
22:4 (*n*-6) [%]	0.00^{e}	0.00^{e}	0.02^{ef}	0.02^{f}
22:5 (*n*-3) [%]	0.08^{e}	0.08^{e}	0.16^{ef}	0.17^{f}
22:6 (*n*-3) [%]	0.00^{e}	0.00^{e}	0.45^{f}	0.54^{f}
SAT [a] [%]	38.79	38.59	38.51	38.96
MUFA [b] [%]	34.38	34.17	33.93	34.10
PUFA [c] [%]	26.22	26.62	26.93	26.33
(M+P)/S [d]	1.56	1.58	1.58	1.55
Total (*n*-3) [%]	3.02^{e}	3.06^{e}	3.84^{ef}	3.95^{f}

[a] SAT, total weight percentage of 14:0, 16:0, 18:0, and 20:0

[b] MUFA, total weight percentage of 16:1 (*n*-7), 18:1 (*n*-9), 18:1 (*n*-7), 20:1 (*n*-9) and 22:1 (*n*-9)

[c] PUFA, total weight percentage of 18:2 (*n*-6), 18:3 (*n*-3), 18:4 (*n*-3), 20:4 (*n*-6), 20:5 (*n*-3), 21:5 (*n*-3), 22:4 (*n*-6), 22:5 (*n*-3), and 22:6 (*n*-3)

[d] (M+P)/S, ratio of (MUFA +PUFA) / SAT

[e, f] For a given fatty acid, means with different superscript differ ($P < 0.05$); means without superscript are not significantly different ($P > 0.05$).

3.1.2 Backfat thickness of pigs

The backfat thickness of pigs (P2) including skin was measured at the 13th or the last real rib, 65 mm from the mid-line, as part of the routine of the commercial abattoir (Hurstbridge Abattoirs (Aust) Pty Ltd, Hurstbridge, Victoria, Australia) where the pigs were slaughtered. Table 3.2 shows the P2 values of pig backfat. Statistical analyses are shown in Appendix 6.2, Tables 6.70 to 6.72. No significant ($P > 0.05$) differences were observed between backfat thickness of all pigs.

Table 3.2 : Backfat thickness (P2) in pigs.

Animal number	Diet	P2 (mm)	Average P2 (mm), n =3
1	Control	23.6	
2	Control	12.8	18.3 [a]
3	Control	18.4	
4	Vitamin E supplemented diet	11.6	
5	Vitamin E supplemented diet	16.0	13.1[a]
6	Vitamin E supplemented diet	11.6	
7	Fishmeal supplemented diet	16.8	
8	Fishmeal supplemented diet	15.2	15.2[a]
9	Fishmeal supplemented diet	13.6	
10	Fishmeal + vitamin E supplemented diet	11.2	
11	Fishmeal + vitamin E supplemented diet	16.0	15.7[a]
12	Fishmeal + vitamin E supplemented diet	20.0	

[a,] Means in column followed by the same letter are not significantly different ($P > 0.05$).

3.1.3 Fatty acid composition of bacon fat

The fatty acid composition of bacon fat is shown in Table 3.3. Statistical analyses are shown in Appendix 6.3, Tables 6.73 to 6.91. No significant differences ($P > 0.05$) were observed for C14:0, C16:1, C18:0, C18:1 *(n-7)*, C20:1 *(n-9)*, and C22:5 *(n-3)* fatty acids. Bacon manufactured from pigs fed a diet supplemented with fishmeal showed a significant ($P < 0.05$) decrease in the level of C18:2 *(n-6)*, C18:*3 (n-3)*, C20:0, C20:3 *(n-6)* and C22:4 *(n-6)* fatty acids. The presence of fishmeal was also found to significantly increase C18:1 *(n-9)* ($P < 0.05$) and C22:6 *(n-3)* ($P < 0.001$) fatty acids. The level of 22:6 (*n*-3) fatty acid obtained in bacon fat (Table 3.3) was much lower than that found by Hertzman et al. (1988) for a similar dietary fish oil content.

No significant changes ($P > 0.05$) were found in the proportions of SAT and MUFAs of the treatment groups and for the ratio of unsaturated (MUFA and PUFA) to SAT fatty acids. However, the bacon produced from pigs on a diet supplemented with vitamin E was found to have a slightly higher ratio. Hence, the bacon produced from pigs on the diet supplemented with vitamin E (with or without fishmeal supplementation) appear to have higher levels of total PUFAs ($P <0.05$) than those containing basal vitamin E. A similar trend was observed by Isabel and co-workers (1999a,b). This change in fatty acid composition could have adverse effect in the rate of lipid oxidation in the processed product as more unsaturation in the fat leads to increased lipid oxidation.

Table 3.3 : Fatty acid composition (% wt/wt of total fatty acids) in bacon fat (n=3 animals/treatment, 3 replicates/sample).

Fatty acid	Control Diet	Vitamin E Supplemented Diet	Fishmeal Supplemented Diet	Fishmeal + Vitamin E Supplemented Diet
14:0 [%]	1.46	1.41	1.38	1.61
16:0 [%]	24.78[e]	23.25[ef]	24.59[efg]	25.48[eg]
16:1 (*n*-7) [%]	3.07	2.60	3.14	3.65
18:0 [%]	11.82	11.05	11.85	11.07
18:1 (*n*-9) [%]	45.08[e]	45.96[ef]	46.06[efg]	44.60[eg]
18:1 (*n*-7) [%]	2.74	2.66	2.76	2.83
18:2 (*n*-6) [%]	7.51[e]	9.35[f]	6.74[e]	7.33[e]
18:3 (*n*-3) [%]	0.56[e]	0.67[ef]	0.48[eg]	0.51[eg]
20:0 [%]	0.32[ef]	0.39[e]	0.29[ef]	0.31[ef]
20:1 (*n*-9) [%]	1.02	0.93	0.99	0.90
20:3 (*n*-6) [%]	0.09[e]	0.10[ef]	0.08[efg]	0.07[eg]
20:4 (*n*-6) [%]	0.19[e]	0.27[f]	0.19[e]	0.18[e]
22:4 (*n*-6) [%]	0.08[e]	0.08[ef]	0.06[ef]	0.05[f]
22:5 (*n*-3) [%]	0.10	0.13	0.13	0.11
22:6 (*n*-3) [%]	0.00[e]	0.00[e]	0.10[f]	0.08[f]
SAT[a] [%]	38.37	36.10	38.10	38.46
MUFA[b] [%]	51.90	52.15	52.96	51.97
PUFA[c] [%]	8.58[e]	10.67[f]	7.78[e]	8.33[e]
(M+P)/S[d]	1.59	1.74	1.59	1.57

[a] SAT, total weight percentage of 14:0, 16:0, 18:0, and 20:0

[b] MUFA, total weight percentage of 16:1 (*n*-7), 18:1 (*n*-9), 18:1 (*n*-7), 20:1 (*n*-9) and 22:1 (*n*-9)

[c] PUFA, total weight percentage of 18:2 (*n*-6), 18:3 (*n*-3), 18:4 (*n*-3), 20:4 (*n*-6), 20:5 (*n*-3), 21:5 (*n*-3), 22:4 (*n*-6), 22:5 (*n*-3), and 22:6 (*n*-3)

[d] (M+P)/S, ratio of (MUFA +PUFA) / SAT

[e, f, g] For a given fatty acid, means with different superscript differ ($P < 0.05$); means without superscript are not significantly different ($P > 0.05$).

3.2 VITAMIN E SUPPLEMENTATION

The average α-tocopherol levels in bacon (for bacon fat and bacon loin muscle) obtained from pigs fed the different experimental diets are shown in Table 3.4. Statistical analyses are shown in Appendix 6.4, Tables 6.92 to 6.93. The level of vitamin E was higher in the fat than in the muscle. Dietary α-tocopherol produced a significant increase in vitamin E content of both bacon fat ($P < 0.001$) and bacon muscle ($P < 0.001$).

The α-tocopherol levels in bacon fat obtained from pigs fed vitamin E supplemented diets increased from 8.0-10.6 μg/g (diet containing basal vitamin E supplement) to 24.3-29.5 μg/g (diet with vitamin E supplementation). The average α-tocopherol levels in bacon fat produced from pigs fed the higher dietary vitamin E level was approximately 3 times higher compared to the level in bacon of the pigs fed the diet containing basal vitamin E.

The α-tocopherol levels in bacon loin muscle increased from 2.2-3.0 μg/g (diet containing basal vitamin E supplement) to 5.0-5.4 μg/g (diet with vitamin E supplementation). The average α-tocopherol levels in bacon loin muscle produced from pigs fed the higher dietary vitamin E level was approximately 2 times higher compared to the level in bacon of the pigs fed the diet containing basal vitamin E.

Fishmeal in the diet did not affect the levels of α-tocopherol for either bacon fat ($P > 0.05$) or bacon loin muscle ($P > 0.05$) but there was a significant interaction ($P < 0.05$) between dietary fishmeal and vitamin E level for the fat. This interaction was such that the vitamin E level in fat from pigs fed vitamin E and fishmeal was higher than in fat from pigs fed vitamin E and no fishmeal (29.5 vs 24.3 μg/g). There was no effect ($p > 0.05$) of fishmeal on vitamin E level in fat from pigs on diets that were not supplemented with vitamin E.

The results of the present study are in agreement with previous reports which demonstrated that the rate of accumulation of α-tocopherol in fat and muscle of pigs was dependent upon the concentration of α-tocopherol in the feed (Ashgar et al., 1991; Monahan et al., 1992b; Morrissey et al., 1996; Isabel et al., 1999a,b; Flaschowsky, 2000).

Table 3.4 : Mean α-tocopherol concentration (μg/g sample) in bacon fat and loin muscle processed with or without liquid smoke. (n=3 animals/treatment, 3 replicates/sample).

Tissue	Diet without vitamin E		Diet with vitamin E		SED [a]	P-Values		
	Diet without fishmeal [μg/g]	Diet with fishmeal [μg/g]	Diet without fishmeal [μg/g]	Diet with fishmeal [μg/g]		Vitamin E effect	Fishmeal effect	Vitamin E – fishmeal interaction
Fat	10.6 [b]	8.0 [b]	24.3 [c]	29.5 [d]	1.69	0.000010 ***	0.888	0.0042 **
Muscle	3.0 [b]	2.2 [b]	5.0 [c]	5.4 [c]	0.32	0.000025 ***	0.404	0.033 *

[a] SED, standard errors of differences of means.

[b, c, d,] Means in the same row followed by the same letter are not significantly different

($LSD_{0.05}$ = 4.137 for fat, $LSD_{0.05}$ = 0.832 for muscle).

3.3 BACON

3.3.1 Effect of wood smoke and a combination of liquid and wood smoke

3.3.1.1 Effect of treatments with smoke on TBARS

The formation of TBARS in bacon processed with or without liquid smoke and stored for 16 weeks at –20°C is shown in Figure 3.1. A summary of data is presented in Appendix 6.5.1, Table 6.94. Statistical analyses are shown in Appendix 6.5.2, Tables 6.105 to 6.111. Bacon processed with wood smoke exhibited higher susceptibility to lipid oxidation than that processed with a combination of liquid and wood smoke. The level of TBARS in bacon processed with wood smoke constantly increased from the initial level up to 8 weeks of frozen storage, then markedly increased up to 16 weeks of frozen storage. A similar trend was observed for bacon processed with a combination of liquid and wood smoke but the TBARS levels were significantly lower (0.11 mg MA/kg sample at production to 1.20 mg MA/kg sample at 16 week of frozen storage). The two processes started to present significant differences ($P < 0.001$) in levels of TBARS after 4 weeks of frozen storage (0.33 mg MA/kg sample for the bacon processed with liquid smoke vs 0.65 mg MA/kg sample for the bacon processed only with wood smoke). Throughout the 16 weeks of frozen storage, TBARS values for bacon processed with wood smoke remained significantly higher ($P < 0.05$ up to 8 weeks; $P < 0.001$ from 10 weeks onwards) than those processed with a combination of liquid and wood smoke.

Freezing bacon has been shown to increase the risk of rancidity, apparently because freezing increases the concentration of salt in the unfrozen liquor with the salt acting as a pro-oxidant (Sheard et al., 2000). From these observations, bacon processed with a combination of liquid and wood smoke was found to be more effective in reducing lipid oxidation than bacon processed only with wood smoke.

3.3.1.2 Effect of treatments with smoke on fluorescence shift

The fluorescence shift data of bacon processed with or without liquid smoke and stored for 16 weeks storage at –20°C is shown in Figure 3.2. A summary of the data is presented in Appendix 6.5.1, Table 6.95. The statistical analyses are shown in Appendix 6.5.2, Tables 6.112 to 6.118. As for TBARS levels, bacon processed with wood smoke had a higher susceptibility to lipid oxidation than that processed with a combination of liquid and wood smoke. The two treatment groups started to present significant differences ($P < 0.001$) in fluorescence shift after 4 weeks of frozen storage. At production, bacon processed with wood smoke had a fluorescence shift level of 3.1, while that processed with a combination of liquid and wood smoke had a fluorescence shift value of 4.0. After 4 weeks of frozen storage, the values increased to 8.2 and 5.2, respectively, for bacon processed with wood smoke and bacon processed with a combination of liquid and wood smoke. Both products showed a constant increase in fluorescence shift values and were significantly different ($P < 0.001$) throughout the frozen storage. The fluorescence shift value at 16 weeks of frozen storage was 13.1 for bacon processed with wood smoke and 8.6 for bacon processed with a combination of liquid and wood smoke.

A fluorescence shift towards higher wavelength maxima was reported as a result of increasing lipid oxidation with time (Aubourg et al., 1997, 1999). The fluorescence shift was due to lipid oxidation products such as peroxides and carbonyls that form interaction compounds, which have fluorescent properties. As for TBARS, the fluorescence shift analysis showed that bacon processed with a combination of liquid and wood smoke was found to be more effective in reducing lipid oxidation than bacon processed only with wood smoke.

3.3.1.3 Interpretation of results on smoke treatments

Maga (1988) and Potthast (1993) demonstrated that liquid smoke protected meat products against lipid oxidation in the same way as wood smoke. Furthermore, liquid smoke presented a similar stability against microorganisms as wood smoke. Maga (1988) reported that fish smoked traditionally with wood smoke contained 5 to 8 times more phenolic substances as compared fish treated

with liquid smoke only. In the present study, TBARS and fluorescence analyses showed that a combination of liquid and wood smoke was more effective in retarding lipid oxidation than wood smoke alone. This could be due to the presence of higher concentrations of phenolic compounds, some phenols being present in the liquid smoke combined with those present in the wood smoking process.

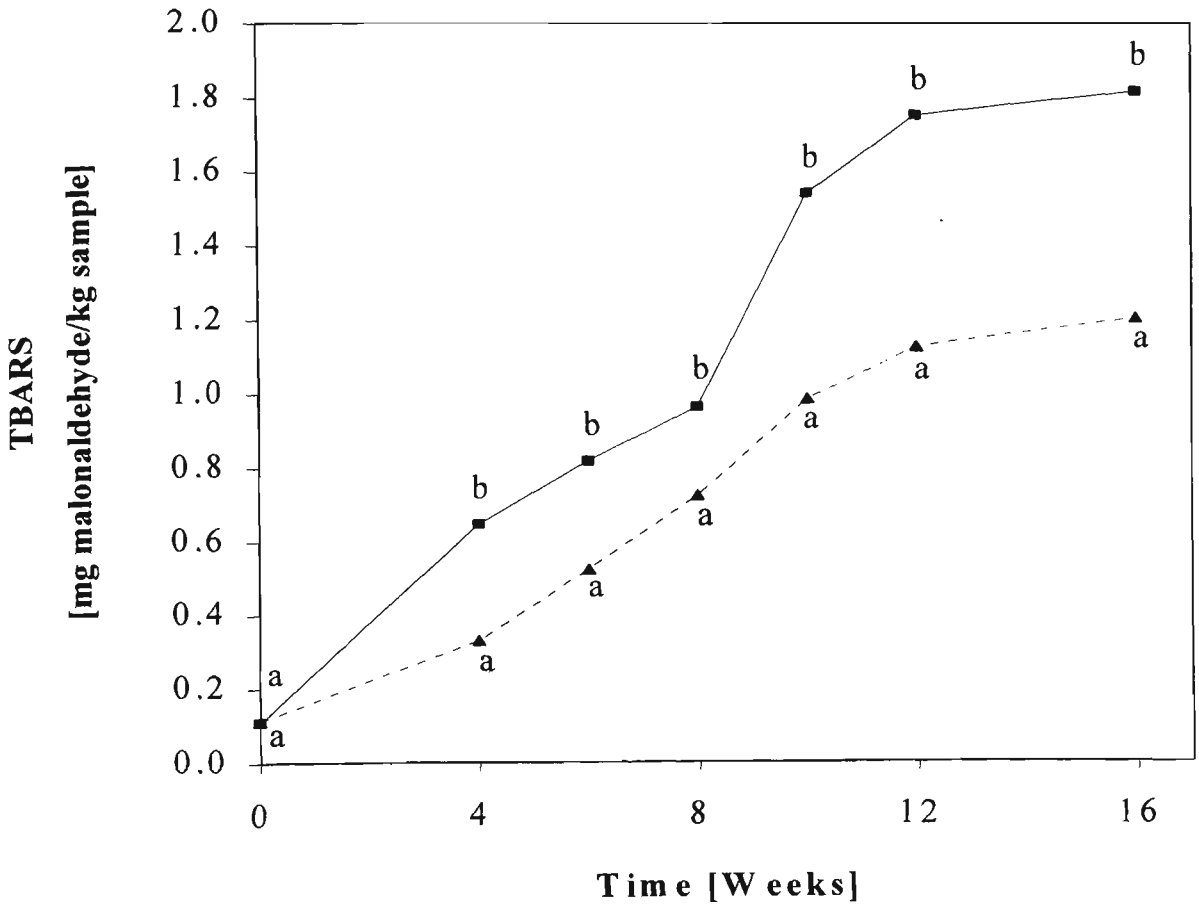

Figure 3.1: The effect of liquid smoke on formation of TBARS in bacon during storage at –20°C (n = 12).

Bacon processed with wood smoke (——■——)

Bacon processed with liquid + wood smoke (----------▲----------)

[a,b] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 0.00919 week 0, $LSD_{0.05}$ = 0.150 week 4, $LSD_{0.05}$ = 0.244 week 6, $LSD_{0.05}$ = 0.163 week 8, $LSD_{0.05}$ = 0.232 week 10, $LSD_{0.05}$ = 0.116 week 12, $LSD_{0.05}$ = 0.0747 week 16).

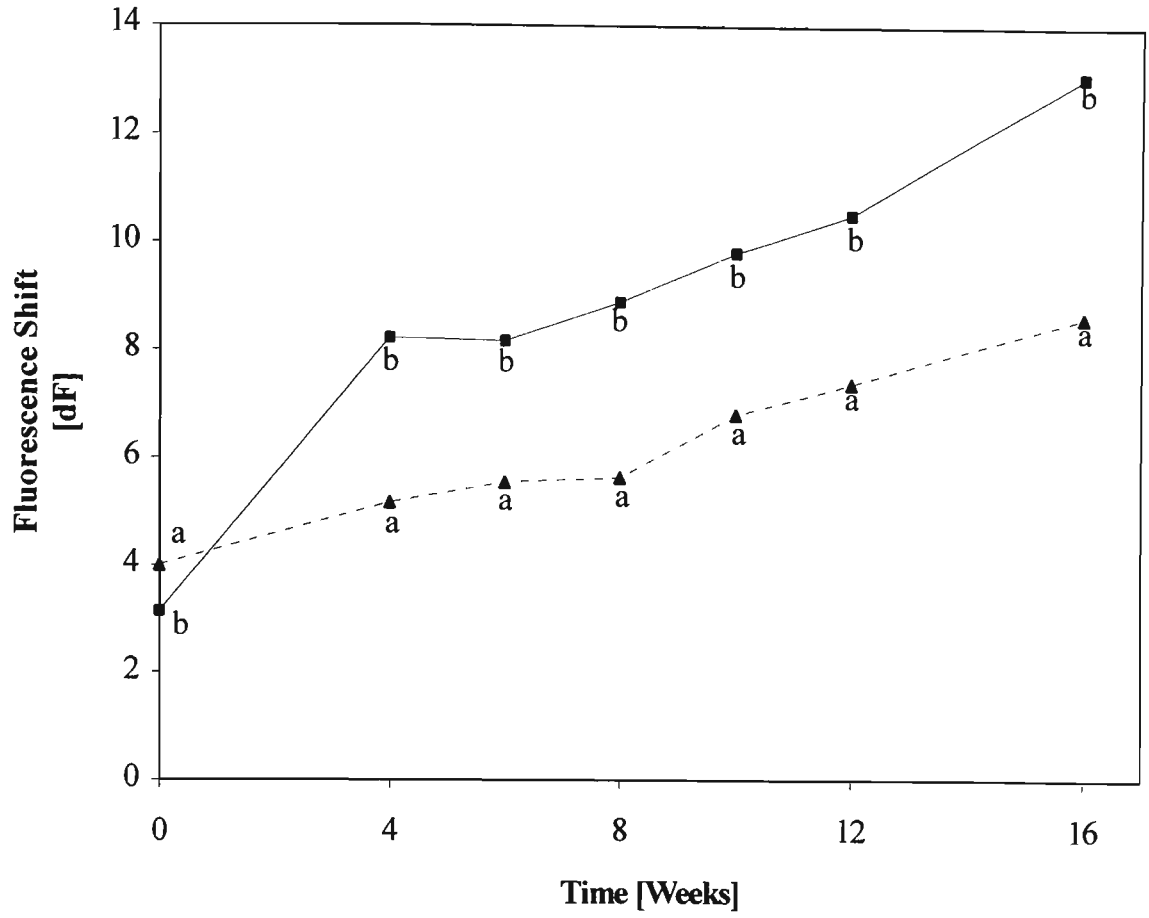

Figure 3.2: The effect of liquid smoke on fluorescence shift in bacon during storage at –20°C (n = 12).

Bacon processed with wood smoke (———■———)

Bacon processed with liquid + wood smoke (----------▲----------)

[a,b] Means in same week followed by the same letter are not significantly different (LSD $_{0.05}$ = 0.181 week 0, LSD $_{0.05}$ = 1.120 week 4, LSD $_{0.05}$ = 0.813 week 6, LSD $_{0.05}$ = 1.558 week 8, LSD $_{0.05}$ = 1.605 week 10, LSD $_{0.05}$ = 1.264 week 12, LSD $_{0.05}$ = 1.570 week 16).

3.3.2 Effect of vitamin E and treatments with smoke

3.3.2.1 Effect of vitamin E and treatments with smoke on TBARS

The formation of TBARS in bacon produced from pigs fed a supplement of 200 mg α-tocopheryl acetate per kilogram of feed, processed with or without liquid smoke and stored for 16 weeks at –20°C is shown in Figure 3.3. A summary of data is presented in Appendix 6.5.1, Table 6.96. Statistical analyses are shown in Appendix 6.5.2, Tables 6.105 to 6.111. The TBARS level of all treatment groups at production was 0.11 mg MA/kg sample. Bacon processed with wood smoke, whether supplemented with vitamin E or not, displayed a higher susceptibility to lipid oxidation ($P < 0.001$) than bacon processed with a combination of liquid and wood smoke with or without vitamin E supplementation.

Bacon processed with wood smoke and supplemented with vitamin E showed significantly lower ($P < 0.05$) TBARS levels for up to 6 weeks of frozen storage than its counterpart processed without vitamin E. Beyond this time of storage, vitamin E had no effect in reducing lipid oxidation and no significant differences ($P > 0.05$) in the levels of TBARS were observed between the product containing vitamin E or not.

Bacon processed with a combination of liquid and wood smoke and supplemented with vitamin E showed significantly lower ($P < 0.001$) levels of TBARS (0.87 mg MA/kg sample after 16 weeks of frozen storage) all throughout the 16 weeks of frozen storage than its counterpart without vitamin E (1.53 mg MA/kg sample after 16 weeks of frozen storage) and the products processed with wood smoke only (1.82 mg MA/kg sample after 16 weeks of frozen storage).

Producing bacon from pigs supplemented with vitamin E (200 mg/kg feed) and processing with a combination of liquid and wood smoke was found to be a very effective means of reducing lipid oxidation.

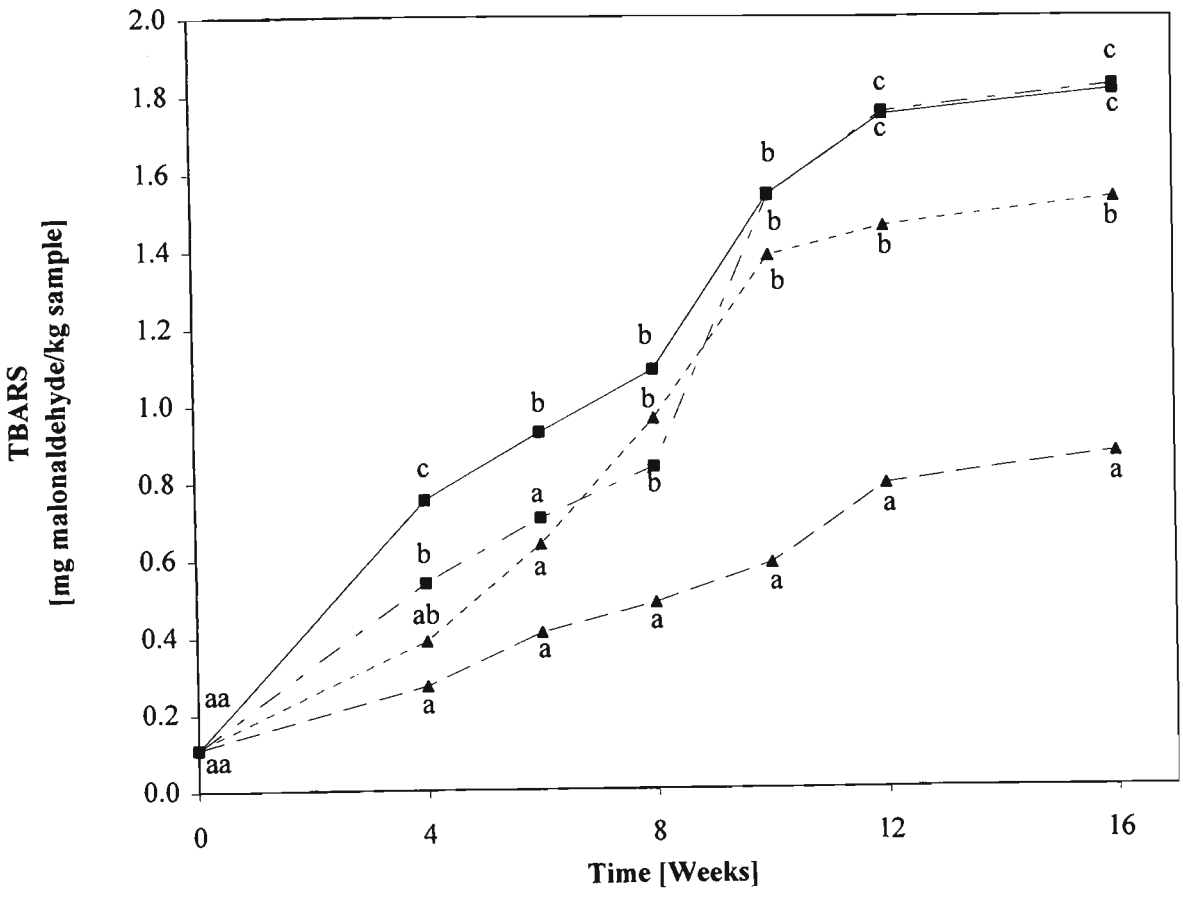

Figure 3.3: The effect of diet with vitamin E and treatments with smoke on formation of TBARS in bacon during storage at –20°C (n = 6).

Diets containing basal vitamin E (10 mg/kg) – Wood smoke (——■——)

Diets supplemented with 200 mg vitamin E – Wood smoke (– — –■— –)

Diets containing basal vitamin E (10 mg/kg) – Liquid + wood smoke (----------▲---------)

Diets supplemented with 200 mg vitamin E – Liquid + wood smoke (— — ▲— —)

[a,b,c] Means in same week followed by the same letter are not significantly different ($LSD_{0.05}$ = 0.0130, 0.0130 [d] week 0, $LSD_{0.05}$ = 0.201, 0.213 [d] week 4, $LSD_{0.05}$ = 0.232, 0.291 [d] week 6, $LSD_{0.05}$ = 0.377, 0.231 [d] week 8, $LSD_{0.05}$ = 0.267, 0.328 [d] week 10, $LSD_{0.05}$ = 0.261, 0.165 [d] week 12, $LSD_{0.05}$ = 0.122, 0.106 [d] week 16)

[d] Except when comparing means with same level of vitamin E.

3.3.2.2 *Effect of vitamin E and treatments with smoke on fluorescence shift*

The fluorescence shift in bacon produced from pigs fed a supplement of 200 mg α-tocopheryl acetate per kilogram of feed, processed with or without liquid smoke and stored during 16 weeks at –20°C is presented in Figure 3.4. A summary of data is presented in Appendix 6.5.1, Table 6.97. Statistical analyses are shown in Appendix 6.5.2, Tables 6.112 to 6.118. Similar to TBARS values, bacon processed with wood smoke, independently of vitamin E supplementation, presented significant higher ($P < 0.001$) susceptibility to lipid oxidation than its counterpart processed with a combination of liquid and wood smoke. The fluorescence shift of all treatment groups steadily increased with time of frozen storage. Fluorescence shifts at production (week 0) varied between 2.96 and 4.10; these values were found to be slightly higher for bacon processed with a combination of liquid and wood smoke (3.90-4.10) compared to bacon processed with wood smoke only (2.96-3.33).

It was observed that bacon processed with wood smoke and supplemented with vitamin E showed significant lower ($P < 0.01$) fluorescence shift (12.05 after 16 weeks of frozen storage) than its counterpart processed without vitamin E (14.13) after 16 weeks of frozen storage. Similarly, bacon processed with a combination of liquid and wood smoke and supplemented with vitamin E showed significant lower ($P < 0.01$) fluorescence shift (7.04 after 16 weeks of frozen storage) than its equivalent without vitamin E (10.22 after 16 weeks of frozen storage).

From these results, a similar observation to TBARS can be made. The oxidative stability of the bacon increased when produced from pigs supplemented with vitamin E (200 mg/kg feed) and processed with a combination of liquid and wood smoke.

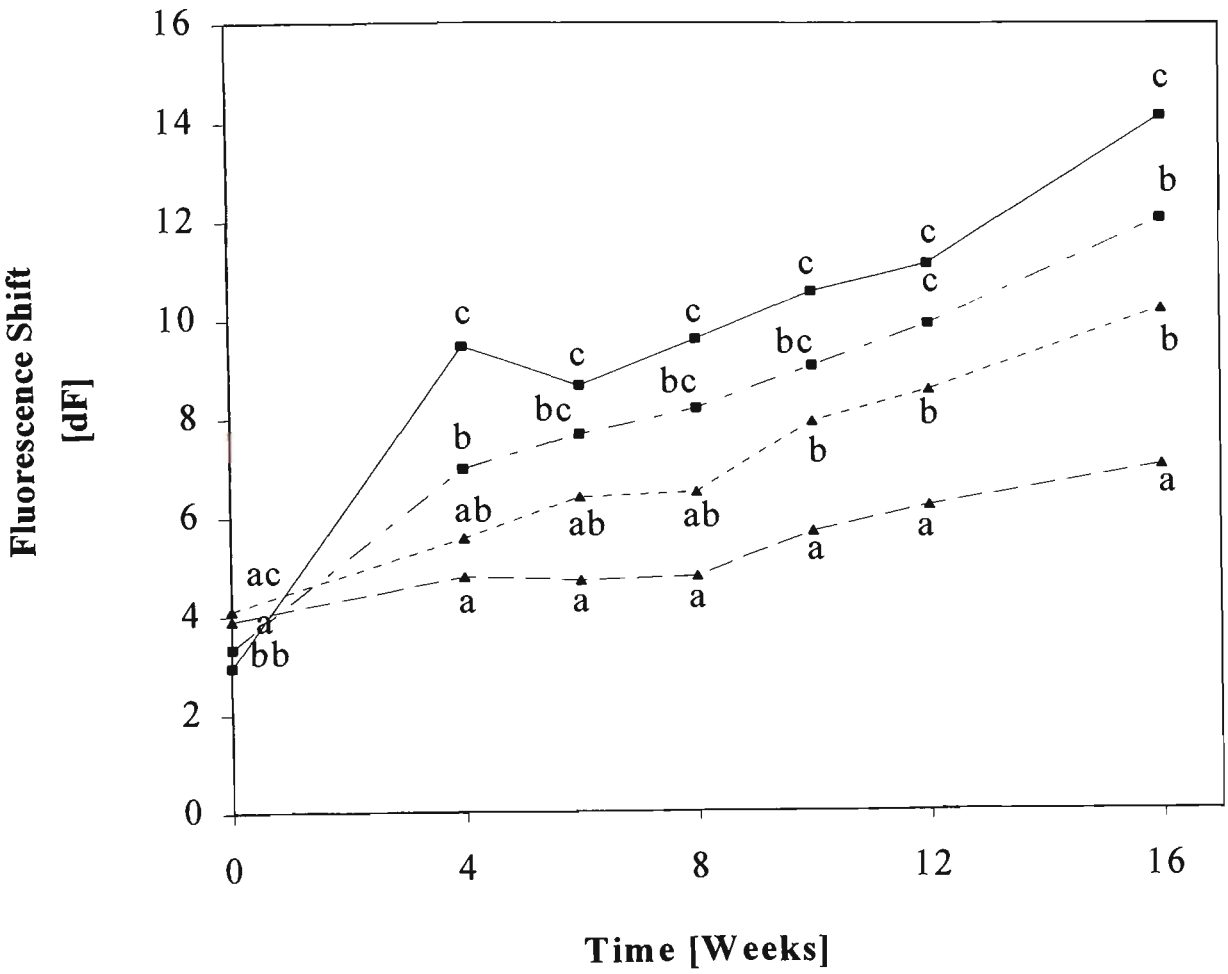

Figure 3.4: The effect of diet with vitamin E and treatments with smoke on fluorescence shift in bacon during storage at –20°C (n = 6).

Diets containing basal vitamin E (10 mg/kg) – Wood smoke (——■——)

Diets supplemented with 200 mg vitamin E – Wood smoke (– — —■— –)

Diets containing basal vitamin E (10 mg/kg) – Liquid + wood smoke (----------▲---------)

Diets supplemented with 200 mg vitamin E – Liquid + wood smoke (— —▲— —)

[a,b,c,] Means in same week followed by the same letter are not significantly different (LSD $_{0.05}$ = 0.503, 0.256 [d] week 0, LSD $_{0.05}$ = 1.729, 1.584 [d] week 4, LSD $_{0.05}$ = 1.726, 1.150[d] week 6, LSD $_{0.05}$ = 2.673, 2.204 [d] week 8, LSD $_{0.05}$ = 2.212, 2.270 [d] week 10, LSD $_{0.05}$ = 1.294, 1.635 [d] week 12, LSD $_{0.05}$ = 1.866, 2.220 [d] week 16)

[d] Except when comparing means with same level of vitamin E.

3.3.2.3 Effect of vitamin E and treatments with smoke on sensory evaluation of bacon

Table 3.5 shows the effect of vitamin E and treatment with smoke on the sensory characteristics of bacon. Statistical analyses are shown in Appendix 6.5.2, Tables 6.119 to 6.124 and Appendix 6.5.3, Tables 6.125 to 6.148. No significant differences ($P > 0.05$) in sensory characteristics were found between bacon processed with wood smoke and liquid and wood smoke with or without vitamin E. The sensory scores appear to increase with time and ranged from 1.85 to 4.71 (out of a maximum score of 9) during the 16 weeks of frozen storage. The panellists detected slight rancid odours after 6 weeks of frozen storage in all the treatment groups.

The reason that there was no significant differences between treatments even though significant differences were shown with TBARS and fluorescence shift could be that the level of rancidity was at the threshold of detection for the panellists. For example, Ke and co-workers (1984) reported that in fish flesh, TBARS values less than 1.76 mg MA/kg was indicative of not being rancid, while TBARS values between 1.98 and 4.40 mg MA/kg was slightly rancid but acceptable. TBARS values greater than 4.62 mg MA/kg were considered rancid and unacceptable. Tarladgis et al. (1960) stated that the threshold range of TBARS values for detecting off-odours in ground pork was approximately 0.5 - 1.0, while, Greene and Cumuze (1981) demonstrated that an untrained panel could note a difference in oxidised odour around a TBARS value of 0.6 - 2.0 in cooked ground beef. Melton (1983) reported that oxidised flavours and odours were detectable at TBARS value of 0.3 - 1.0 in beef or pork.

However, these ranges of TBARS values should not be considered as a general reference for thresholds of rancid odour in meats because TBARS values are influenced by factors including animal species, dietary status and age of animal prior to slaughtering, whether the meat is raw or cooked and the type of TBA methods used for the analyses.

Table 3.5: The effect of vitamin E and treatments with smoke on sensory evaluation of bacon during storage at –20°C (n = 6).

Storage time in weeks	Wood Smoke		Liquid + Wood Smoke		SED[a]	
	Diet without vitamin E	Diet with vitamin E	Diet without vitamin E	Diet with vitamin E	Same level of vitamin E	Different levels of vitamin E
0	[b]2.38[c]	2.40[c]	2.23[c]	1.85[c]	0.344	0.345
4	2.43[c]	2.09[c]	2.52[c]	2.20[cd]	0.273	0.288
6	3.80[d]	3.40[d]	3.50[d]	2.63[d]	0.327	0.392
10	3.60[d]	3.90[d]	4.07[de]	3.85[e]	0.399	0.376
12	3.35[d]	4.71[d]	4.04[e]	3.94[e]	0.490	0.670
16	3.24[d]	3.87[d]	4.04[de]	3.35[e]	0.439	0.367

[a] SED; Standard errors of differences of means

[b] Sensory score, where 1 represents not rancid bacon and 9 represents extremely rancid bacon

[c, d, e,] Means in the same column with different superscripts differ ($P < 0.05$).

3.3.2.4 Interpretation of results on the effect of vitamin E and treatment with smoke

The results of the present investigation on the effect of vitamin E and treatments with smoke based on TBARS and fluorescence shift values (Figures 3.3 and 3.4), it appears that bacon produced from pigs supplemented with vitamin E (200 mg/kg feed) had a higher oxidative stability than that without the vitamin E supplementation. These results are in agreement with those of De Winne and Dirinck (1997) and Isabel et al. (1999a,b) who have reported that hams from animals supplemented with vitamin E were more stable and less susceptible to lipid oxidation than those without any vitamin E supplementation. A protective effect of vitamin E against lipid oxidation was also reported by Buckley et al. (1995); Monahan et al. (1990) and Morrissey et al. (1996) in raw pork muscle and by Monahan et al., (1990) and Leskanich et al., (1997) in cooked pork muscle. Furthermore, producing bacon from pigs supplemented with vitamin E (200 mg/kg feed) and processing with a combination of liquid and wood smoke was

found to be a very effective way means in reducing lipid oxidation. It seems that the combination vitamin E and liquid + wood smoke works synergistically in reducing lipid oxidation. In the present study, TBARS values did not exceed 1.82 mg MA/kg of sample (Table 6.96); therefore, rancidity was at the threshold of being detected by the panellists. Panellists did observe a slight increase of rancid odour with time but the identification of such low levels of rancidity was probably difficult for untrained panellists to perceive. However, the intensity of rancidity detected in the product by untrained panellists reflects how consumers would judge the product quality.

3.3.3 Effect of fishmeal

3.3.3.1 Effect of fishmeal on TBARS

The effect of fishmeal on the formation of TBARS in bacon produced from pigs fed 5% fishmeal and stored for 16 weeks at –20°C is shown in Figure 3.5. A summary of data is presented in Appendix 6.5.1, Table 6.99. Statistical analyses are shown in Appendix 6.5.2, Tables 6.105 to 6.111. Bacon obtained from pigs fed diets containing no fishmeal, independent of the treatment with wood smoke or liquid and wood smoke, presented lower TBARS levels compared to bacon obtained from pigs fed diets containing fishmeal. The differences were significant ($P < 0.05$) at week 4, 6 and 16 of frozen storage; however, at other time intervals of frozen storage, there were no significant differences ($P > 0.05$).

3.3.3.2 Effect of fishmeal on fluorescence shift

The effect of fishmeal supplementation on fluorescence shift in bacon stored for 16 weeks at –20°C is presented in Figure 3.6. A summary of data is presented in Appendix 6.5.1, Table 6.100. Statistical analyses are shown in Appendix 6.5.2, Tables 6.112 to 6.118. A similar oxidation pattern to TBARS appeared when using fluorescence shift. Bacon made from pigs without the fishmeal supplementation, regardless of the treatment with wood smoke or liquid and wood smoke, generally presented a smaller fluorescence shift compared to

bacon made from pigs fed the fishmeal supplementation. The differences in fluorescence shift were significant ($P < 0.05$) at 4, 6,12 and 16 weeks of frozen storage; however, at other time intervals of frozen storage there were no significant differences ($P > 0.05$).

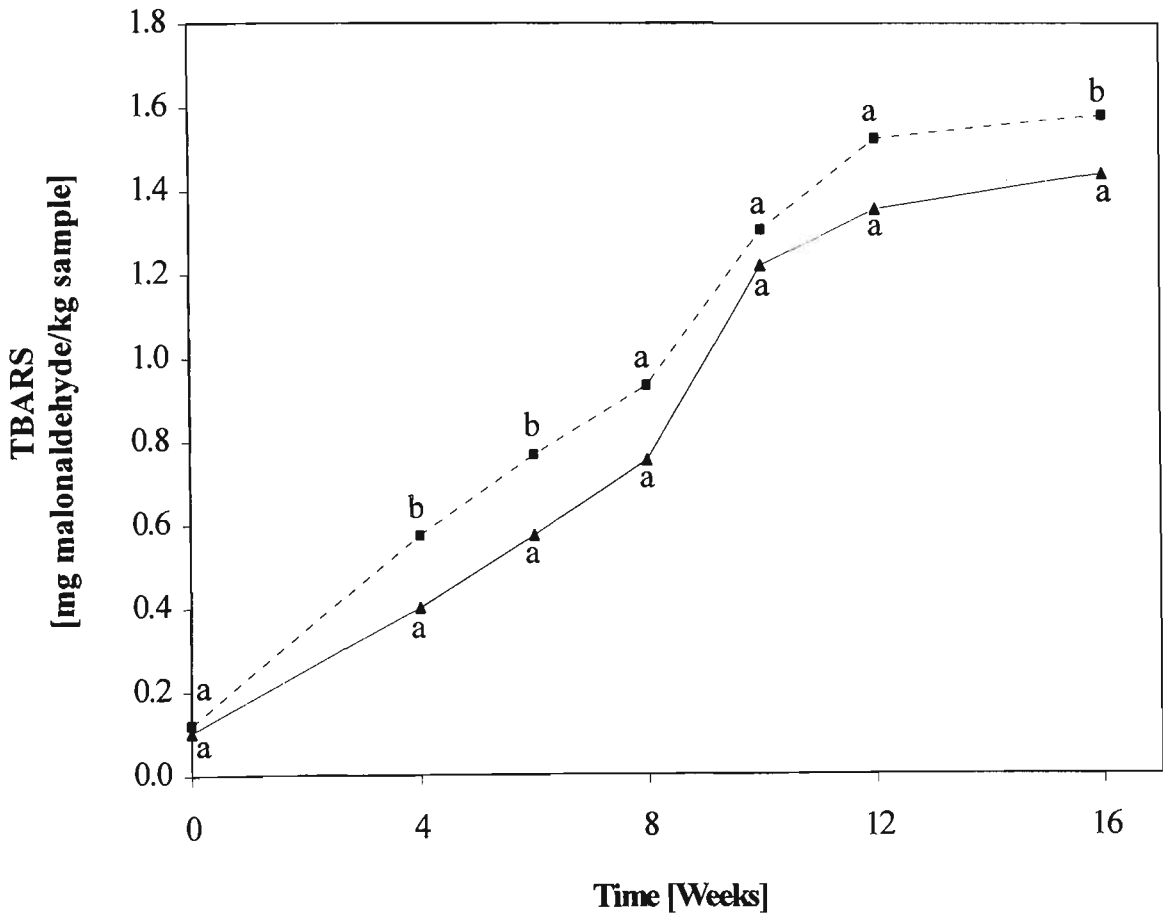

Figure 3.5: The effect of diets with fishmeal on formation of TBARS in bacon during storage at –20°C (n = 12).

Diet without fishmeal (——▲——)

Diet with 5% fishmeal (----------■----------)

[a,b] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 0.0111 week 0, $LSD_{0.05}$ = 0.164 week 4, $LSD_{0.05}$ = 0.209 week 6, $LSD_{0.05}$ = 0.367 week 8, $LSD_{0.05}$ = 0.177 week 10, $LSD_{0.05}$ = 0.253 week 12, $LSD_{0.05}$ = 0.110 week 16).

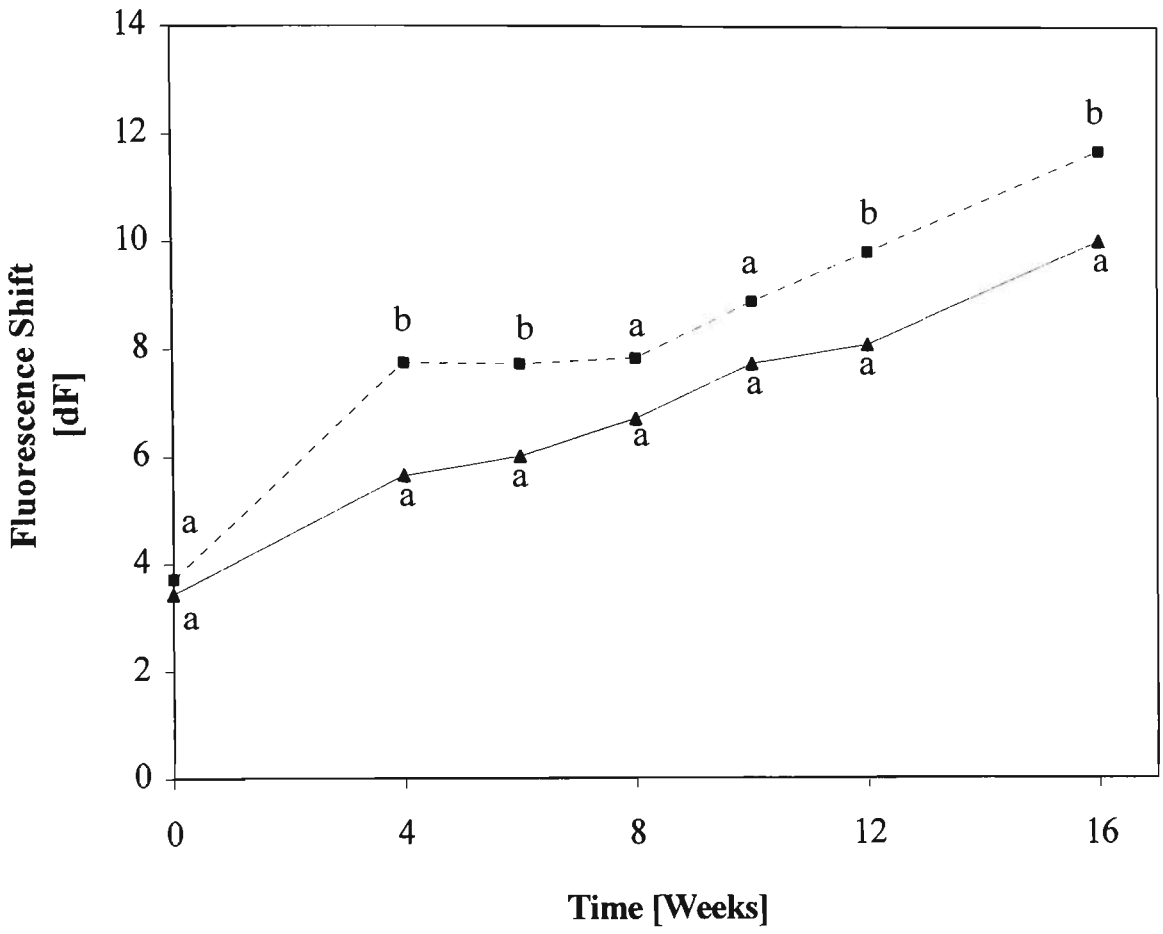

Figure 3.6: The effect of diets with fishmeal on fluorescence shift in bacon during storage at –20°C (n = 12).

Diet without fishmeal (——▲——)

Diet with 5% fishmeal (----------■----------)

[a,b] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 0.495 week 0, $LSD_{0.05}$ = 1.537 week 4, $LSD_{0.05}$ = 1.661 week 6, $LSD_{0.05}$ = 2.467 week 8, $LSD_{0.05}$ = 1.845 week 10, $LSD_{0.05}$ = 1.294 week 12, $LSD_{0.05}$ = 1.323 week 16).

3.3.3.3 Effect of fishmeal on sensory evaluation of bacon

Table 3.6 shows the effect of fishmeal on the sensory characteristics of bacon during 16 weeks of storage at -20°C. Statistical analyses are shown in Appendix 6.5.2, Tables 6.119 to 6.124 and Appendix 6.5.3, Tables 6.149 to 6.160. No significant differences ($P > 0.05$) were found between the two treatment groups. The sensory scores appear to increase with time and ranged from 2.05 to 4.37 (out of a maximum score of 9) through the 16 weeks of frozen storage. The panellists detected slight rancid odours after 6 weeks of frozen storage in both treatment groups. The untrained panellists probably found it difficult to perceive rancidity as the highest TBARS value obtained was 1.58 mg MA/kg of sample (Table 6.99), which had been considered as the threshold level for detection of rancid odour by panellists (Tarladgis et al., 1960; Greene and Cumuze, 1981; Melton, 1983; Ke et al., 1984).

Table 3.6: The effect of diets with fishmeal on sensory evaluation of bacon during storage at –20°C (n = 12).

storage time in weeks	Diet without fishmeal	Diet with fishmeal	SED [a]
0	[b] 2.38 [c]	2.05 [c]	0.244
4	2.36 [c]	2.26 [c]	0.214
6	3.58 [df]	3.08 [d]	0.317
10	4.10 [e]	3.61 [d]	0.248
12	4.37 [eg]	3.65 [d]	0.573
16	3.70 [efg]	3.55 [d]	0.197

[a] SED; Standard errors of differences of means

[b] Sensory score, where 1 represents not rancid bacon and 9 represents extremely rancid bacon

[c, d, e, f, g,] Means in the same column with different superscripts differ ($P < 0.05$).

3.3.3.4 Interpretation of results on the effect of fishmeal

The present study on the effect of dietary fishmeal, TBARS and fluorescence shift analyses (Figures 3.5 and 3.6) showed similar patterns of oxidation. The effect of dietary fishmeal supplementation on TBARS and fluorescence shift can be explained on the basis of the fatty acid composition. As described in the fatty acid composition of the bacon (Section 3.1.3), no significant changes in proportions of total SAT and total MUFAs were observed between bacon produced from the different diets (Table 3.3). Although non-significant, the total PUFAs were found to unusually decrease with the presence of fishmeal. Normally the presence of fishmeal in the diet will cause the fat to become more unsaturated, thus accelerating lipid oxidation (Hertzman et al., 1988; Irie and Sakimoto, 1992; Morgan et al., 1992; Taugbøl, 1993; Howe et al., 1996; Leskanich et al., 1997; Wood and Enser, 1997). Pigs are monogastric animals and lack the ability to saturate the fatty acids present in their diets. Therefore, the degree of saturation of porcine fat will reflect the degree of saturation of their dietary fats (Gillett, 1987). In the present study, the experimental diets did not show significant differences in the proportions of total SAT, total MUFA and total PUFAs fatty acids (Table 3.1). This was reflected in bacon fat (Table 3.3) where the total fatty acids did not differ between each other.

This may explain why there was no significant difference in the levels of TBARS at 8, 10 and 12 weeks of frozen storage between bacon containing dietary fishmeal or no fishmeal (Figures 3.5). The adverse effect of fishmeal on TBARS levels at 4, 6 and 16 weeks of frozen storage could possibly be caused by the presence of C22:6 (*n-3*) fatty acid present in fat from bacon containing dietary fishmeal but absent in the case of bacon without dietary fishmeal (Table 3.3). Due to its six double bonds, the C22:6 (*n-3*) fatty acid is more prone to oxidation than less unsaturated fatty acids and saturated fatty acids. Bacon with dietary fishmeal also had a higher proportion of higher unsaturated fatty acid (including fatty acids with 4, 5 and 6 double bonds, Table 3.3) than its counterpart without fishmeal. These fatty acids are readily oxidised and can account for the difference in TBARS values.

TBARS values did not exceed 1.58 mg MA/kg of sample (Table 6.99), therefore, rancidity was at the threshold of being detected by the panellists. Panellists did observe a slight increase of rancid odour with time but the identification of such low levels of rancidity was probably difficult for untrained panellists to perceive.

3.3.4 Combined effects of dietary fishmeal and vitamin E and bacon treatments with smoke

3.3.4.1 Effect of dietary fishmeal and vitamin E and bacon treatments with smoke on TBARS

The effect of fishmeal, vitamin E supplementation and treatments with smoke on the formation of TBARS in bacon produced from pigs fed 5% fishmeal during 16 weeks storage at –20°C is shown in Figure 3.7. A summary of data is presented in Appendix 6.5.1, Table 6.102. Statistical analyses are shown in Appendix 6.5.2, Tables 6.105 to 6.111. It was observed that bacon obtained from pigs fed diets containing fishmeal but no vitamin E supplementation and processed with wood smoke only was more prone to lipid oxidation than the other treatment groups and presented significant differences ($P < 0.05$) in TBARS after 4 weeks of frozen storage. No significant differences ($P > 0.05$) were observed between bacon obtained from pigs fed diets containing fishmeal and vitamin E supplementation and processed with wood smoke only and bacon obtained from pigs fed diets containing fishmeal but no vitamin E supplementation and processed with liquid and wood smoke. Furthermore, after 6 weeks of frozen storage, those treatment groups were not significantly different ($P > 0.05$) to bacon obtained from pigs fed diets containing fishmeal and no vitamin E supplement and processed with wood smoke only.

Bacon obtained from pigs fed diets containing fishmeal and vitamin E supplementation, independent of the treatments with wood smoke or liquid and wood smoke, consistently presented lower TBARS levels compared to bacon obtained from pigs fed diets containing fishmeal but no vitamin E

supplementation. Dietary vitamin E helped retard lipid oxidation up to 8 weeks ($P < 0.05$) (TBARS = 0.88 mg MA/kg sample) in bacon processed with wood smoke than oxidation increase to 1.81 mg MA/kg sample after 16 weeks of frozen storage. A marked reduction in lipid oxidation was observed throughout the 16 weeks of frozen storage (0.94 mg MA/kg sample after 16 weeks of frozen storage) for bacon obtained from pigs fed diets containing fishmeal and vitamin E supplementation and processed with liquid and wood smoke. A similar trend was previously mentioned in the case of bacon obtained from pigs fed diets containing with vitamin E supplementation and processed with liquid and wood smoke (Section 3.3.2.1, Figure 3.3). Dietary supplementation of vitamin E appeared to be working synergistically with a combination of liquid and wood smoke.

3.3.4.2 Effect of dietary fishmeal and vitamin E and bacon treatments with smoke on fluorescence shift

The effect of fishmeal, vitamin E supplementation and treatments with smoke on fluorescence shift values in bacon during 16 weeks storage at –20°C is presented in Figure 3.8. A summary of data is presented in Appendix 6.5.1, Table 6.103. Statistical analyses are shown in Appendix 6.5.2, Tables 6.112 to 6.118. A similar trend to TBARS appeared with fluorescence shift measurements. It was observed that bacon obtained from pigs fed diets containing fishmeal but no vitamin E supplementation and processed with wood smoke only was more prone to lipid oxidation than the other treatment groups and presented significant differences ($P < 0.05$) throughout the 16 weeks of frozen storage. No significant differences ($P > 0.05$) were observed between bacon produced from pigs fed diets containing fishmeal and vitamin E supplementation and processed with wood smoke only and bacon obtained from pigs fed diets containing fishmeal but no vitamin E supplement and processed with liquid and wood smoke.

Bacon containing dietary vitamin E supplementation, independent of the treatment with wood smoke or liquid and wood smoke, reduced lipid oxidation significantly ($P < 0.05$) as measured by TBARS throughout the 16 weeks of frozen storage compared to its counterpart without the vitamin E supplement.

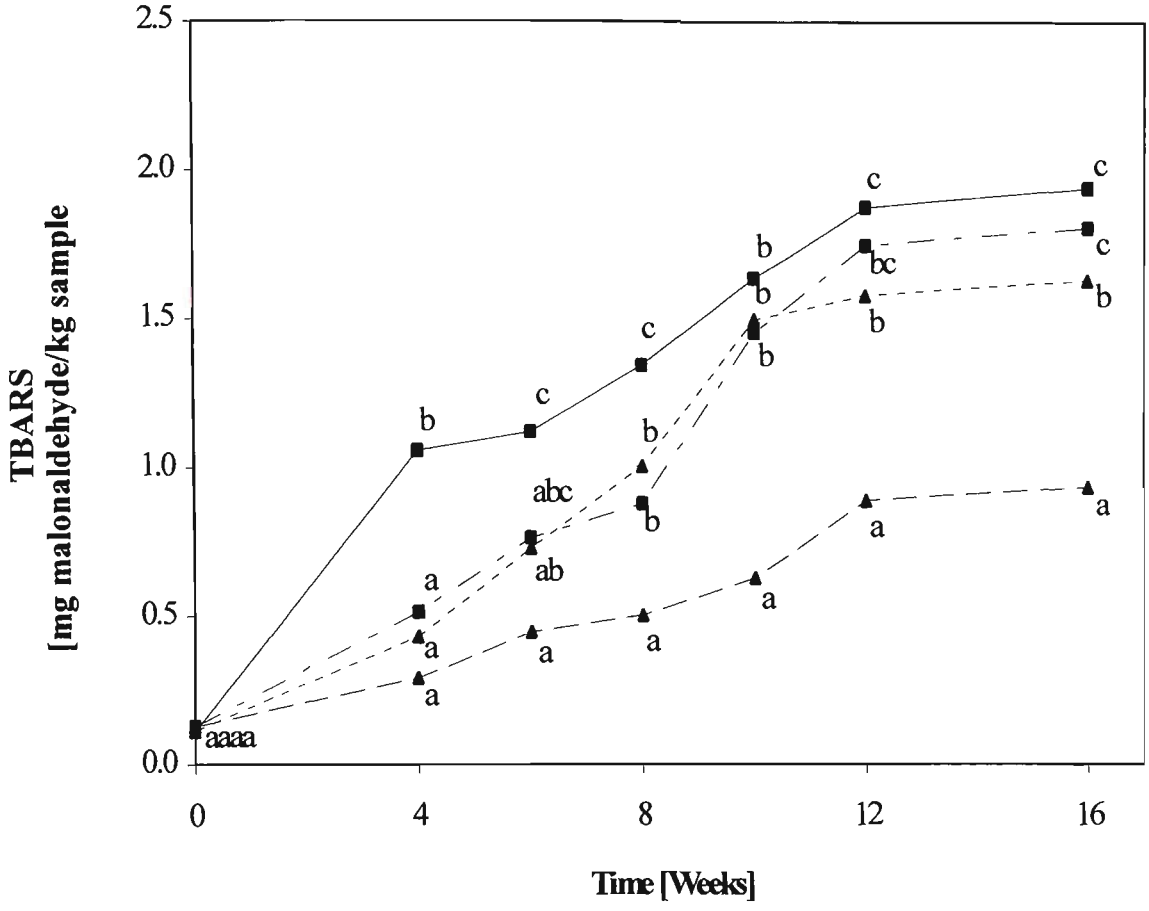

Figure 3.7: The effect of dietary fishmeal, vitamin E and treatments with smoke on formation of TBARS in bacon during storage at –20°C (n = 3).

Diets containing fishmeal and 10 mg/kg vitamin E – Wood smoke (——■——)

Diets containing fishmeal and 200 mg vitamin E – Wood smoke (– — –■ — –)

Diets containing fishmeal and 10 mg/kg vitamin E – Liquid + wood smoke (----------▲---------)

Diets containing fishmeal and 200 mg vitamin E – Liquid + wood smoke (— —▲— —)

[a,b,c,] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 0.018 week 0, $LSD_{0.05}$ = 0.301 week 4, $LSD_{0.05}$ = 0.354 week 6, $LSD_{0.05}$ = 0.327 week 8, $LSD_{0.05}$ = 0.464 week 10, $LSD_{0.05}$ = 0.233 week 12, $LSD_{0.05}$ = 0.149 week 16).

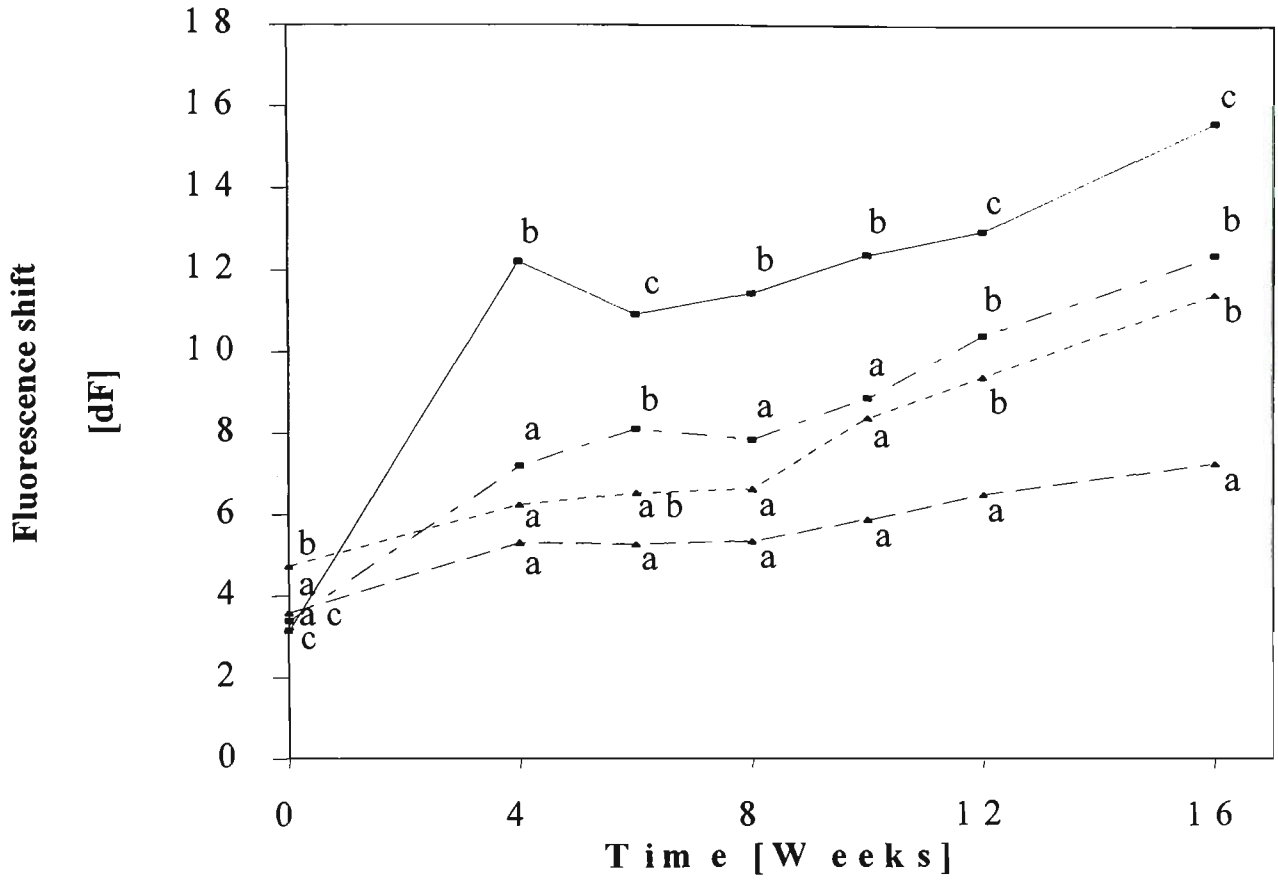

Figure 3.8: The effect of dietary fishmeal, vitamin E and treatments with smoke on fluorescence shift in bacon during storage at –20°C (n = 3).

Diets containing fishmeal and 10 mg/kg vitamin E – Wood smoke (_____■_____)

Diets containing fishmeal and 200 mg vitamin E – Wood smoke (– — –■ — –)

Diets containing fishmeal and 10 mg/kg vitamin E – Liquid + wood smoke (----------▲---------)

Diets containing fishmeal and 200 mg vitamin E – Liquid + wood smoke (— —▲— —)

[a,b,c,] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 0.361 week 0, $LSD_{0.05}$ = 2.240 week 4, $LSD_{0.05}$ = 1.626 week 6, $LSD_{0.05}$ = 3.117 week 8, $LSD_{0.05}$ = 3.211 week 10, $LSD_{0.05}$ = 2.528 week 12, $LSD_{0.05}$ = 3.139 week 16).

3.3.4.3 Effect of dietary fishmeal and vitamin E and bacon treatments with smoke on sensory evaluation of bacon

Table 3.7 shows the effect of fishmeal on the sensory characteristics of bacon during 16 weeks storage at -20°C. Statistical analyses are shown in Appendix 6.5.2, Tables 6.119 to 6.124 and Appendix 6.5.3, Tables 6.161 to 6.184. No significant differences ($P > 0.05$) were found between the treatment groups. The sensory scores appear to increase with time and ranged from 1.70 to 4.29 (out of a maximum score of 9) during the 16 weeks of frozen storage. The panellists detected slight rancid odours after 6 weeks of frozen storage in all treatment groups. The highest TBARS value obtained was 1.94 mg MA/kg of sample (Table 6.102), which has been reported as the threshold value for the detection of rancid odour through sensory evaluation (Tarladgis et al., 1960; Greene and Cumuze, 1981; Melton, 1983; Ke et al., 1984). Thus, the detection of rancidity was difficult for the untrained panellists.

Table 3.7: The effect of dietary fishmeal and vitamin E and treatments with smoke on sensory evaluation of bacon during storage at –20°C (n = 3).

Storage time in weeks	Wood smoke		Liquid + wood smoke		SED [a]
	Diet with fishmeal	Diet with fishmeal + vitamin E	Diet wit fishmeal	Diet with fishmeal + vitamin E	
0	[b]2.30 [ce]	2.03 [c]	2.13 [c]	1.73 [c]	0.244
4	2.04 [ce]	1.70 [c]	2.93 [d]	2.37 [cdef]	0.214
6	4.07 [d]	2.73 [df]	3.23 [cde]	2.30 [df]	0.317
10	3.23 [de]	3.93 [cdg]	3.90 [e]	3.37 [e]	0.248
12	3.17 [de]	4.29 [eg]	3.71 [cde]	3.42 [ef]	0.573
16	3.81 [d]	3.63 [fg]	3.85 [de]	2.89 [ef]	0.197

[a] SED; Standard errors of differences of means

[b] Sensory score, where 1 represents not rancid bacon and 9 represents extremely rancid bacon

[c, d, e, f, g,] Means in the same column with different superscripts differ ($P < 0.05$).

3.3.4.4 Interpretation of results on the effect of dietary fishmeal and vitamin E and bacon treatments with smoke

The present study on the effect of dietary fishmeal, vitamin E supplementation and treatments with smoke showed similar trends of oxidation based on TBARS and fluorescence shift values (Figures 3.7 and 3.8).

A marked reduction in lipid oxidation was observed throughout the 16 weeks of frozen storage for bacon produced from pigs fed diets containing fishmeal and vitamin E supplementation and processed with liquid and wood smoke. A similar observation was previously made in the case of bacon obtained from pigs fed diets supplemented with vitamin E and processed with liquid and wood smoke (Section 3.3.2.1, Figure 3.3), where dietary supplementation of vitamin E appeared to be working synergistically with a combination of liquid and wood smoke.

As previously mentioned, fishmeal has a tendency to oxidise faster due to the presence of C22:6 (*n-3*) fatty acid and a higher proportion of higher unsaturated fatty acid (including fatty acids with 4, 5 and 6 double bonds (Table 3.3). However, the inclusion of α-tocopherol (200 mg/kg feed) in pig feed can help counteract the negative influence of fishmeal on lipid oxidation in bacon produced from those pigs. Furthermore, processing bacon that contained dietary fishmeal and vitamin E supplementation with a combination of liquid and wood smoke retarded lipid oxidation considerably as measured by TBARS and fluorescence shift over a 16 weeks of frozen storage period.

The highest TBARS value obtained was 1.94 mg MA/kg of sample (Table 6.102), which has been regarded as the threshold value for the detection of rancid odour by panellists (Tarladgis et al., 1960; Greene and Cumuze, 1981; Melton, 1983; Ke et al., 1984). As previously reported, the untrained panellists possibly had difficulties in assessing rancidity; however, they did observe that rancidity increased slightly with time.

3.4 WIENER SAUSAGES

3.4.1 Effect of dietary treatments on TBARS

The effect of dietary treatments on TBARS in wieners is shown in Figure 3.9. A summary of data is presented in Appendix 6.6.2, Table 6.224. No significant differences ($P > 0.05$) were observed between the diets. The TBARS level declined during the first 4 weeks of frozen storage. Then gradually increased to reach a constant level of approximately 0.16 mg malonaldehyde per kilogram of wieners after 12 weeks frozen storage (Table 6.224). A similar trend was observed by Shackelford et al. (1991) in Frankfurters. It can be observed that in most cases, dietary supplementation of vitamin E presented lower but non-significant ($P > 0.05$) levels of TBARS, suggesting vitamin E helped reduce oxidation. Dietary vitamin E supplementation was effective in stabilising muscle tissues containing elevated levels of unsaturated fatty acids, thus retarding the onset of lipid oxidation. This result is in agreement with the findings of Buckley et al. (1995), Monahan et al. (1990), Morrissey et al. (1996), De Winne and Dirinck (1997), Leskanich et al. (1997) and Isabel et al. (1999a). No differences ($P > 0.05$) in TBARS levels were apparent between wieners produced from pigs fed the fishmeal supplementation and those that did not contain any fishmeal.

3.4.2 Effect of exogenous antioxidants

The effect of antioxidants added to wieners is shown in Figures 3.10 and 3.11 and Table 3.8. A summary of data is presented in Appendix 6.6.2, Table 6.225 for TBARS and Table 6.226 for fluorescence. Statistical analyses are shown in Appendix 6.6.1, Tables 6.185 to 6.197 for TBARS, Tables 6.198 to 6.210 for fluorescence shift and Tables 6.211 to 6.223 for sensory evaluation. The experiment was terminated after 10 months of frozen storage, which is more than the recommended shelf life of 4 months.

At ten months of frozen storage, no differences ($P > 0.05$) were observed among wieners formulations, the levels of TBARS, fluorescence shift and sensory remained almost unchanged. The TBARS level declined during the first 4 weeks

of frozen storage, then gradually increased to reach a constant level of approximately 0.16 mg malonaldehyde per kilogram of wieners after 16 weeks of frozen storage (Figure 3.10, Table 6.225). A similar pattern was observed for fluorescence shift analysis; however, the fluorescence shift first increased after 4 weeks frozen storage, decreased after 8 weeks of frozen storage and gradually continued to increase (Figure 3.11, Table 6.226). A similar trend was observed by Shackelford et al. (1991) in Frankfurters.

Oxidation in wieners took place very slowly. This can be observed from the slope results (Table 3.8). The slope may be interpreted as a measure of oxidation with time. It is expressed as mg of MA/kg feed per week of frozen storage for TBARS, as fluorescence shift per week of frozen storage for the fluorescence method and as sensory score per week of frozen storage for the sensory analysis. In all cases, the slope was very small and slightly positive which showed the product had a tendency to oxidise with time. The average TBARS value was not significant (Table 3.8). The TBARS slopes did not differ significantly; however, it was observed that wieners produced without additional antioxidants had the highest slope value followed by those containing rosemary extract and whey powder. Thus it appears that rosemary extract and whey powder presented some antioxidant activity.

It appeared that the fluorescence shift method was not satisfactory for measuring lipid oxidation in wiener sausages containing rosemary extract. Figure 3.11 shows wieners containing rosemary extract had significant higher values ($P < 0.05$) of fluorescence shift than those containing whey powder. Although non-significant, they also had higher values than the wieners without antioxidants. This trend was not observed in the case of TBARS, thus, it appears that rosemary extract contained compounds that fluoresced and interfered with the measurements of fluorescence shift. Since the day of production, it was observed that fluorescence shift values for wieners containing rosemary extract were consistently higher than the wieners without antioxidant or the wieners containing whey powder. After discarding the wieners results containing rosemary extract, it was observed that wieners without added antioxidants had a higher fluorescence shift average than wieners containing whey powder but the values were

non-significant ($P > 0.05$) at those levels. A similar trend appeared when analysing the fluorescence shift slope (Table 3.8), where wieners produced without antioxidant had higher fluorescence shift slope than those containing whey powder, suggesting that wieners without antioxidant may oxidise faster.

The low sensory scores (Table 3.8) indicated that panellists were unable to detect ($P > 0.05$) any rancid odour between the products throughout the 40 weeks of frozen storage, suggesting no or little oxidation took place during the 40 weeks of frozen storage. This was consistent with the results obtained with TBARS and fluorescence. The sensory score average showed a non-significant ($P > 0.05$) higher value, for wieners containing rosemary extract than for those without antioxidant or whey powder. This suggested that the presence of rosemary in the wieners may have interfered with assessment of rancidity by the panellists. It was further observed that from the day of production, sensory scores for wieners containing rosemary extract were consistently higher than for wieners without antioxidant or those containing whey powder. The sensory score slope revealed that wieners without antioxidant had higher slope values, although non-significant ($P > 0.05$), than wieners produced with rosemary extract or whey powder. This implied that rosemary extract and whey powder could have acted as antioxidants and have reduced the rate of oxidation.

3.4.3 Interpretation of the results on the effect of exogenous antioxidants in wieners

Ho et al. (1995) stated that rosemary extract contained many compounds with antioxidant properties. These compounds probably function as free radical scavengers, thus reducing lipid oxidation. Barbut et al. (1985) reported that the addition of rosemary in refrigerated turkey sausage was an effective way to suppress lipid oxidation and to increase the shelf life. Similar results were obtained by Mc Carthy et al. (2001) in pork patties, by Murphy et al. (1998) in precooked roast beef slices and by Stoik et al. (1991) in restructured beef during refrigerated storage. Murphy et al. (1998) observed that TBARS values were higher than the products without rosemary during frozen storage. Stoik et al. (1991) observed that samples containing salt, phosphate and rosemary had a

comparable antioxidative effect to samples containing salt, phosphate and BHA/BHT. In the present study, the lower slope values for TBARS and sensory scores suggested slower rate of oxidation for wieners containing rosemary extract than those without additional antioxidant. Furthermore, as no phosphates were added in the present study, the presence of sodium erythorbate could have acted as an additional antioxidant, thus may have played a role in the oxidative stability of the product when stored under frozen conditions.

Browdy and Harris (1997) demonstrated that whey powder suppressed the formation of hydroperoxides and TBARS in model systems and could be useful as an antioxidant in some processed foods. Colbert and Decker (1991) showed that antioxidants were present in the ultrafiltration permeate of acid and sweet whey. Those antioxidants were heat stable and had a molecular weight of 500 – 5000 daltons. However, further research is required to identify and characterise those low molecular weight antioxidants. Methods to isolate these compounds commercially are also needed in order to bring these antioxidants to the market (Decker et al. 1998a). In the present study, the results obtained were not significantly different enough to confirm that whey powder acted as antioxidant. However, whey powder was found to lower TBARS and fluorescence shift values in wieners and improved sensory scores as compared to those without additional antioxidant. This suggested that whey powder could have acted as a potential antioxidant.

Overall it appeared that long term frozen storage did not significantly ($P > 0.05$) affect lipid oxidation. TBARS values, fluorescence shift and sensory scores were not affected by treatments. The wieners were acceptable even after storage at –20°C for 40 weeks. Wieners containing rosemary and whey powder appeared to have slower rate of oxidation than those without antioxidant.

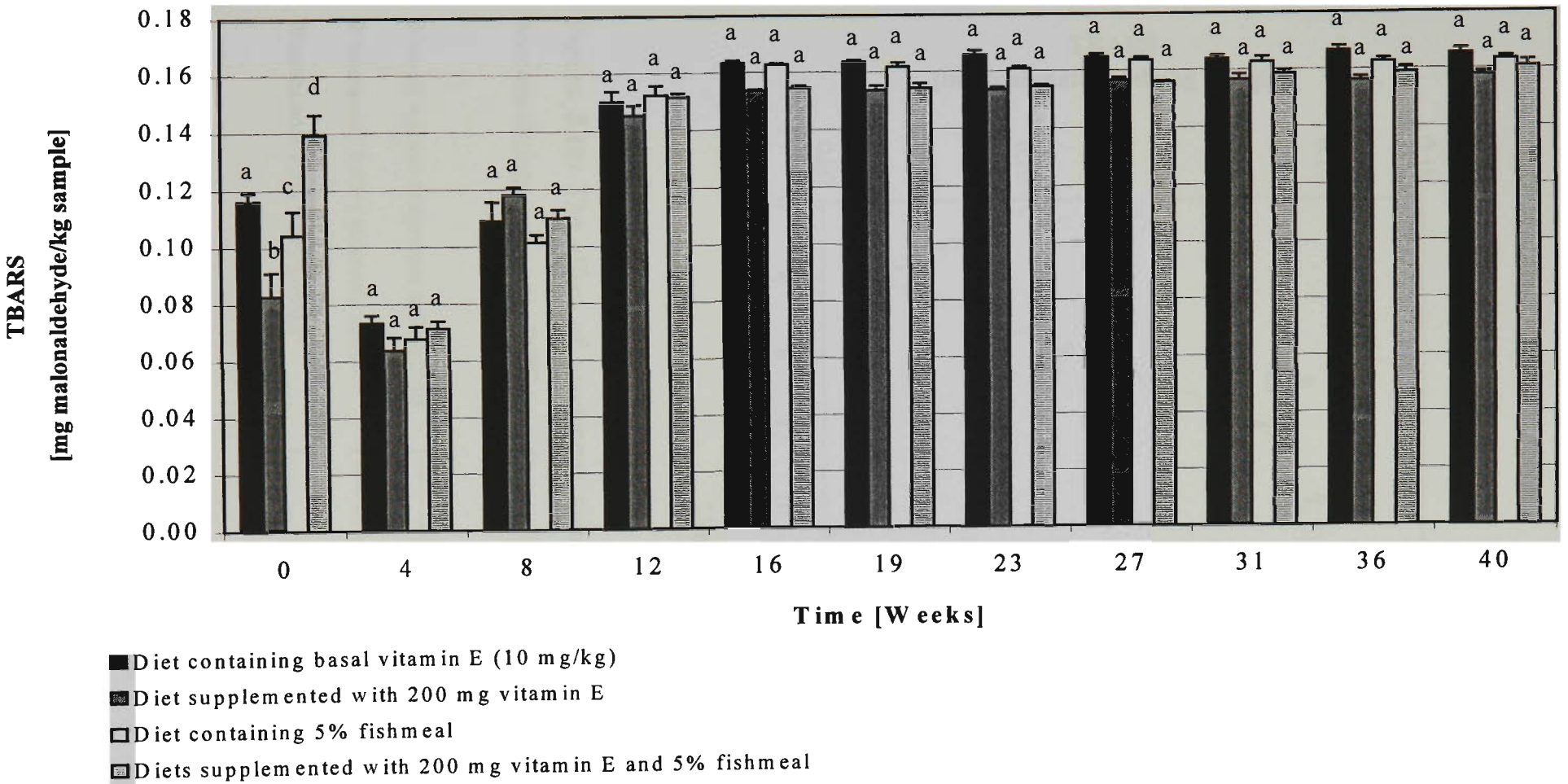

Figure 3.9: The effect of diets on formation of TBARS in wieners during storage at –20°C (n = 3). (Means ± SEM (standard error of the mean))

[a, b, c, d,] Means in same week followed by the same letter are not significantly different (P > 0.05).

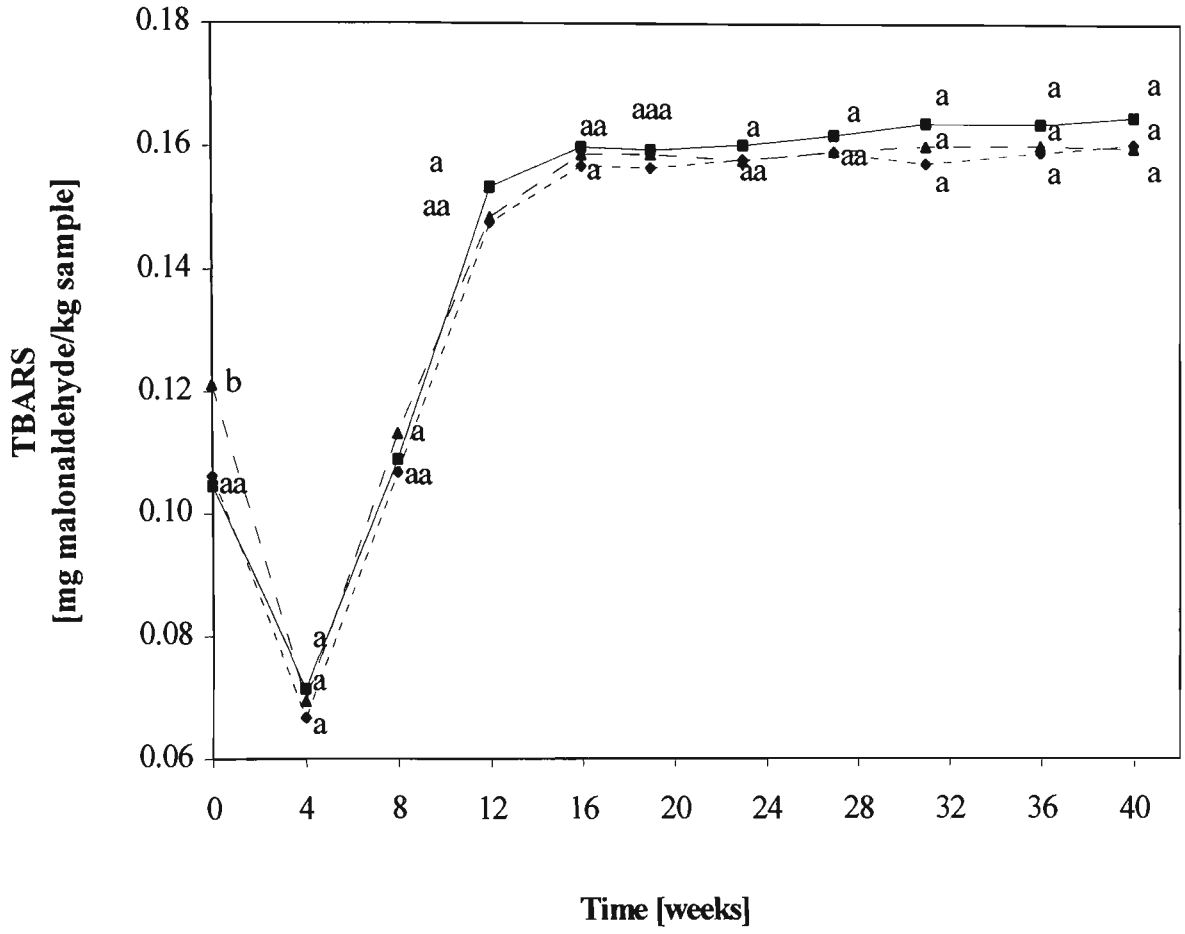

Figure 3.10: The effect of exogenous antioxidants on formation of TBARS in wieners during storage at –20°C (n = 4).

Wieners without exogenous antioxidants

Wieners with 0.03% rosemary extract

Wieners with 2.5% whey powder

[a,] Means in same week followed by the same letter are not significantly different (LSD $_{0.05}$ = 0.00386 week 0, LSD $_{0.05}$ = 0.01908 week 4, LSD $_{0.05}$ = 0.00620 week 8, LSD $_{0.05}$ = 0.00266 week 12, LSD $_{0.05}$ = 0.003738 week 16, LSD $_{0.05}$ = 0.001054 week 19, LSD $_{0.05}$ = 0.00304 week 23, LSD $_{0.05}$ = 0.002207 week 27, LSD $_{0.05}$ = 0.001478 week 31, LSD $_{0.05}$ = 0.000249 week 36, LSD $_{0.05}$ = 0.000915 week40).

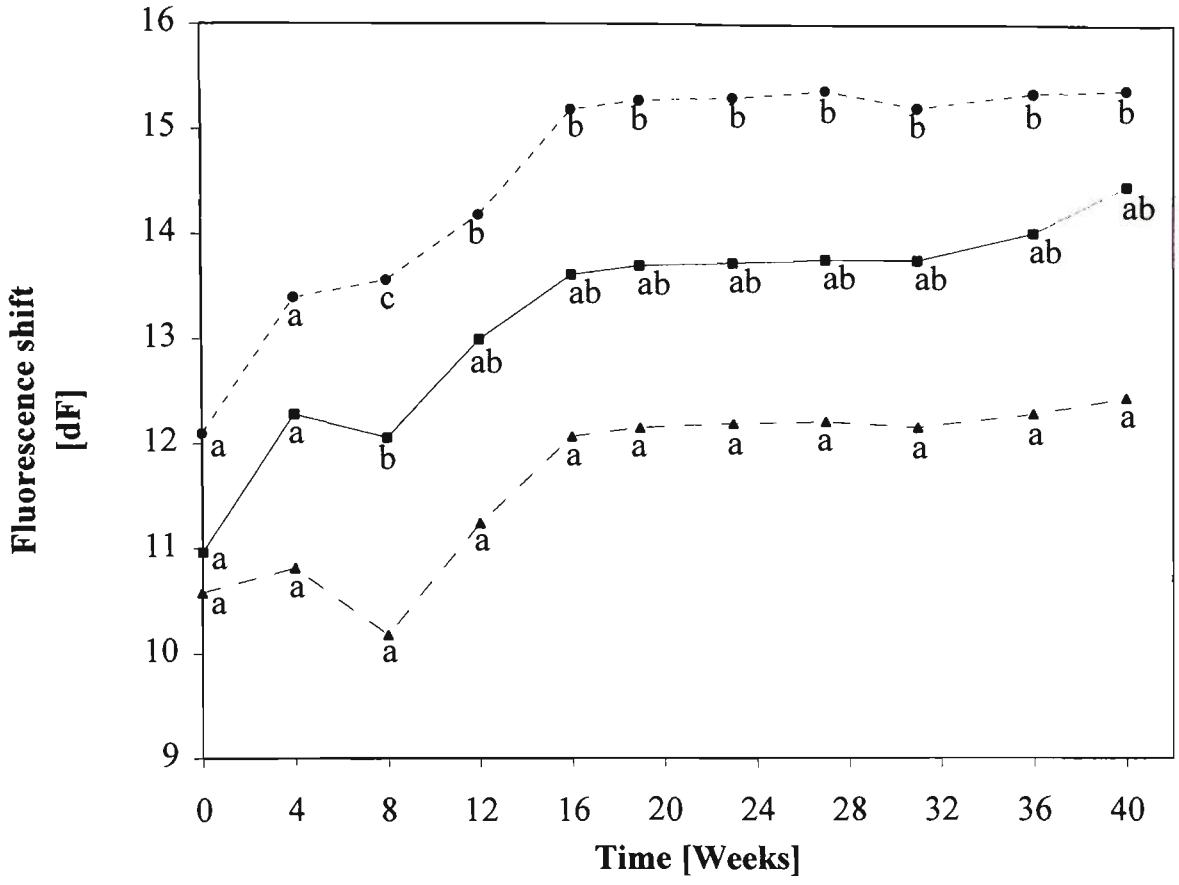

Figure 3.11: The effect of exogenous antioxidants on fluorescence shift in wieners during storage at –20°C (n = 4).

Wieners without exogenous antioxidants (—■—)

Wieners with 0.03% rosemary extract (----●----)

Wieners with 2.5% whey powder (__ __ ▲__ __)

[a,b,c,] Means in same week followed by the same letter are not significantly different

($LSD_{0.05}$ = 1.945 week 0, $LSD_{0.05}$ = 4.943 week 4, $LSD_{0.05}$ = 0.5442 week 8, $LSD_{0.05}$ = 2.145 week 12, $LSD_{0.05}$ = 1.878 week 16, $LSD_{0.05}$ = 1.796 week 19, $LSD_{0.05}$ = 1.876 week 23, $LSD_{0.05}$ = 1.631 week 27, $LSD_{0.05}$ = 1.859week 31, $LSD_{0.05}$ = 2.120 week 36, $LSD_{0.05}$ = 0.9778 week 40).

Table 3.8: The effect of antioxidants on TBARS, fluorescence shift and sensory scores for wieners stored at –20°C for 10 months.

Analysis	No antioxidants	Rosemary extract	Whey powder	SED	P-value
TBARS average [mg MA/kg sample]	0.143 [a]	0.140 [a]	0.142 [a]	0.0014	0.211
TBARS slope [(mg MA/kg sample) / week]	0.00195 [a]	0.00186 [a]	0.00164 [a]	0.000076	0.102
Fluorescence shift average	13.2 [ab]	14.6 [a]	11.7 [b]	0.38	0.033
Fluorescence shift slope [fluorescence shift / week]	0.072 [a]	0.072 [a]	0.053 [a]	0.0081	0.217
Sensory score average	2.18 [a]	2.30 [a]	1.82 [a]	0.143	0.139
Sensory score slope [sensory score / week]	0.019 [a]	0.011 [a]	0.012 [a]	0.0121	0.820

[a, b,] Means in same row followed by the same letter are not significantly different ($LSD_{0.05} = 0.005872$ for TBARS average, $LSD_{0.05} = 0.0003280$ for TBARS slope, $LSD_{0.05} = 1.634$ for fluorescence average, $LSD_{0.05} = 0.03475$ for fluorescence slope, $LSD_{0.05} = 0.6156$ for sensory average, $LSD_{0.05} = 0.05187$ for sensory slope).

CHAPTER 4

OVERALL CONCLUSIONS AND INDUSTRY IMPLICATIONS

4.1 CONCLUSIONS

Twelve pigs were allotted to four dietary treatments containing fishmeal at 0 or 5% and vitamin E at 10 or 200 mg/kg of feed and two products (bacon and wieners sausages) were manufactured from the meat obtained from the pigs. The oxidative stability of bacon processed with wood smoke or with a combination of liquid and wood smoke and the oxidative stability of bacon manufactured from pork obtained from pigs fed the experimental diets were investigated. The oxidative stability of wieners manufactured from pork obtained from pigs fed the experimental diets and of those manufactured with or without the addition of exogenous antioxidant (0.03% rosemary extract or 2.5% whey powder) was also studied.

4.1.1 Bacon conclusions

The fatty acid composition of pigs feed showed lower levels of C18:0 fatty acid and higher levels of C14:0, C16:1 *(n-7)*, C20:0, C18:4 *(n-3)*, C20:4 *(n-6)*, C22:1 *(n-9)*, C22:4 *(n-6)*, C22:5 *(n-3)* and C22:6 *(n-3)* fatty acids when fishmeal was present.

The fatty acid composition of bacon fat was only slightly affected by the diet. No significant changes ($P > 0.05$) were found in the proportions of SAT and MUFAs. Diets supplemented with vitamin E had higher levels ($P < 0.05$) of total PUFAs than the other experimental diets. No significant differences ($P > 0.05$) were observed for the ratio of unsaturated (MUFA and PUFA) to SAT fatty acids.

Diets supplemented with 200 mg vitamin E per kilogram of feed increased α-tocopherol levels by 3 folds in bacon fat and by 2 folds in bacon loin muscle compared to bacon produced from pigs fed a basal level of vitamin E (10 mg vitamin E per kilogram of feed).

The addition of liquid smoke in bacon combined with wood smoke was a very effective means of retarding lipid oxidation. Dietary supplementation of fishmeal produced adverse effects on lipid oxidation in bacon. This was possibly caused by the presence of highly unsaturated fatty acids. Dietary supplementation of vitamin E was shown to be effectively incorporated in fat and muscle tissues and it helped reduce lipid oxidation in bacon whether fishmeal was present in the diet or not. Lipid oxidation in frozen bacon was most successfully reduced when bacon was manufactured from pigs fed a diet supplemented with 200 mg of α-tocopherol per kilogram of feed and processed with a combination of liquid and wood smoke. The combination dietary vitamin E and processing with a combination of liquid and wood smoke was also very effective in reducing lipid oxidation in the case of bacon produced from pigs fed fishmeal. Sensory evaluation showed a slight increase in oxidation with time but no overall differences were observed between the dietary treatment groups.

4.1.2 Wieners conclusions

No lipid oxidation was observed in wieners stored frozen at –20°C for ten months. Lipid oxidation, as measured by TBARS, fluorescence shift and sensory analysis, was not affected by dietary treatments or by the addition of antioxidants. Dietary vitamin E appeared to lower TBARS values, suggesting vitamin E helped retard lipid oxidation. The low TBARS, fluorescence shift and sensory scores obtained for wieners containing whey powder showed that lipid oxidation was taking place slower than in wieners without antioxidants. A similar trend was observed with rosemary extract. The high oxidative stability of the wieners, even in the absence of antioxidants could be due to sodium erythorbate present in the formulation as an additional antioxidant and preservative. The panellists were unable to detect any rancid odour between the products throughout the 40 weeks of frozen storage. This was consistent with results obtained with TBARS. The TBARS values remained very low (~0.16 mg malonaldehyde per kilogram of wieners) throughout the 40 weeks of frozen storage. These values were much under the threshold for detection of rancid odour through sensory evaluation (0.5-2.0 mg malonaldehyde per kilogram sample).

4.2 IMPLICATIONS TO THE INDUSTRY

Lipid oxidation is one of the major problems encountered in meat processing following cooking and subsequent refrigerated or frozen storage. It affects the quality of the product by the loss of desirable colour, odour and flavour and a reduced shelf life. The rate of lipid oxidation can be effectively retarded by the use of antioxidants. Natural antioxidants are of main interest nowadays. Synthetic antioxidants were widely used in the meat industry but consumers concern over their safety and toxicity pressed the food industry to find natural sources of antioxidant. Rosemary extract and whey powder are natural antioxidants. Rosemary is used commercially as an antioxidant; however, it is a costly ingredient. Whey is a cheap by-product from the cheese industry and is currently being investigated for its antioxidant activity. Whey was shown to contain antioxidant compounds that were heat stable and had a molecular weight of 500 – 5000 daltons. Whey and whey ultrafiltration permeate have been proposed to be used as a natural antioxidant in foods. Their application into other products would help the cheese industry to partially solve the problem of whey disposal. The present study could not conclusively show the antioxidative effects of rosemary extract and whey powder but it appeared that both antioxidants reduced the rate of oxidation.

Lipid oxidation can also be reduced by supplementing antioxidants to the diet of the animals. α-Tocopherol (vitamin E) is a lipid soluble antioxidant that contains a phenolic structure which scavenges lipid and oxygen radicals through the formation of a tocopheryl quinone radical that does not promote further oxidation of lipids (Decker and Xu, 1998b). Tocopherols are not synthesised by the animal tissues and their sources are therefore dependent on the diet. It was noted that 200mg of α-tocopherol per kilogram of feed significantly increased the levels of vitamin E in bacon fat and bacon muscle. This in turn significantly reduced lipid oxidation in bacon over a 6 weeks frozen storage. However, the effect of vitamin E was not effective for longer frozen storage period.

The present study also examined the effect of processing bacon with wood smoke and a combination of liquid and wood smoke. The preservation effect of smoke on meat is well recognised. However, a combination of liquid and wood

smoke was found more effective in retarding lipid oxidation in frozen bacon than wood smoke only. Furthermore, it was observed that dietary supplementation of vitamin E worked synergistically with a combination of liquid and wood smoke. During 16 weeks of frozen storage, lipid oxidation was markedly reduced when bacon was manufactured from pigs fed a diet supplemented with 200 mg of α-tocopherol per kilogram of feed and processed with a combination of liquid and wood smoke. This can be of importance for products requiring a longer shelf life.

4.3 FUTURE RESEARCH

Applications of whey or whey powder as an antioxidant into smallgoods products should be further investigated. Whey has been shown to contain antioxidant compounds that were heat stable and had a molecular weight of 500 – 5000 daltons. However, further research is required to identify and characterise the low molecular weight antioxidants. Methods to isolate these compounds commercially are also needed in order to bring these antioxidants to the market. Whey powder can be used as a suitable additive in sausages. It has the following functionalities: nutritional extender, flavour enhancer, processing aid, formulation aid, binder, texturiser, stabiliser and thickener. If these properties can be combined together with an antioxidant effect, whey would become a cheap and useful natural additive.

Numerous studies on the effect of dietary α-tocopherol supplementation in animals on meat quality have been concluded and many are still underway. Dietary α-tocopherol supplementation was found to improve oxidative stability in fresh and processed pork products.

The preservation effect of smoke on meat is well recognised. Phenols have been clearly demonstrated to be the main antioxidant compound associated with smoke (Maga, 1988). Wood smoke has been reported to contain higher amounts of phenolic substances, carbonyls and total acid than liquid smoke (Maga, 1988). Liquid smoke was found to have antioxidant properties at levels from 0.2 to 2%; however, high levels (10% and above) of liquid smoke used for antioxidant purposes were shown to act as prooxidant. Presence of polycyclic aromatic hydrocarbons (PAHs) in smoked meats is of major concern, as some of the PAHs

are considered carcinogenic. Studies involving various commercial products showed that processed products with natural wood smoke had higher total carcinogenic PAHs content than those processed with liquid smoke flavouring. The present study examined the effect of processing bacon with wood smoke and a combination of liquid and wood smoke. The combination of liquid and wood smoke was found more effective in retarding lipid oxidation in frozen bacon than wood smoke only. Furthermore, during 16 weeks of frozen storage, lipid oxidation in bacon manufactured from pigs fed a diet supplemented with 200 mg of α-tocopherol per kilogram of feed and processed with a combination of liquid and wood smoke was markedly reduced compared to bacon manufactured from pigs without any vitamin E supplementation. It appeared that dietary supplementation of vitamin E worked synergistically with a combination of liquid and wood smoke. The effect of feeding vitamin E to animals and manufacturing smoked processed products from them using wood smoke or liquid smoke or a combination of liquid and wood smoke should be further investigated.

CHAPTER 5

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CHAPTER 6

APPENDICES

6.1 STATISTICAL ANALYSIS OF THE FATTY ACID COMPOSITION OF PIG FEED

Table 6.1: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C14:0 fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control Diet versus:**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C14:0							
SD	0.066	0.013		0.121		0.026	
Mean	2.054	2.055		2.290		2.426	
Calculated t-statistics			-0.03		-2.43		-7.42
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.2: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C14:0 fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C14:0					
SD	0.013	0.121		0.026	
Mean	2.055	2.290		2.426	
Calculated t-statistics			-2.74		-17.90
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.3: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C14:0 fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C14:0			
SD	0.121	0.026	
Mean	2.290	2.426	
Calculated t-statistics			-1.56
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.4: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C16:0 fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:0							
SD	0.160	0.250		0.225		0.201	
Mean	23.339	23.319		23.632		23.823	
Calculated t-statistics			0.10		-1.50		-2.66
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.5: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C16:0 fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:0					
SD	0.250	0.225		0.201	
Mean	23.319	23.632		23.823	
Calculated t-statistics			-0.02		-2.22
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.6: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C16:0 fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:0			
SD	0.225	0.201	
Mean	23.632	23.823	
Calculated t-statistics			-0.89
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion	No difference in mean value		

Table 6.7: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C16:1 *(n-7)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:1 *(n-7)*							
SD	0.056	0.027		0.040		0.056	
Mean	1.866	1.861		2.013		2.047	
Calculated t-statistics			0.12		-3.03		-3.24
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion	No difference in mean value			No difference in mean value		No difference in mean value	

Table 6.8: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C16:1 *(n-7)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:1 *(n-7)*					
SD	0.027	0.040		0.056	
Mean	1.861	2.013		2.047	
Calculated t-statistics			-4.51		-4.26
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		Difference in mean value		No difference in mean value	

Table 6.9: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C16:1 *(n-7)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C16:1 *(n-7)*			
SD	0.040	0.056	
Mean	2.013	2.047	
Calculated t-statistics			-0.704
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.10: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:0 fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:0							
SD	0.146	0.179		0.211		0.182	
Mean	13.144	12.970		12.292		12.388	
Calculated t-statistics			1.07		4.70		4.59
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value		Difference in mean value	

Table 6.11: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:0 fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:0					
SD	0.170	0.211		0.182	
Mean	12.970	12.292		12.388	
Calculated t-statistics			3.47		3.23
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.12: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:0 fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:0			
SD	0.211	0.182	
Mean	12.292	12.388	
Calculated t-statistics			-0.489
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.13: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:1 *(n-9)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-9)*							
SD	0.800	0.854		0.058		0.421	
Mean	31.067	30.850		29.673		29.571	
Calculated t-statistics			0.26		2.46		2.34
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.14: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:1 *(n-9)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-9)*					
SD	0.854	0.058		0.421	
Mean	30.850	29.673		29.571	
Calculated t-statistics			1.95		1.90
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.15: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:1 *(n-9)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-9)*			
SD	0.058	0.421	
Mean	29.673	29.571	
Calculated t-statistics			0.34
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.16: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:1 *(n-7)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-7)*							
SD	0.342	0.317		0.050		0.018	
Mean	1.153	1.155		1.671		1.674	
Calculated t-statistics			-0.01		-2.12		-2.15
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.17: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:1 *(n-7)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-7)*					
SD	0.317	0.050		0.018	
Mean	1.155	1.671		1.674	
Calculated t-statistics			-2.28		-2.31
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.18: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:1 *(n-7))* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:1 *(n-7)*			
SD	0.058	0.421	
Mean	1.671	1.674	
Calculated t-statistics			-0.08
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.19: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:2 *(n-6)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:2 *(n-6)*							
SD	0.539	0.138		0.526		0.281	
Mean	23.084	23.442		22.915		22.186	
Calculated t-statistics			-0.91		0.32		2.09
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.20: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:2 *(n-6)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:2 *(n-6)*					
SD	0.138	0.526		0.281	
Mean	23.442	22.915		22.186	
Calculated t-statistics			1.37		5.67
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.21: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:2 *(n-6)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:2 *(n-6)*			
SD	0.526	0.281	
Mean	22.915	22.186	
Calculated t-statistics			1.73
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.22: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:3 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:3 *(n-3)*							
SD	0.068	0.008		0.030		0.008	
Mean	2.223	2.255		2.045		2.014	
Calculated t-statistics			-0.67		3.38		4.30
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.23: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:3 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:3 *(n-3)*					
SD	0.008	0.030		0.008	
Mean	2.255	2.045		2.014	
Calculated t-statistics			9.70		30.41
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		Difference in mean value		Difference in mean value	

Table 6.24: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:3 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:3 *(n-3)*			
SD	0.030	0.008	
Mean	2.045	2.014	
Calculated t-statistics			1.45
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.25: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C20:0 fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:0							
SD	0.004	0.004		0.048		0.013	
Mean	0.249	0.247		0.300		0.324	
Calculated t-statistics			0.62		-1.48		-7.96
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.26: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:0 fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:0					
SD	0.004	0.048		0.013	
Mean	0.247	0.300		0.324	
Calculated t-statistics			-1.55		-8.21
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.27: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:0 fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:0			
SD	0.048	0.013	
Mean	0.300	0.324	
Calculated t-statistics			-0.70
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.28: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C18:4 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty Acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:4 *(n-3)*							
SD	0.003	0.004		0.156		0.046	
Mean	0.482	0.480		0.928		1.003	
Calculated t-statistics			0.49		-4.04		-16.03
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.29: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:4 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:4 *(n-3)*					
SD	0.004	0.156		0.046	
Mean	0.480	0.928		1.003	
Calculated t-statistics			-4.05		-16.07
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.30: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C18:4 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C18:4 *(n-3)*			
SD	0.156	0.046	
Mean	0.928	1.003	
Calculated t-statistics			-0.65
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.31: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C20:1 *(n-9)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:1 *(n-9)*							
SD	0.030	0.024		0.009		0.035	
Mean	0.181	0.186		0.189		0.179	
Calculated t-statistics			-0.20		-0.38		0.06
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.32: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:1 *(n-9)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:1 *(n-9)*					
SD	0.024	0.009		0.035	
Mean	0.186	0.189		0.179	
Calculated t-statistics			-0.17		0.24
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.33: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:1 *(n-9)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:1 *(n-9)*			
SD	0.009	0.035	
Mean	0.189	0.179	
Calculated t-statistics			0.41
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.34: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C20:4 *(n-6)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:4 *(n-6)*							
SD	0.002	0.002		0.000		0.010	
Mean	0.110	0.114		0.161		0.154	
Calculated t-statistics			-2.01		-40.06		-6.20
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value		Difference in mean value	

Table 6.35: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:4 *(n-6)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:4 *(n-6)*					
SD	0.002	0.000		0.010	
Mean	0.114	0.161		0.154	
Calculated t-statistics			-38.12		-5.71
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		Difference in mean value		Difference in mean value	

Table 6.36: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:4 *(n-6)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:4 *(n-6)*			
SD	0.000	0.010	
Mean	0.161	0.154	
Calculated t-statistics			0.92
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion	No difference in mean value		

Table 6.37: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C20:5 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:5 *(n-3)*							
SD	0.009	0.001		0.043		0.022	
Mean	0.133	0.139		0.140		0.122	
Calculated t-statistics			-0.95		-0.20		0.69
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion	No difference in mean value			No difference in mean value		No difference in mean value	

Table 6.38: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:5 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:5 *(n-3)*					
SD	0.001	0.043		0.022	
Mean	0.139	0.140		0.122	
Calculated t-statistics			-0.003		1.14
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.39: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C20:5 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C20:5 *(n-3)*			
SD	0.043	0.022	
Mean	0.140	0.122	
Calculated t-statistics			0.52
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.40: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C22:1 *(n-9)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:1 *(n-9)*							
SD	0.011	0.005		0.375		0.046	
Mean	0.109	0.119		0.387		0.625	
Calculated t-statistics			-1.18		-1.05		-15.56
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.41: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:1 *(n-9)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:1 *(n-9)*					
SD	0.005	0.375		0.046	
Mean	0.119	0.387		0.625	
Calculated t-statistics			-1.01		-15.57
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.42: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:1 *(n-9)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:1 *(n-9)*			
SD	0.375	0.046	
Mean	0.387	0.625	
Calculated t-statistics			-0.89
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.43: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C21:5 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C21:5 *(n-3)*							
SD	0.015	0.014		0.018		0.005	
Mean	0.101	0.101		0.108		0.102	
Calculated t-statistics			0.01		-0.42		-0.04
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.44: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C21:5 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C21:5 *(n-3)*					
SD	0.014	0.018		0.005	
Mean	0.101	0.108		0.102	
Calculated t-statistics			-0.45		-0.06
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.45: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C21:5 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C21:5 *(n-3)*			
SD	0.018	0.005	
Mean	0.108	0.102	
Calculated t-statistics			0.49
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.46: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C22:4 *(n-6)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:4 *(n-6)*							
SD	0.000	0.000		0.031		0.002	
Mean	0.000	0.000		0.022		0.039	
Calculated t-statistics			0.00		-1.00		-35.68
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.47: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:4 *(n-6)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:4 *(n-6)*					
SD	0.000	0.031		0.002	
Mean	0.000	0.022		0.039	
Calculated t-statistics			-1.00		-36.68
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.48: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:4 *(n-6)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:4 *(n-6)*			
SD	0.031	0.002	
Mean	0.022	0.039	
Calculated t-statistics			-0.76
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.49: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C22:5 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:5 *(n-3)*							
SD	0.001	0.001		0.027		0.011	
Mean	0.083	0.085		0.160		0.173	
Calculated t-statistics			-2.24		-3.96		-11.16
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.50: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:5 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:5 *(n-3)*					
SD	0.001	0.027		0.011	
Mean	0.085	0.160		0.173	
Calculated t-statistics			-3.88		-10.99
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.51: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:5 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:5 *(n-3)*			
SD	0.027	0.011	
Mean	0.160	0.173	
Calculated t-statistics			-0.62
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.52: Statistical analysis of fatty acid composition of pig feed by Student's t-test – C22:6 *(n-3)* fatty acid (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:6 *(n-3)*							
SD	0.000	0.000		0.144		0.027	
Mean	0.000	0.000		0.455		0.536	
Calculated t-statistics			0.00		-4.47		-28.36
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value		Difference in mean value	

Table 6.53: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:6 *(n-3)* fatty acid (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:6 *(n-3)*					
SD	0.000	0.144		0.027	
Mean	0.000	0.455		0.536	
Calculated t-statistics			-4.47		-28.36
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		Difference in mean value		Difference in mean value	

Table 6.54: Statistical analysis of fatty acid composition of pig feed by Student's t-test - C22:6 *(n-3)* fatty acid (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
C22:6 *(n-3)*			
SD	0.144	0.027	
Mean	0.455	0.536	
Calculated t-statistics			-0.78
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion	No difference in mean value		

Table 6.55: Statistical analysis of fatty acid composition of pig feed by Student's t-test – total saturated (SAT) fatty acids (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total SAT							
SD	0.085	0.438		0.267		0.343	
Mean	38.786	38.590		38.515		38.962	
Calculated t-statistics			0.62		1.37		-0.70
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.56: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total saturated (SAT) fatty acids (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total SAT					
SD	0.438	0.267		0.343	
Mean	38.590	38.515		38.962	
Calculated t-statistics			0.21		-0.94
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.57: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total saturated (SAT) fatty acids (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total SAT			
SD	0.267	0.343	
Mean	38.515	38.962	
Calculated t-statistics			-1.45
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.58: Statistical analysis of fatty acid composition of pig feed by Student's t-test – total monounsaturated (MUFA) fatty acids (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total MUFA							
SD	0.534	0.583		0.532		0.576	
Mean	34.376	34.171		33.933		34.095	
Calculated t-statistics			0.37		0.83		0.51
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.59: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total monounsaturated (MUFA) fatty acids (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + Vitamin E Suppl. diet**	**t-test**
Total MUFA					
SD	0.583	0.532		0.576	
Mean	34.171	33.933		34.095	
Calculated t-statistics			0.43		0.13
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.60: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total monounsaturated (MUFA) fatty acids (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total MUFA			
SD	0.532	0.576	
Mean	33.933	34.095	
Calculated t-statistics			-0.29
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.61: Statistical analysis of fatty acid composition of pig feed by Student's t-test – total polyunsaturated (PUFA) fatty acids (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total PUFA							
SD	0.620	0.147		0.261		0.224	
Mean	26.217	26.617		26.933		26.328	
Calculated t-statistics			-0.89		-1.51		-0.24
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.62: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total polyunsaturated (PUFA) fatty acids (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + Vitamin E Suppl. diet**	**t-test**
Total PUFA					
SD	0.147	0.261		0.224	
Mean	26.617	26.933		26.328	
Calculated t-statistics			-1.49		1.53
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.63: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total polyunsaturated (PUFA) fatty acids (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total PUFA			
SD	0.261	0.224	
Mean	26.933	26.328	
Calculated t-statistics			2.49
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.64: Statistical analysis of fatty acid composition of pig feed by Student's t-test – M+P/S (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid M+P/S	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
SD	0.006	0.029		0.018		0.023	
Mean	1.562	1.575		1.580		1.551	
Calculated t-statistics			-0.63		-1.37		0.68
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.65: Statistical analysis of fatty acid composition of pig feed by Student's t-test - M+P/S (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid M+P/S	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + Vitamin E Suppl. diet**	**t-test**
SD	0.029	0.018		0.023	
Mean	1.575	1.580		1.551	
Calculated t-statistics			-0.21		0.94
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value	

Table 6.66: Statistical analysis of fatty acid composition of pig feed by Student's t-test - M+P/S (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
M+P/S			
SD	0.018	0.023	
Mean	1.580	1.551	
Calculated t-statistics			1.44
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

Table 6.67: Statistical analysis of fatty acid composition of pig feed by Student's t-test – total *n-3* fatty acids (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total *n-3*							
SD	0.079	0.008		0.296		0.049	
Mean	3.023	3.061		3.835		3.949	
Calculated t-statistics			-0.68		-3.75		-14.10
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		No difference in mean value		Difference in mean value	

Table 6.68: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total *n-3* fatty acids (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Vitamin E Suppl. Diet versus:**	**Fishmeal Suppl. diet**	**t-test**	**Fishmeal + Vitamin E Suppl. diet**	**t-test**
Total *n-3*					
SD	0.008	0.296		0.049	
Mean	3.061	3.835		3.949	
Calculated t-statistics			-3.70		-25.29
Tabulated t-value ($t_{0.050}$)			+/- 4.30		+/- 4.30
Conclusion		No difference in mean value		Difference in mean value	

Table 6.69: Statistical analysis of fatty acid composition of pig feed by Student's t-test - total *n-3* fatty acids (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 2).

Fatty acid	**Fishmeal suppl. diet versus:**	**Fishmeal + vitamin E suppl. diet**	**t-test**
Total *n-3*			
SD	0.296	0.049	
Mean	3.835	3.949	
Calculated t-statistics			-0.54
Tabulated t-value ($t_{0.050}$)			+/- 4.30
Conclusion		No difference in mean value	

6.2 STATISTICAL ANALYSIS OF THE BACKFAT THICKNESS (P2) IN PIGS

Table 6.70: Statistical analysis of backfat thickness in pigs by Student's t-test (control versus vitamin E supplemented diet, control versus fishmeal supplemented diet and control versus fishmeal + vitamin E supplemented diet), (n = 3).

Fatty acid	**Control diet**	**Vitamin E suppl. diet**	**t-test**	**Fishmeal suppl. diet**	**t-test**	**Fishmeal + vitamin E suppl. diet**	**t-test**
P2 value							
SD	5.401	2.540		1.600		4.406	
Mean	18.267	13.067		15.200		15.733	
Calculated t-statistics			1.51		0.94		0.63
Tabulated t-value ($t_{0.050}$)			+/- 2.92		+/- 2.92		+/- 2.92
Conclusion		No difference in mean value		No difference in mean value		No difference in mean value	

Table 6.71: Statistical analysis of backfat thickness in pigs by Student's t-test (vitamin E supplemented diet versus fishmeal supplemented diet and vitamin E supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 3).

Fatty acid P2 value	Vitamin E Suppl. Diet versus:	Fishmeal Suppl. diet	t-test	Fishmeal + Vitamin E Suppl. diet	t-test
SD	2.540	1.600		4.406	
Mean	13.067	15.200		15.733	
Calculated t-statistics			-1.23		-0.91
Tabulated t-value ($t_{0.050}$)			+/- 2.92		+/- 2.92
Conclusion		No difference in mean value		No difference in mean value	

Table 6.72: Statistical analysis of backfat thickness in pigs by Student's t-test (fishmeal supplemented diet versus fishmeal + vitamin E supplemented diet), (n = 3).

Fatty acid P2 value	Fishmeal suppl. diet versus:	Fishmeal + vitamin E suppl. diet	t-test
SD	1.600	4.406	
Mean	15.200	15.733	
Calculated t-statistics			-0.20
Tabulated t-value ($t_{0.050}$)			+/- 2.92
Conclusion		No difference in mean value	

6.3 STATISTICAL ANALYSIS OF THE FATTY ACID COMPOSITION OF BACON FAT

Table 6.73: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C14:0 fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.08585	0.04292	2.99	
Block. Animal Stratum					
Fishmeal	1	0.01226	0.01226	0.85	0.391
Vitamin E	1	0.02466	0.02466	1.72	0.238
Fishmeal. Vitamin E	1	0.05981	0.05981	4.17	0.087
Residual	6	0.08607	0.01435		
Total	11	0.26865			

Table 6.74: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C16:0 fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.9254	1.4627	2.14	
Block. Animal Stratum					
Fishmeal	1	3.1201	3.1201	4.56	0.077
Vitamin E	1	0.2984	0.2984	0.44	0.534
Fishmeal. Vitamin E	1	4.3937	4.3937	6.42	**0.044**
Residual	6	4.1074	0.6846		
Total	11	14.8450			

Table 6.75: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C16:1 *(n-7)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.1992	0.0996	0.27	
Block. Animal Stratum					
Fishmeal	1	0.9491	0.9491	2.57	0.160
Vitamin E	1	0.0011	0.0011	0.00	0.958
Fishmeal. Vitamin E	1	0.7052	0.7052	1.91	0.216
Residual	6	2.2171	0.3695		
Total	11	4.0717			

Table 6.76: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C18:0 fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.842	1.421	0.76	
Block. Animal Stratum					
Fishmeal	1	0.002	0.002	0.00	0.977
Vitamin E	1	1.816	1.816	0.97	0.363
Fishmeal. Vitamin E	1	0.000	0.000	0.00	0.991
Residual	6	11.268	1.878		
Total	11	15.928			

Table 6.77: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C18:1 *(n-9)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.7017	1.3509	4.16	
Block. Animal Stratum					
Fishmeal	1	0.1062	0.1062	0.33	0.588
Vitamin E	1	0.2588	0.2588	0.80	0.406
Fishmeal. Vitamin E	1	4.1185	4.1185	12.70	**0.012**
Residual	6	1.9463	0.3244		
Total	11	9.1315			

Table 6.78: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C18:1 *(n-7)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.08123	0.04062	0.49	
Block. Animal Stratum					
Fishmeal	1	0.02736	0.02736	0.33	0.588
Vitamin E	1	0.00015	0.00015	0.00	0.968
Fishmeal. Vitamin E	1	0.01361	0.01361	0.16	0.701
Residual	6	0.50244	0.08374		
Total	11	0.62479			

Table 6.79: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C18:2 *(n-6)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.9817	0.4909	0.67	
Block. Animal Stratum					
Fishmeal	1	5.8568	5.8568	7.97	**0.030**
Vitamin E	1	4.4440	4.4440	6.05	**0.049**
Fishmeal. Vitamin E	1	1.1884	1.1884	1.62	0.251
Residual	6	4.4081	0.7347		
Total	11	16.8790			

Table 6.80: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C18:3 *(n-3)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.003997	0.001998	0.61	
Block. Animal Stratum					
Fishmeal	1	0.041725	0.041725	12.64	**0.012**
Vitamin E	1	0.013475	0.013475	4.08	0.090
Fishmeal. Vitamin E	1	0.005488	0.005488	1.66	0.245
Residual	6	0.019805	0.003301		
Total	11	0.084490			

Table 6.81: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C20:0 fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.003510	0.001755	0.78	
Block. Animal Stratum					
Fishmeal	1	0.011878	0.011878	5.30	0.061
Vitamin E	1	0.006530	0.006530	2.92	0.139
Fishmeal. Vitamin E	1	0.002142	0.002142	0.96	0.366
Residual	6	0.013437	0.002240		
Total	11	0.037497			

Table 6.82: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C20:1 *(n-9)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.041391	0.020695	2.30	
Block. Animal Stratum					
Fishmeal	1	0.002550	0.002550	0.28	0.614
Vitamin E	1	0.025654	0.025654	2.85	0.142
Fishmeal. Vitamin E	1	0.000007	0.000007	0.00	0.979
Residual	6	0.054052	0.009009		
Total	11	0.123654			

Table 6.83: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C20:3 *(n-6)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0005491	0.0002745	2.04	
Block. Animal Stratum					
Fishmeal	1	0.0009667	0.0009667	7.19	**0.044**
Vitamin E	1	0.0001194	0.0001194	0.89	0.389
Fishmeal. Vitamin E	1	0.0003699	0.0003699	2.75	0.158
Residual	6	0.0006718	0.0001344		
Total	11	0.0018721			

Table 6.84: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C20:4 *(n-6)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0003461	0.0001730	0.19	
Block. Animal Stratum					
Fishmeal	1	0.0060713	0.0060713	6.69	**0.041**
Vitamin E	1	0.0025518	0.0025518	2.81	0.145
Fishmeal. Vitamin E	1	0.0058742	0.0058742	6.47	**0.044**
Residual	6	0.0054455	0.0009076		
Total	11	0.0202888			

Table 6.85: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C22:4 *(n-6)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0001244	0.0000622	0.37	
Block. Animal Stratum					
Fishmeal	1	0.0016762	0.0016762	9.93	**0.020**
Vitamin E	1	0.0001987	0.0001987	1.18	0.320
Fishmeal. Vitamin E	1	0.0000121	0.0000121	0.07	0.797
Residual	6	0.0010129	0.0001688		
Total	11	0.0030244			

Table 6.86: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C22:5 *(n-3)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0002680	0.0001340	0.34	
Block. Animal Stratum					
Fishmeal	1	0.0000877	0.0000877	0.22	0.656
Vitamin E	1	0.0001178	0.0001178	0.29	0.607
Fishmeal. Vitamin E	1	0.0014660	0.0014660	3.66	0.104
Residual	6	0.0024001	0.0004000		
Total	11	0.0043397			

Table 6.87: Analysis of variance of the fatty acid composition of bacon fat.

Variate: C22: 6 *(n-3)* fatty acid.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0001061	0.0000531	0.37	
Block. Animal Stratum					
Fishmeal	1	0.0046173	0.0046173	32.12	**0.0013**
Vitamin E	1	0.0002570	0.0002570	1.79	0.230
Fishmeal. Vitamin E	1	0.0002850	0.0002850	1.98	0.209
Residual	6	0.0008624	0.0001437		
Total	11	0.0061279			

Table 6.88: Analysis of variance of the fatty acid composition of bacon fat.

Variate: SAT fatty acids

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	6.162	3.081	1.09	
Block. Animal Stratum					
Fishmeal	1	3.277	3.277	1.16	0.323
Vitamin E	1	2.742	2.742	0.97	0.362
Fishmeal. Vitamin E	1	5.190	5.190	1.84	0.224
Residual	6	16.937	2.823		
Total	11	34.308			

Table 6.89: Analysis of variance of the fatty acid composition of bacon fat.

Variate: MUFA

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	3.2288	1.6144	3.52	
Block. Animal Stratum					
Fishmeal	1	0.5826	0.5826	1.27	0.303
Vitamin E	1	0.4197	0.4197	0.91	0.376
Fishmeal. Vitamin E	1	1.1457	1.1457	2.50	0.165
Residual	6	2.7527	0.4588		
Total	11	8.1295			

Table 6.90: Analysis of variance of the fatty acid composition of bacon fat.

Variate: PUFA

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	1.2280	0.6140	0.68	
Block. Animal Stratum					
Fishmeal	1	7.3924	7.3924	8.24	**0.028**
Vitamin E	1	5.2360	5.2360	5.83	0.052
Fishmeal. Vitamin E	1	1.7973	1.7973	2.00	0.207
Residual	6	5.3843	0.8974		
Total	11	21.0380			

Table 6.91: Analysis of variance of the fatty acid composition of bacon fat.

Variate: (M+P)/S

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.02920	0.01460	1.30	
Block. Animal Stratum					
Fishmeal	1	0.01968	0.01968	1.75	0.234
Vitamin E	1	0.01271	0.01271	1.13	0.328
Fishmeal. Vitamin E	1	0.02386	0.02386	2.13	0.195
Residual	6	0.06737	0.01123		
Total	11	0.15282			

6.4 STATISTICAL ANALYSIS OF VITAMIN E

Table 6.92: Analysis of variance of the vitamin E content in bacon fat.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	34.8493	17.4246	2.03	
Block. Animal Stratum					
Fishmeal	1	0.1843	0.1843	0.02	0.888
Vitamin E	1	1553.6791	1553.6791	181.14	**< 0.001**
Fishmeal. Vitamin E	1	172.4435	172.4435	20.10	**0.004**
Residual	6	51.4632	8.5772	11.12	
Block. Animal. Side stratum					
Process	1	7.1474	7.1474	9.27	**0.016**
Fishmeal. Process	1	27.3338	27.3338	33.44	**< 0.001**
Vitamin E. Process	1	14.2925	14.2925	18.53	**0.003**
Fishmeal. Vitamin E. Process	1	6.9719	6.9719	9.04	**0.017**
Residual	8	6.1696	0.7712		
Total	23	1874.5345			

Table 6.93: Analysis of variance of the vitamin E content in bacon loin muscle.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.2250	1.1125	3.65	
Block. Animal Stratum					
Fishmeal	1	0.2462	0.2462	0.81	0.404
Vitamin E	1	40.6173	40.6173	133.18	**< 0.001**
Fishmeal. Vitamin E	1	2.2989	2.2989	7.54	**0.033**
Residual	6	1.8299	0.3050	0.80	
Block. Animal. Side stratum					
Process	1	0.1473	0.1473	0.38	0.552
Fishmeal. Process	1	0.1578	0.1578	0.41	0.539
Vitamin E. Process	1	0.0033	0.0033	0.01	0.928
Fishmeal. Vitamin E. Process	1	0.0010	0.0010	0.00	0.960
Residual	8	3.0632	0.3829		
Total	23	50.5900			

6.5 BACON

6.5.1 Summary of results

Table 6.94: Effects of treatments with smoke on formation of TBARS in bacon during storage at –20°C (n = 12).

Time of storage in weeks	Bacon processed with wood smoke	Bacon processed with liquid and wood smoke	SED [a]
0	0.11	0.11	0.004
4	0.65	0.33	0.065
6	0.82	0.52	0.106
8	0.96	0.72	0.071
10	1.54	0.99	0.101
12	1.75	1.13	0.050
16	1.82	1.20	0.032

[a] SED; Standard errors of differences of means

Table 6.95: Effects of treatments with smoke on fluorescence shift in bacon during storage at –20°C (n = 12).

Time of storage in weeks	Bacon processed with wood smoke	Bacon processed with liquid and wood smoke	SED [a]
0	3.41	4.00	0.078
4	8.22	5.18	0.486
6	8.17	5.56	0.353
8	8.90	5.65	0.676
10	9.81	6.82	0.696
12	10.52	7.39	0.548
16	13.09	8.63	0.681

[a] SED; Standard errors of differences of means

Table 6.96: Effects of vitamin E and treatments with smoke on formation of TBARS in bacon during storage at –20°C (n = 6).

Time of storage	Wood Smoke		Liquid + Wood Smoke		SED [a]	
[Weeks]	Diet without vitamin E	Diet with vitamin E	Diet without vitamin E	Diet with vitamin E	Same level of vitamin E	Different levels of vitamin E
0	0.11	0.11	0.11	0.11	0.006	0.006
4	0.75	0.54	0.39	0.27	0.092	0.094
6	0.94	0.70	0.64	0.41	0.149	0.145
8	1.09	0.84	0.96	0.49	0.100	0.166
10	1.54	1.54	1.38	0.59	0.142	0124
12	1.75	1.76	1.46	0.79	0.071	0.115
16	1.81	1.82	1.53	0.87	0.046	0.056

[a] SED; Standard errors of differences of means

Table 6.97: Effects of vitamin E and treatments with smoke on fluorescence shift in bacon during storage at –20°C (n = 6).

Time of storage	Wood Smoke		Liquid + Wood Smoke		SED [a]	
[Weeks]	Diet without vitamin E	Diet with vitamin E	Diet without vitamin E	Diet with vitamin E	Same level of vitamin E	Different levels of vitamin E
0	2.96	3.33	4.10	3.90	0.11	0.217
4	9.46	6.98	5.57	4.78	0.687	0.794
6	8.67	7.68	6.41	4.72	0.499	0.765
8	9.60	8.19	6.51	4.79	0.956	1.214
10	10.57	9.06	7.94	5.70	0.985	1.026
12	11.13	9.91	8.56	6.22	0.775	0.762
16	14.13	12.05	10.22	7.04	0.963	0.869

[a] SED; Standard errors of differences of means

Table 6.98: Effects of vitamin E and treatments with smoke on sensory evaluation of bacon during storage at –20°C (n = 6).

Time of storage	Wood Smoke		Liquid + Wood Smoke		SED [a]	
[Weeks]	Diet without vitamin E	Diet with vitamin E	Diet without vitamin E	Diet with vitamin E	Same level of vitamin E	Different levels of vitamin E
0	2.38	2.40	2.23	1.85	0.344	0.345
4	2.43	2.09	2.52	2.20	0.273	0.288
6	3.80	3.40	3.50	2.63	0.327	0.392
10	3.60	3.90	4.07	3.85	0.399	0.376
12	3.35	4.71	4.04	3.94	0.490	0.670
16	3.24	3.87	4.04	3.35	0.439	0.367

[a] SED; Standard errors of differences of means

Table 6.99: Effects of fishmeal on formation of TBARS in bacon during storage at –20°C (n = 12).

Time of storage in weeks	Diet without fishmeal	Diet with fishmeal	SED [a]
0	0.10	0.11	0.005
4	0.40	0.57	0.067
6	0.57	0.78	0.100
8	0.75	0.93	0.150
10	1.22	1.31	0.073
12	1.36	1.52	0.103
16	1.44	1.58	0.045

[a] SED; Standard errors of differences of means

Table 6.100: Effects of fishmeal on fluorescence shift in bacon during storage at –20°C (n = 12).

Time of storage in weeks	Diet without fishmeal	Diet with fishmeal	SED [a]
0	3.43	3.71	0.202
4	5.65	7.75	0.628
6	6.02	7.72	0.679
8	6.71	7.83	1.008
10	7.74	8.89	0.754
12	8.09	9.82	0.529
16	10.03	11.69	0.541

[a] SED; Standard errors of differences of means

Table 6.101: Effects of fishmeal on sensory evaluation of bacon during storage at –20°C (n = 12).

Time of storage in weeks	Diet without fishmeal	Diet with fishmeal	SED [a]
0	2.38	2.05	0.244
4	2.36	2.26	0.214
6	3.58	3.08	0.317
10	4.10	3.61	0.248
12	4.37	3.65	0.573
16	3.70	3.55	0.197

[a] SED; Standard errors of differences of means

Table 6.102: Effect of feeding dietary fishmeal and vitamin E and treatments with smoke on formation of TBARS in bacon during storage at –20°C (n = 3).

	Wood smoke		Liquid + wood smoke		
Time of storage in weeks	Diet with fishmeal	Diet with fishmeal + vitamin E	Diet with fishmeal	Diet with fishmeal + vitamin E	SED [a]
0	0.11	0.13	0.11	0.13	0.036
4	1.06	0.51	0.43	0.29	0.593
6	1.12	0.77	0.73	0.45	0.960
8	1.34	0.88	1.01	0.50	0.644
10	1.64	1.50	1.45	0.63	0.916
12	1.88	1.75	1.58	0.89	0.459
16	1.94	1.81	1.63	0.94	0.295

[a] SED; Standard errors of differences of means

Table 6.103: Effect of dietary fishmeal and vitamin E and treatments with smoke on fluorescence shift in bacon during storage at –20°C (n = 3).

	Wood smoke		Liquid + wood smoke		
Time of storage in weeks	Diet with fishmeal	Diet with fishmeal + vitamin E	Diet wit fishmeal	Diet with fishmeal + vitamin E	SED [a]
0	3.15	3.38	4.73	3.58	0.157
4	12.21	7.20	6.25	5.32	0.971
6	10.93	8.12	6.55	5.29	0.705
8	11.46	7.86	6.65	5.37	1.351
10	12.39	8.89	8.40	5.90	1.392
12	12.96	10.41	9.40	6.51	1.096
16	15.63	11.45	12.40	7.29	1.361

[a] SED; Standard errors of differences of means

Table 6.104: Effect of dietary fishmeal and vitamin E and treatments with smoke on sensory evaluation of bacon during storage at –20°C (n = 3).

	Wood smoke		Liquid + wood smoke		
Time of storage in weeks	Diet with fishmeal	Diet with fishmeal + vitamin E	Diet wit fishmeal	Diet with fishmeal + vitamin E	SED [a]
0	2.30	2.03	2.13	1.73	0.486
4	2.04	1.70	2.93	2.37	0.385
6	4.07	2.73	3.23	2.30	0.463
10	3.23	3.93	3.90	3.37	0.564
12	3.17	4.29	3.71	3.42	0.693
16	3.81	3.63	3.85	2.89	0.620

[a] SED; Standard errors of differences of means

6.5.2 Statistical analysis of bacon – Analysis of variance

Table 6.105: Analysis of variance of the formation of TBARS in bacon after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.000419	0.000210	1.71	
Block. Animal Stratum					
Fishmeal	1	0.001923	0.001923	15.67	**0.007**
Vitamin E	1	9.293×10^{-6}	9.293×10^{-6}	0.08	0.792
Fishmeal. Vitamin E	1	0.001839	0.001839	14.99	**0.008**
Residual	6	0.000736	0.000123	1.29	
Block. Animal. Side stratum					
Process	1	7.192×10^{-5}	7.192×10^{-5}	0.75	0.410
Fishmeal. Process	1	3.761×10^{-5}	3.761×10^{-5}	0.39	0.548
Vitamin E. Process	1	3.388×10^{-6}	3.388×10^{-6}	0.04	0.855
Fishmeal. Vitamin E. Process	1	1.936×10^{-7}	1.936×10^{-7}	0.00	0.966
Residual	8	0.000763	9.530×10^{-5}		
Total	23	0.005804			

Table 6.106: Analysis of variance of the formation of TBARS in bacon after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.003194	0.001597	0.06	
Block. Animal Stratum					
Fishmeal	1	0.176583	0.176583	6.54	**0.043**
Vitamin E	1	0.165296	0.165296	6.13	**0.048**
Fishmeal. Vitamin E	1	0.182802	0.182802	6.77	**0.041**
Residual	6	0.161898	0.026983	1.06	
Block. Animal. Side stratum					
Process	1	0.600712	0.600712	23.50	**0.001**
Fishmeal. Process	1	0.071085	0.071085	2.78	0.134
Vitamin E. Process	1	0.014249	0.014249	0.56	0.477
Fishmeal. Vitamin E. Process	1	0.141289	0.141289	5.53	0.047
Residual	8	0.204538	0.025565		
Total	23	1.721656			

Table 6.107: Analysis of variance of the formation of TBARS in bacon after 6 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.139489	0.069744	1.17	
Block. Animal Stratum					
Fishmeal	1	0.225447	0.225447	3.78	0.100
Vitamin E	1	0.304339	0.304339	5.11	0.065
Fishmeal. Vitamin E	1	0.053385	0.053385	0.90	0.380
Residual	6	0.357482	0.059580	0.89	
Block. Animal. Side stratum					
Process	1	0.515944	0.515944	7.70	**0.024**
Fishmeal. Process	1	0.021441	0.021441	0.32	0.587
Vitamin E. Process	1	9.680×10^{-5}	9.680×10^{-5}	0.00	0.973
Fishmeal. Vitamin E. Process	1	0.009535	0.009535	0.14	0.716
Residual	8	0.535740	0.066986		
Total	23	2.162851			

Table 6.108: Analysis of variance of the formation of TBARS in bacon after 8 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.018794	0.009399	0.07	
Block. Animal Stratum					
Fishmeal	1	0.192990	0.192990	1.43	0.276
Vitamin E	1	0.795498	0.795498	5.91	0.051
Fishmeal. Vitamin E	1	0.084840	0.084840	0.63	0.458
Residual	6	0.807859	0.134644	4.47	
Block. Animal. Side stratum					
Process	1	0.344274	0.344274	11.43	**0.010**
Fishmeal. Process	1	0.084748	0.084748	2.81	0.132
Vitamin E. Process	1	0.076467	0.076467	2.54	0.150
Fishmeal. Vitamin E. Process	1	0.053477	0.053477	1.78	0.219
Residual	8	0.240988	0.030124		
Total	23	2.699936			

Table 6.109: Analysis of variance of the formation of TBARS in bacon after 10 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.021732	0.010890	0.34	
Block. Animal Stratum					
Fishmeal	1	0.043221	0.043221	1.37	0.286
Vitamin E	1	0.962628	0.962628	30.52	**0.001**
Fishmeal. Vitamin E	1	0.092202	0.092202	2.92	0.138
Residual	6	0.189292	0.031557	0.52	
Block. Animal. Side stratum					
Process	1	1.849170	1.849170	30.38	< **0.001**
Fishmeal. Process	1	0.032864	0.032864	0.54	0.483
Vitamin E. Process	1	0.947333	0.947333	15.56	**0.004**
Fishmeal. Vitamin E. Process	1	0.018392	0.018392	0.30	0.597
Residual	8	0.486952	0.060887		
Total	23	4.643786			

Table 6.110: Analysis of variance of the formation of TBARS in bacon after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.076375	0.038188	0.60	
Block. Animal Stratum					
Fishmeal	1	0.172299	0.172299	2.69	0.152
Vitamin E	1	0.654823	0.654823	10.21	**0.019**
Fishmeal. Vitamin E	1	0.035201	0.035201	0.55	0.487
Residual	6	0.384736	0.064120	4.20	
Block. Animal. Side stratum					
Process	1	2.353755	2.353755	153.99	< **0.001**
Fishmeal. Process	1	0.014704	0.014704	0.96	0.355
Vitamin E. Process	1	0.685068	0.685068	44.82	< **0.001**
Fishmeal. Vitamin E. Process	1	0.019254	0.019254	1.26	0.294
Residual	8	0.122278	0.015285		
Total	23	4.518498			

Table 6.111: Analysis of variance of the formation of TBARS in bacon after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.000876	0.000436	0.04	
Block. Animal Stratum					
Fishmeal	1	0.117399	0.117399	9.61	**0.021**
Vitamin E	1	0.632656	0.632656	51.79	< **0.001**
Fishmeal. Vitamin E	1	0.047350	0.047350	3.88	0.097
Residual	6	0.073302	0.012216	1.94	
Block. Animal. Side stratum					
Process	1	2.264486	2.264486	359.49	< **0.001**
Fishmeal. Process	1	0.003282	0.003282	0.52	0.491
Vitamin E. Process	1	0.378462	0.378462	107.71	< **0.001**
Fishmeal. Vitamin E. Process	1	0.018842	0.018842	2.99	0.122
Residual	8	0.050394	0.006297		
Total	23	3.887043			

Table 6.112: Analysis of variance of the fluorescence shift in bacon after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.33899	0.16950	0.69	
Block. Animal Stratum					
Fishmeal	1	0.47041	0.47041	1.91	0.216
Vitamin E	1	0.04139	0.04139	0.17	0.696
Fishmeal. Vitamin E	1	1.74082	1.74082	7.08	**0.037**
Residual	6	1.17178	0.24580	6.68	
Block. Animal. Side stratum					
Process	1	4.44782	4.44782	120.81	< **0.001**
Fishmeal. Process	1	0.00571	0.00571	0.16	0.704
Vitamin E. Process	1	0.49920	0.49920	13.56	**0.006**
Fishmeal. Vitamin E. Process	1	0.96919	0.96919	26.32	< **0.001**
Residual	8	0.29454	0.03682		
Total	23	10.28285			

Table 6.113: Analysis of variance of the fluorescence shift in bacon after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.135	0.067	0.03	
Block. Animal Stratum					
Fishmeal	1	26.250	26.250	11.09	**0.016**
Vitamin E	1	16.027	16.027	6.77	**0.041**
Fishmeal. Vitamin E	1	10.703	10.703	4.52	**0.078**
Residual	6	14.205	2.368	1.67	
Block. Animal. Side stratum					
Process	1	55.630	55.630	39.31	**< 0.001**
Fishmeal. Process	1	4.582	4.582	3.24	0.110
Vitamin E. Process	1	4.312	4.312	3.05	0.119
Fishmeal. Vitamin E. Process	1	8.528	8.528	6.03	**0.040**
Residual	8	11.322	1.415		
Total	23	151.694			

Table 6.114: Analysis of variance of the fluorescence shift in bacon after 6 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.8793	1.4397	0.52	
Block. Animal Stratum					
Fishmeal	1	17.4695	17.4695	6.32	**0.046**
Vitamin E	1	10.7428	10.7428	3.88	0.096
Fishmeal. Vitamin E	1	2.8915	2.8915	1.05	0.346
Residual	6	16.5977	2.7663	3.71	
Block. Animal. Side stratum					
Process	1	40.8927	40.8927	54.81	**< 0.001**
Fishmeal. Process	1	5.9088	5.9088	7.92	0.023
Vitamin E. Process	1	0.7401	0.7401	0.99	0.348
Fishmeal. Vitamin E. Process	1	7.5524	7.5524	10.12	**0.013**
Residual	8	5.9683	0.7460		
Total	23	111.6433			

Table 6.115: Analysis of variance of the fluorescence shift in bacon after 8 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	2.727	1.363	0.22	
Block. Animal Stratum					
Fishmeal	1	7.599	7.599	1.25	0.307
Vitamin E	1	14.587	14.587	2.39	0.173
Fishmeal. Vitamin E	1	4.643	4.643	0.76	0.417
Residual	6	36.607	6.101	2.23	
Block. Animal. Side stratum					
Process	1	63.290	6.290	23.10	**0.001**
Fishmeal. Process	1	0.963	0.963	0.35	0.570
Vitamin E. Process	1	0.145	0.145	0.05	0.824
Fishmeal. Vitamin E. Process	1	10.357	10.357	3.78	0.088
Residual	8	21.918	2.740		
Total	23	162.837			

Table 6.116: Analysis of variance of the fluorescence shift in bacon after 10 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	6.288	3.144	0.92	
Block. Animal Stratum					
Fishmeal	1	8.034	8.034	2.36	0.176
Vitamin E	1	21.040	21.040	6.17	**0.048**
Fishmeal. Vitamin E	1	7.639	7.639	2.24	0.185
Residual	6	20.464	3.411	1.17	
Block. Animal. Side stratum					
Process	1	53.777	53.777	18.49	**0.003**
Fishmeal. Process	1	1.510	1.510	0.52	0.492
Vitamin E. Process	1	0.783	0.783	0.27	0.618
Fishmeal. Vitamin E. Process	1	4.458	4.458	1.53	0.251
Residual	8	23.267	2.908		
Total	23	147.260			

Table 6.117: Analysis of variance of the fluorescence shift in bacon after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	14.919	7.459	4.45	
Block. Animal Stratum					
Fishmeal	1	17.909	17.909	10.67	**0.017**
Vitamin E	1	19.039	19.039	11.35	**0.015**
Fishmeal. Vitamin E	1	5.309	5.309	3.16	0.126
Residual	6	10.068	1.678	0.93	
Block. Animal. Side stratum					
Process	1	58.689	58.689	32.57	**< 0.001**
Fishmeal. Process	1	2.151	2.151	1.19	0.306
Vitamin E. Process	1	1.872	1.872	1.04	0.338
Fishmeal. Vitamin E. Process	1	0.896	0.896	0.50	0.501
Residual	8	14.416	1.802		
Total	23	145.270			

Table 6.118: Analysis of variance of the fluorescence shift in bacon after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.295	0.147	0.08	
Block. Animal Stratum					
Fishmeal	1	16.657	16.657	9.49	0.022
Vitamin E	1	41.462	41.462	23.63	**0.003**
Fishmeal. Vitamin E	1	6.891	6.891	3.93	0.095
Residual	6	10.527	1.754	0.63	
Block. Animal. Side stratum					
Process	1	119.102	119.102	42.84	**< 0.001**
Fishmeal. Process	1	0.215	0.215	0.08	0.788
Vitamin E. Process	1	1.789	1.789	0.64	0.446
Fishmeal. Vitamin E. Process	1	0.037	0.037	0.01	0.911
Residual	8	22.241	2.780		
Total	23	219.214			

Table 6.119: Analysis of variance of the sensory evaluation of bacon after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.0408	0.0204	0.06	
Block. Animal Stratum					
Fishmeal	1	0.6667	0.6667	1.86	0.221
Vitamin E	1	0.2017	0.2017	0.56	0.481
Fishmeal. Vitamin E	1	0.1350	0.1350	0.38	0.562
Residual	6	2.1492	0.3582	1.01	
Block. Animal. Side stratum					
Process	1	0.7350	0.7350	2.07	0.188
Fishmeal. Process	1	0.0817	0.0817	0.23	0.644
Vitamin E. Process	1	0.2400	0.2400	0.68	0.435
Fishmeal. Vitamin E. Process	1	0.1067	0.1067	0.30	0.598
Residual	8	2.8367	0.3546		
Total	23	7.1933			

Table 6.120: Analysis of variance of the sensory evaluation of bacon after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.2356	0.1178	0.43	
Block. Animal Stratum					
Fishmeal	1	0.0622	0.0622	0.23	0.651
Vitamin E	1	0.6301	0.6301	2.30	0.180
Fishmeal. Vitamin E	1	0.0869	0.0869	0.32	0.594
Residual	6	1.6451	0.2742	1.23	
Block. Animal. Side stratum					
Process	1	0.0622	0.0622	0.28	0.611
Fishmeal. Process	1	2.7413	2.7413	12.31	**0.008**
Vitamin E. Process	1	0.0005	0.0005	0.00	0.963
Fishmeal. Vitamin E. Process	1	0.0869	0.0869	0.39	0.550
Residual	8	1.7919	0.2227		
Total	23	7.3328			

Table 6.121: Analysis of variance of the sensory evaluation of bacon after 6 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	1.7658	0.8829	1.47	
Block. Animal Stratum					
Fishmeal	1	1.5000	1.5000	2.49	0.165
Vitamin E	1	2.4067	2.4067	4.00	0.092
Fishmeal. Vitamin E	1	1.5000	1.5000	2.49	0.165
Residual	6	3.6108	0.6018	1.87	
Block. Animal. Side stratum					
Process	1	1.7067	1.7067	5.31	0.050
Fishmeal. Process	1	0.0600	0.0600	0.19	0.677
Vitamin E. Process	1	0.3267	0.3267	1.02	0.343
Fishmeal. Vitamin E. Process	1	1.1267	1.1267	3.51	0.098
Residual	8	2.5700	0.3212		
Total	23	16.5733			

Table 6.122: Analysis of variance of the sensory evaluation of bacon after 10 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	4.1033	2.0517	5.55	
Block. Animal Stratum					
Fishmeal	1	1.4504	1.4504	3.92	0.095
Vitamin E	1	0.0104	0.0104	0.03	0.872
Fishmeal. Vitamin E	1	0.0104	0.0104	0.03	0.872
Residual	6	2.2200	0.3700	0.77	
Block. Animal. Side stratum					
Process	1	0.2604	0.2604	0.54	0.481
Fishmeal. Process	1	0.1504	0.1504	0.31	0.590
Vitamin E. Process	1	0.4004	0.4004	0.84	0.387
Fishmeal. Vitamin E. Process	1	0.7704	0.7704	1.61	0.240
Residual	8	3.8233	0.4779		
Total	23	13.1996			

Table 6.123: Analysis of variance of the sensory evaluation of bacon after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	3.2122	1.6061	0.81	
Block. Animal Stratum					
Fishmeal	1	3.1901	3.1901	1.62	0.251
Vitamin E	1	2.3438	2.3438	1.19	0.318
Fishmeal. Vitamin E	1	0.2604	0.2604	0.13	0.729
Residual	6	11.8346	1.9724	2.74	
Block. Animal. Side stratum					
Process	1	0.0104	0.0104	0.01	0.907
Fishmeal. Process	1	0.0938	0.0938	0.13	0.728
Vitamin E. Process	1	3.1901	3.1901	4.43	0.069
Fishmeal. Vitamin E. Process	1	0.0026	0.0026	0.00	0.954
Residual	8	5.7656	0.7207		
Total	23	29.9036			

Table 6.124: Analysis of variance of the sensory evaluation of bacon after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Block stratum	2	0.3179	0.1590	0.68	
Block. Animal Stratum					
Fishmeal	1	0.1487	0.1487	0.64	0.454
Vitamin E	1	0.0046	0.0046	0.02	0.892
Fishmeal. Vitamin E	1	1.7906	1.7906	7.71	**0.032**
Residual	6	1.3940	0.2323	0.40	
Block. Animal. Side stratum					
Process	1	0.1157	0.1157	0.20	0.666
Fishmeal. Process	1	1.4450	1.4450	2.50	0.152
Vitamin E. Process	1	2.5931	2.5931	4.49	0.067
Fishmeal. Vitamin E. Process	1	0.4326	0.4326	0.75	0.412
Residual	8	4.6173	0.5772		
Total	23	12.8596			

6.5.3 Statistical analysis of sensory evaluation of bacon – Student's t-test

Table 6.125: Sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke. (Standard deviation, SD and mean), (n = 6).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.601	0.598	0.465	0.775	0.644	0.672
Mean	2.383	2.426	3.800	3.600	3.354	3.241

Table 6.126: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 6).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-0.54	-3.38	-2.91	-2.70	-2.57
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.127: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 6).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-3.46	-2.95	-2.73	-2.54
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	*	*	*	*

* significant at $t_{0.050}$

Table 6.128: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke, Week 6 versus Week 10, 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	0.72	1.76	2.00
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02
Conclusions	NS	NS	NS

NS, not significant

Table 6.129: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke, Week 10 versus Week 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	0.79	1.06
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02
Conclusions	NS	NS

NS, not significant

Table 6.130: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – wood smoke, Week 12 versus Week 16. (n = 6).

	Week 6 versus Week 10
Calculated t-statistics	0.36
Tabulate t-value ($t_{0.050}$)	± 2.02
Conclusions	NS

NS, not significant

Table 6.131: Sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke. (Standard deviation, SD and mean), (n = 6).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.615	0.552	1.147	0.242	1.359	0.687
Mean	2.233	2.519	3.500	4.067	4.042	4.037

Table 6.132: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 6).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-1.06	-2.41	-3.37	-2.85	-3.36
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.133: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 6).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-2.18	-3.46	-2.77	-3.42
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	*	*	*	*

* significant at $t_{0.050}$

Table 6.134: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke, Week 6 versus Week 10, 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-1.50	-0.90	-1.17
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02
Conclusions	NS	NS	NS

NS, not significant

Table 6.135: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke, Week 10 versus Week 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	0.068	0.14
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02
Conclusions	NS	NS

NS, not significant

Table 6.136: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – wood smoke, Week 12 versus Week 16. (n = 6).

	Week 6 versus Week 10
Calculated t-statistics	0.010
Tabulate t-value ($t_{0.050}$)	± 2.02
Conclusions	NS

NS, not significant

Table 6.137: Sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke. (Standard deviation, SD and mean), (n = 6).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.613	0.568	0.837	1.094	1.332	0.824
Mean	2.400	2.093	3.400	3.900	4.708	3.870

Table 6.138: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 6).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	1.27	-2.42	-2.76	-3.26	-3.18
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.139: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 6).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-3.08	-3.07	-3.35	-3.46
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	*	*	*	*

* significant at $t_{0.050}$

Table 6.140: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke, Week 6 versus Week 10, 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-1.08	-2.34	-1.26
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	NS

NS, not significant
* significant at $t_{0.050}$

Table 6.141: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke, Week 10 versus Week 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-1.44	0.074
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02
Conclusions	NS	NS

NS, not significant

Table 6.142: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, control diet – liquid and wood smoke, Week 12 versus Week 16. (n = 6).

	Week 6 versus Week 10
Calculated t-statistics	1.57
Tabulate t-value ($t_{0.050}$)	± 2.02
Conclusions	NS

NS, not significant

Table 6.143: Sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke. (Standard deviation, SD and mean), (n = 6).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.295	0.585	0.441	0.807	0.914	0.635
Mean	1.850	2.204	2.633	3.850	3.938	3.352

Table 6.144: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 6).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-1.71	-3.02	-3.46	-3.32	-3.27
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.145: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 6).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-1.68	-3.33	-3.11	-2.75
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02	± 2.02
Conclusions	NS	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.146: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke, Week 6 versus Week 10, 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-3.28	-2.95	-2.32
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02	± 2.02
Conclusions	*	*	*

* significant at $t_{0.050}$

Table 6.147: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke, Week 10 versus Week 12, 16. (n = 6).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-0.23	1.57
Tabulate t-value ($t_{0.050}$)	± 2.02	± 2.02
Conclusions	NS	NS

NS, not significant

Table 6.148: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, vitamin E supplemented diet – liquid and wood smoke, Week 12 versus Week 16. (n = 6).

	Week 6 versus Week 10
Calculated t-statistics	1.73
Tabulate t-value ($t_{0.050}$)	± 2.02
Conclusions	NS

NS, not significant

Table 6.149: Sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. (Standard deviation, SD and mean), (n = 12).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.616	0.467	0.693	0.619	1.147	0.828
Mean	2.383	2.361	3.583	4.100	4.375	3.704

Table 6.150: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. Week 0 versus Week 4, 6, 10, 12, 16. (n = 12).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	0.13	-3.92	-4.73	-4.79	-3.95
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80	± 1.80	± 1.80
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.151: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. Week 4 versus Week 6, 10, 12, 16. (n = 12).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-4.33	-4.84	-4.80	-4.28
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80	± 1.80
Conclusions	*	*	*	*

* significant at $t_{0.050}$

Table 6.152: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. Week 6 versus Week 10, 12, 16. (n = 12).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-2.15	-2.46	-0.48
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80
Conclusions	*	*	NS

NS, not significant
* significant at $t_{0.050}$

Table 6.153: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. Week 10 versus Week 12, 16. (n = 12).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-0.95	1.66
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80
Conclusions	NS	NS

NS, not significant

Table 6.154: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets without fishmeal. Week 12 versus Week 16. (n = 12).

	Week 6 versus Week 10
Calculated t-statistics	2.06
Tabulate t-value ($t_{0.050}$)	± 1.80
Conclusions	*

* significant at $t_{0.050}$

Table 6.155: Sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. (Standard deviation, SD and mean), (n = 12).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.462	0.666	0.944	0.827	1.055	0.686
Mean	2.050	2.259	3.083	3.608	3.646	3.546

Table 6.156: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. Week 0 versus Week 4, 6, 10, 12, 16. (n = 12).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-1.05	-3.78	-4.49	-4.28	-4.66
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80	± 1.80	± 1.80
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.157: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. Week 4 versus Week 6, 10, 12, 16. (n = 12).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-2.89	-4.07	-3.82	-4.23
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80	± 1.80
Conclusions	*	*	*	*

* significant at $t_{0.050}$

Table 6.158: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. Week 6 versus Week 10, 12, 16. (n = 12).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-1.71	-1.61	-1.65
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80	± 1.80
Conclusions	NS	NS	NS

NS, not significant

Table 6.159: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. Week 10 versus Week 12, 16. (n = 12).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-0.13	0.27
Tabulate t-value ($t_{0.050}$)	± 1.80	± 1.80
Conclusions	NS	NS

NS, not significant

Table 6.160: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diets containing fishmeal. Week 12 versus Week 16. (n = 12).

	Week 6 versus Week 10
Calculated t-statistics	0.37
Tabulate t-value ($t_{0.050}$)	± 1.80
Conclusions	NS

NS, not significant

Table 6.161: Sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke. (Standard deviation, SD and mean), (n = 3).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.693	0.570	0.451	0.808	0.946	0.231
Mean	2.300	2.037	4.067	3.233	3.167	3.815

Table 6.162: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 3).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	0.62	-2.45	-2.06	-2.08	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	NS	*	NS	NS	*

NS, not significant
* significant at $t_{0.050}$

Table 6.163: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 3).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-2.45	-2.24	-2.23	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.035	± 2.35	± 2.35
Conclusions	*	NS	NS	*

NS, not significant
* significant at $t_{0.050}$

Table 6.164: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke, Week 6 versus Week 10, 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	1.75	1.65	1.19
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35
Conclusions	NS	NS	NS

NS, not significant

Table 6.165: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke, Week 10 versus Week 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	0.13	-1.48
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35
Conclusions	NS	NS

NS, not significant

Table 6.166: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – wood smoke, Week 12 versus Week 16. (n = 3).

	Week 6 versus Week 10
Calculated t-statistics	-1.38
Tabulate t-value ($t_{0.050}$)	± 2.35
Conclusions	NS

NS, not significant

Table 6.167: Sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke. (Standard deviation, SD and mean), (n = 3).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.462	0.501	0.416	1.557	0.722	0.570
Mean	2.033	1.704	2.733	3.933	4.292	3.630

Table 6.168: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 3).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	1.00	-2.45	-1.98	-2.45	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.169: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 3).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-2.44	-2.12	-2.45	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	*	NS	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.170: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke, Week 6 versus Week 10, 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-1.56	-2.45	-2.13
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35
Conclusions	NS	*	NS

NS, not significant
* significant at $t_{0.050}$

Table 6.171: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke, Week 10 versus Week 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-0.57	0.49
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35
Conclusions	NS	NS

NS, not significant

Table 6.172: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – wood smoke, Week 12 versus Week 16. (n = 3).

	Week 6 versus Week 10
Calculated t-statistics	1.85
Tabulate t-value ($t_{0.050}$)	± 2.35
Conclusions	NS

NS, not significant

Table 6.173: Sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke. (Standard deviation, SD and mean), (n = 3).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.462	0.257	0.365	0.200	1.774	0.996
Mean	2.133	2.926	3.233	3.900	3.708	3.852

Table 6.174: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 3).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-2.45	-1.73	-2.45	-1.83	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	*	NS	*	NS	*

NS, not significant
* significant at $t_{0.050}$

Table 6.175: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 3).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	-0.64	-2.45	-1.22	-2.11
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	NS	*	NS	NS

NS, not significant
* significant at $t_{0.050}$

Table 6.176: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke, Week 6 versus Week 10, 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-1.15	-0.52	-0.87
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35
Conclusions	NS	NS	NS

NS, not significant

Table 6.177: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke, Week 10 versus Week 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	0.32	0.13
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35
Conclusions	NS	NS

NS, not significant

Table 6.178: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, diet containing fishmeal – liquid and wood smoke, Week 12 versus Week 16. (n = 3).

	Week 6 versus Week 10
Calculated t-statistics	1.57
Tabulate t-value ($t_{0.050}$)	± 2.02
Conclusions	NS

NS, not significant

Table 6.179: Sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke. (Standard deviation, SD and mean), (n = 3).

	Week 0	Week 4	Week 6	Week 10	Week 12	Week 16
SD	0.115	0.756	0.265	0.252	0.711	0.556
Mean	1.733	2.370	2.300	3.367	3.417	2.889

Table 6.180: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke, Week 0 versus Week 4, 6, 10, 12, 16. (n = 3).

	Week 0 versus Week 4	Week 0 versus Week 6	Week 0 versus Week 10	Week 0 versus Week 12	Week 0 versus Week 16
Calculated t-statistics	-1.90	-2.45	-2.45	-2.45	-2.45
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	NS	*	*	*	*

NS, not significant
* significant at $t_{0.050}$

Table 6.181: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke, Week 4 versus Week 6, 10, 12, 16. (n = 3).

	Week 4 versus Week 6	Week 4 versus Week 10	Week 4 versus Week 12	Week 4 versus Week 16
Calculated t-statistics	0.22	-1.98	-1.77	-1.14
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35	± 2.35
Conclusions	NS	NS	NS	NS

NS, not significant

Table 6.182: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke, Week 6 versus Week 10, 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12	Week 6 versus Week 16
Calculated t-statistics	-2.45	-2.15	-1.88
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35	± 2.35
Conclusions	*	NS	NS

NS, not significant
* significant at $t_{0.050}$

Table 6.183: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke, Week 10 versus Week 12, 16. (n = 3).

	Week 6 versus Week 10	Week 6 versus Week 12
Calculated t-statistics	-0.17	1.65
Tabulate t-value ($t_{0.050}$)	± 2.35	± 2.35
Conclusions	NS	NS

NS, not significant

Table 6.184: Student's t-test of the sensory evaluation of bacon after 16 weeks storage at –20°C, fishmeal and vitamin E supplemented diet – liquid and wood smoke, Week 12 versus Week 16. (n = 3).

	Week 6 versus Week 10
Calculated t-statistics	1.20
Tabulate t-value ($t_{0.050}$)	± 2.35
Conclusions	NS

NS, not significant

6.6 WIENERS

6.6.1 Statistical analysis of wieners

Table 6.185: Analysis of variance of the formation of TBARS in wieners after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.147×10^{-2}	0.147×10^{-2}		
Vitamin E	1	0.191×10^{-5}	0.191×10^{-5}		
Fishmeal. Vitamin E	1	0.349×10^{-2}	0.349×10^{-2}		
Group. Batch stratum					
Antioxidant	2	0.674×10^{-3}	0.337×10^{-3}	209.55	**0.005**
Fishmeal. Antioxidant	2	0.218×10^{-4}	0.109×10^{-4}	6.78	0.129
Vitamin E. Antioxidant	2	0.144×10^{-3}	0.720×10^{-4}	44.75	**0.022**
Residual	2	0.322×10^{-5}	0.161×10^{-5}		
Total	11	0.581×10^{-2}			

Table 6.186: Analysis of variance of the formation of TBARS in wieners after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.189×10^{-5}	0.189×10^{-5}		
Vitamin E	1	0.267×10^{-4}	0.267×10^{-4}		
Fishmeal. Vitamin E	1	0.134×10^{-3}	0.134×10^{-3}		
Group. Batch stratum					
Antioxidant	2	0.447×10^{-4}	0.223×10^{-4}	0.57	0.638
Fishmeal. Antioxidant	2	0.638×10^{-4}	0.319×10^{-4}	0.81	0.552
Vitamin E. Antioxidant	2	0.575×10^{-4}	0.288×10^{-4}	0.73	0.578
Residual	2	0.787×10^{-4}	0.393×10^{-4}		
Total	11	0.407×10^{-3}			

Table 6.187: Analysis of variance of the formation of TBARS in wieners after 8 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.189×10^{-3}	0.189×10^{-3}		
Vitamin E	1	0.230×10^{-3}	0.230×10^{-3}		
Fishmeal. Vitamin E	1	0.290×10^{-6}	0.290×10^{-6}		
Group. Batch stratum					
Antioxidant	2	0.855×10^{-4}	0.428×10^{-4}	0.56	0.643
Fishmeal. Antioxidant	2	0.642×10^{-4}	0.321×10^{-4}	0.42	0.706
Vitamin E. Antioxidant	2	0.502×10^{-4}	0.251×10^{-4}	0.33	0.754
Residual	2	0.154×10^{-3}	0.769×10^{-4}		
Total	11	0.773×10^{-3}			

Table 6.188: Analysis of variance of the formation of TBARS in wieners after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.499×10^{-4}	0.499×10^{-4}		
Vitamin E	1	0.190×10^{-4}	0.190×10^{-4}		
Fishmeal. Vitamin E	1	0.111×10^{-4}	0.111×10^{-4}		
Group. Batch stratum					
Antioxidant	2	0.760×10^{-4}	0.380×10^{-4}	2.69	0.271
Fishmeal. Antioxidant	2	0.503×10^{-4}	0.252×10^{-4}	1.78	0.359
Vitamin E. Antioxidant	2	0.224×10^{-4}	0.112×10^{-4}	0.79	0.557
Residual	2	0.282×10^{-4}	0.141×10^{-4}		
Total	11	0.257×10^{-3}			

Table 6.189: Analysis of variance of the formation of TBARS in wieners after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.911×10^{-7}	0.911×10^{-7}		
Vitamin E	1	0.233×10^{-3}	0.233×10^{-3}		
Fishmeal. Vitamin E	1	0.221×10^{-5}	0.221×10^{-5}		
Group. Batch stratum					
Antioxidant	2	0.196×10^{-4}	0.978×10^{-5}	6.48	0.134
Fishmeal. Antioxidant	2	0.639×10^{-5}	0.319×10^{-5}	2.12	0.321
Vitamin E. Antioxidant	2	0.692×10^{-5}	0.346×10^{-5}	2.29	0.304
Residual	2	0.302×10^{-5}	0.151×10^{-5}		
Total	11	0.271×10^{-3}			

Table 6.190: Analysis of variance of the formation of TBARS in wieners after 19 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.165×10^{-5}	0.165×10^{-5}		
Vitamin E	1	0.225×10^{-3}	0.225×10^{-3}		
Fishmeal. Vitamin E	1	0.432×10^{-5}	0.432×10^{-5}		
Group. Batch stratum					
Antioxidant	2	0.189×10^{-4}	0.947×10^{-5}	4.27	0.190
Fishmeal. Antioxidant	2	0.223×10^{-4}	0.112×10^{-4}	5.03	0.166
Vitamin E. Antioxidant	2	0.532×10^{-5}	0.266×10^{-5}	1.20	0.455
Residual	2	0.444×10^{-5}	0.222×10^{-5}		
Total	11	0.282×10^{-3}			

Table 6.191: Analysis of variance of the formation of TBARS in wieners after 23 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.136×10^{-4}	0.136×10^{-4}		
Vitamin E	1	0.251×10^{-3}	0.251×10^{-3}		
Fishmeal. Vitamin E	1	0.273×10^{-4}	0.273×10^{-4}		
Group. Batch stratum					
Antioxidant	2	0.164×10^{-4}	0.819×10^{-5}	0.44	0.693
Fishmeal. Antioxidant	2	0.757×10^{-5}	0.378×10^{-5}	0.20	0.830
Vitamin E. Antioxidant	2	0.204×10^{-4}	0.102×10^{-4}	0.55	0.644
Residual	2	0.369×10^{-4}	0.185×10^{-4}		
Total	11	0.374×10^{-3}			

Table 6.192: Analysis of variance of the formation of TBARS in wieners after 27 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.233×10^{-5}	0.233×10^{-5}		
Vitamin E	1	0.191×10^{-3}	0.191×10^{-3}		
Fishmeal. Vitamin E	1	0.169×10^{-6}	0.169×10^{-6}		
Group. Batch stratum					
Antioxidant	2	0.193×10^{-4}	0.964×10^{-5}	0.99	0.503
Fishmeal. Antioxidant	2	0.148×10^{-5}	0.741×10^{-6}	0.08	0.929
Vitamin E. Antioxidant	2	0.125×10^{-4}	0.623×10^{-5}	0.64	0.610
Residual	2	0.195×10^{-4}	0.974×10^{-5}		
Total	11	0.246×10^{-3}			

Table 6.193: Analysis of variance of the formation of TBARS in wieners after 31 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.249×10^{-6}	0.249×10^{-6}		
Vitamin E	1	0.986×10^{-4}	0.986×10^{-4}		
Fishmeal. Vitamin E	1	0.752×10^{-5}	0.752×10^{-5}		
Group. Batch stratum					
Antioxidant	2	0.866×10^{-4}	0.433×10^{-4}	9.92	0.092
Fishmeal. Antioxidant	2	0.246×10^{-5}	0.123×10^{-5}	0.28	0.780
Vitamin E. Antioxidant	2	0.214×10^{-5}	0.107×10^{-5}	0.25	0.803
Residual	2	0.874×10^{-5}	0.437×10^{-5}		
Total	11	0.206×10^{-3}			

Table 6.194: Analysis of variance of the formation of TBARS in wieners after 36 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.148×10^{-5}	0.148×10^{-5}		
Vitamin E	1	0.154×10^{-3}	0.154×10^{-3}		
Fishmeal. Vitamin E	1	0.315×10^{-4}	0.315×10^{-4}		
Group. Batch stratum					
Antioxidant	2	0.452×10^{-4}	0.226×10^{-4}	244.50	**0.004**
Fishmeal. Antioxidant	2	0.261×10^{-5}	0.131×10^{-5}	14.14	**0.066**
Vitamin E. Antioxidant	2	0.100×10^{-4}	0.501×10^{-5}	51.21	**0.018**
Residual	2	0.185×10^{-6}	0.924×10^{-7}		
Total	11	0.245×10^{-3}			

Table 6.195: Analysis of variance of the formation of TBARS in wieners after 40 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.240×10^{-6}	0.240×10^{-6}		
Vitamin E	1	0.754×10^{-4}	0.754×10^{-4}		
Fishmeal. Vitamin E	1	0.187×10^{-4}	0.187×10^{-4}		
Group. Batch stratum					
Antioxidant	2	0.592×10^{-4}	0.296×10^{-4}	17.64	**0.054**
Fishmeal. Antioxidant	2	0.577×10^{-5}	0.288×10^{-5}	1.72	0.368
Vitamin E. Antioxidant	2	0.838×10^{-5}	0.419×10^{-5}	2.50	0.286
Residual	2	0.335×10^{-5}	0.168×10^{-5}		
Total	11	0.171×10^{-3}			

Table 6.196: Analysis of variance of the formation of TBARS in wieners, average TBARS of all weeks.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.584×10^{-5}	0.584×10^{-5}		
Vitamin E	1	0.582×10^{-4}	0.582×10^{-4}		
Fishmeal. Vitamin E	1	0.751×10^{-4}	0.751×10^{-4}		
Group. Batch stratum					
Antioxidant	2	0.278×10^{-4}	0.139×10^{-4}	17.64	0.211
Fishmeal. Antioxidant	2	0.701×10^{-6}	0.351×10^{-6}	1.72	0.914
Vitamin E. Antioxidant	2	0.459×10^{-5}	0.230×10^{-5}	2.50	0.619
Residual	2	0.745×10^{-5}	0.372×10^{-5}		
Total	11	0.180×10^{-3}			

Table 6.197: Analysis of variance of the formation of TBARS in wieners, TBARS slope (slope of linear regression line through the given data points (TBARS data of all weeks – week number)).

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.165×10^{-6}	0.165×10^{-6}		
Vitamin E	1	0.154×10^{-6}	0.154×10^{-6}		
Fishmeal. Vitamin E	1	0.439×10^{-6}	0.439×10^{-6}		
Group. Batch stratum					
Antioxidant	2	0.206×10^{-6}	0.103×10^{-6}	8.85	0.102
Fishmeal. Antioxidant	2	0.575×10^{-7}	0.288×10^{-7}	2.48	0.288
Vitamin E. Antioxidant	2	0.329×10^{-8}	0.164×10^{-8}	0.14	0.876
Residual	2	0.232×10^{-7}	0.116×10^{-7}		
Total	11	0.105×10^{-5}			

Table 6.198: Analysis of variance of the fluorescence shift in wieners after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.4692	0.4692		
Vitamin E	1	2.3054	2.3054		
Fishmeal. Vitamin E	1	0.1113	0.1113		
Group. Batch stratum					
Antioxidant	2	4.9507	2.4754	6.06	0.142
Fishmeal. Antioxidant	2	0.9431	0.4716	1.15	0.464
Vitamin E. Antioxidant	2	0.0057	0.0029	0.01	0.993
Residual	2	0.8174	0.4087		
Total	11	9.6029			

Table 6.199: Analysis of variance of the fluorescence shift in wieners after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	2.293	2.293		
Vitamin E	1	6.376	6.376		
Fishmeal. Vitamin E	1	0.511	0.511		
Group. Batch stratum					
Antioxidant	2	13.378	6.689	2.53	0.283
Fishmeal. Antioxidant	2	0.531	0.266	0.10	0.909
Vitamin E. Antioxidant	2	4.486	0.243	0.85	0.541
Residual	2	5.279	0.640		
Total	11	33.854			

Table 6.200: Analysis of variance of the fluorescence shift in wieners after 8 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.00530	0.00530		
Vitamin E	1	0.74251	0.74251		
Fishmeal. Vitamin E	1	1.02598	1.02598		
Group. Batch stratum					
Antioxidant	2	22.95381	11.47691	358.75	**0.003**
Fishmeal. Antioxidant	2	1.20685	0.60342	18.86	0.050
Vitamin E. Antioxidant	2	3.63131	1.81565	56.75	0.017
Residual	2	0.06398	0.03199		
Total	11	29.62975			

Table 6.201: Analysis of variance of the fluorescence shift in wieners after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.8549	0.8549		
Vitamin E	1	0.8516	0.8516		
Fishmeal. Vitamin E	1	0.3063	0.3063		
Group. Batch stratum					
Antioxidant	2	17.4376	8.7188	17.53	0.054
Fishmeal. Antioxidant	2	1.6715	0.8357	1.68	0.373
Vitamin E. Antioxidant	2	1.1696	0.5848	1.18	0.460
Residual	2	0.9945	0.4973		
Total	11	23.2859			

Table 6.202: Analysis of variance of the fluorescence shift in wieners after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0276	0.0276		
Vitamin E	1	2.0485	2.0485		
Fishmeal. Vitamin E	1	0.0000	0.0000		
Group. Batch stratum					
Antioxidant	2	19.4379	9.7190	25.51	**0.038**
Fishmeal. Antioxidant	2	0.8561	0.4281	1.12	0.471
Vitamin E. Antioxidant	2	0.5543	0.2772	0.73	0.579
Residual	2	0.7621	0.3810		
Total	11	23.6866			

Table 6.203: Analysis of variance of the fluorescence shift in wieners after 19 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0135	0.0135		
Vitamin E	1	1.9420	1.9420		
Fishmeal. Vitamin E	1	0.0062	0.0062		
Group. Batch stratum					
Antioxidant	2	19.4285	9.7143	27.88	**0.035**
Fishmeal. Antioxidant	2	0.8208	0.4104	1.18	0.479
Vitamin E. Antioxidant	2	0.7412	0.3706	1.06	0.485
Residual	2	0.6968	0.3484		
Total	11	23.6491			

Table 6.204: Analysis of variance of the fluorescence shift in wieners after 23 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0079	0.0079		
Vitamin E	1	1.6944	1.6944		
Fishmeal. Vitamin E	1	0.0019	0.0019		
Group. Batch stratum					
Antioxidant	2	19.2912	9.6456	25.37	**0.038**
Fishmeal. Antioxidant	2	0.8306	0.4153	1.09	0.478
Vitamin E. Antioxidant	2	0.6475	0.3237	0.85	0.540
Residual	2	0.7603	0.3801		
Total	11	23.2337			

Table 6.205: Analysis of variance of the fluorescence shift in wieners after 27 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0065	0.0065		
Vitamin E	1	1.1726	1.1726		
Fishmeal. Vitamin E	1	0.1927	0.1927		
Group. Batch stratum					
Antioxidant	2	19.8871	9.9436	34.58	**0.028**
Fishmeal. Antioxidant	2	0.9021	0.4510	1.57	0.389
Vitamin E. Antioxidant	2	0.8878	0.4439	1.54	0.393
Residual	2	0.5751	0.2875		
Total	11	23.6238			

Table 6.206: Analysis of variance of the fluorescence shift in wieners after 31 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0155	0.0155		
Vitamin E	1	1.1107	1.1107		
Fishmeal. Vitamin E	1	0.5152	0.5152		
Group. Batch stratum					
Antioxidant	2	18.5391	9.2695	24.83	**0.039**
Fishmeal. Antioxidant	2	0.4952	0.2476	0.66	0.601
Vitamin E. Antioxidant	2	0.7960	0.3980	1.07	0.484
Residual	2	0.7468	0.3734		
Total	11	22.2185			

Table 6.207: Analysis of variance of the fluorescence shift in wieners after 36 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1577	0.1577		
Vitamin E	1	0.1894	0.1894		
Fishmeal. Vitamin E	1	0.2994	0.2994		
Group. Batch stratum					
Antioxidant	2	18.7185	9.3592	19.27	**0.049**
Fishmeal. Antioxidant	2	0.0522	0.0261	0.05	0.949
Vitamin E. Antioxidant	2	1.8527	0.9264	1.91	0.344
Residual	2	0.9713	0.4857		
Total	11	22.2413			

Table 6.208: Analysis of variance of the fluorescence shift in wieners after 40 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.4705	0.4705		
Vitamin E	1	0.0362	0.0362		
Fishmeal. Vitamin E	1	0.5774	0.5774		
Group. Batch stratum					
Antioxidant	2	17.9659	8.9829	86.96	**0.011**
Fishmeal. Antioxidant	2	0.0924	0.0462	0.45	0.691
Vitamin E. Antioxidant	2	3.1207	1.5603	15.11	**0.062**
Residual	2	0.2066	0.1033		
Total	11	22.4696			

Table 6.209: Analysis of variance of the fluorescence shift in wieners, average fluorescence shift of all weeks.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0019	0.0019		
Vitamin E	1	1.2577	1.2577		
Fishmeal. Vitamin E	1	0.0450	0.0450		
Group. Batch stratum					
Antioxidant	2	16.8671	8.4335	29.25	**0.033**
Fishmeal. Antioxidant	2	0.5201	0.2601	0.90	0.526
Vitamin E. Antioxidant	2	0.1890	0.0945	0.33	0.753
Residual	2	0.5767	0.2884		
Total	11	19.4576			

Table 6.210: Analysis of variance of fluorescence shift in wieners, fluorescence shift slope (slope of linear regression line through the given data points (fluorescence shift data of all weeks – week number)).

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.748×10^{-4}	0.748×10^{-4}		
Vitamin E	1	0.143×10^{-2}	0.143×10^{-2}		
Fishmeal. Vitamin E	1	0.230×10^{-3}	0.230×10^{-3}		
Group. Batch stratum					
Antioxidant	2	0.941×10^{-3}	0.471×10^{-3}	3.61	0.217
Fishmeal. Antioxidant	2	0.436×10^{-3}	0.218×10^{-3}	1.67	0.375
Vitamin E. Antioxidant	2	0.381×10^{-2}	0.190×10^{-2}	14.59	0.064
Residual	2	0.261×10^{-3}	0.131×10^{-3}		
Total	11	0.718×10^{-2}			

Table 6.211: Analysis of variance of the sensory evaluation of wieners after 0 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1169	0.1169		
Vitamin E	1	0.0344	0.0344		
Fishmeal. Vitamin E	1	0.1265	0.1265		
Group. Batch stratum					
Antioxidant	2	0.9550	0.4775	1.15	0.465
Fishmeal. Antioxidant	2	0.1114	0.0557	0.13	0.882
Vitamin E. Antioxidant	2	0.4797	0.2399	0.58	0.634
Residual	2	0.8302	0.4151		
Total	11	2.6542			

Table 6.212: Analysis of variance of the sensory evaluation of wieners after 4 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1499	0.1499		
Vitamin E	1	0.0000	0.0000		
Fishmeal. Vitamin E	1	0.0001	0.0001		
Group. Batch stratum					
Antioxidant	2	0.2752	0.1376	0.45	0.688
Fishmeal. Antioxidant	2	0.6910	0.3455	1.14	0.468
Vitamin E. Antioxidant	2	0.1344	0.0672	0.22	0.819
Residual	2	0.6079	0.3040		
Total	11	1.8586			

Table 6.213: Analysis of variance of the sensory evaluation of wieners after 8 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.00149	0.00149		
Vitamin E	1	0.00331	0.00331		
Fishmeal. Vitamin E	1	0.40969	0.40969		
Group. Batch stratum					
Antioxidant	2	0.51308	0.25654	7.69	0.115
Fishmeal. Antioxidant	2	0.78161	0.39080	11.72	0.079
Vitamin E. Antioxidant	2	0.22100	0.11050	3.31	0.232
Residual	2	0.06672	0.03336		
Total	11	1.99690			

Table 6.214: Analysis of variance of the sensory evaluation of wieners after 12 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.3756	0.3756		
Vitamin E	1	0.0894	0.0894		
Fishmeal. Vitamin E	1	0.2037	0.2037		
Group. Batch stratum					
Antioxidant	2	1.5106	0.7553	6.05	0.142
Fishmeal. Antioxidant	2	0.7905	0.3953	3.17	0.240
Vitamin E. Antioxidant	2	0.0199	0.0099	0.08	0.926
Residual	2	0.2496	0.1248		
Total	11	3.2393			

Table 6.215: Analysis of variance of the sensory evaluation of wieners after 16 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.00629	0.00629		
Vitamin E	1	0.01372	0.01372		
Fishmeal. Vitamin E	1	0.03286	0.03286		
Group. Batch stratum					
Antioxidant	2	0.27119	0.13560	2.90	0.257
Fishmeal. Antioxidant	2	0.03250	0.01625	0.35	0.742
Vitamin E. Antioxidant	2	0.19065	0.09533	2.04	0.329
Residual	2	0.09362	0.04681		
Total	11	0.64084			

Table 6.216: Analysis of variance of the sensory evaluation of wieners after 19 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.22573	0.22573		
Vitamin E	1	0.00509	0.00509		
Fishmeal. Vitamin E	1	0.05828	0.05828		
Group. Batch stratum					
Antioxidant	2	0.12121	0.06061	1.89	0.346
Fishmeal. Antioxidant	2	0.01992	0.00996	0.31	0.763
Vitamin E. Antioxidant	2	0.13688	0.06844	2.13	0.319
Residual	2	0.06422	0.03211		
Total	11	0.63133			

Table 6.217: Analysis of variance of the sensory evaluation of wieners after 23 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1178	0.1178		
Vitamin E	1	0.0278	0.0278		
Fishmeal. Vitamin E	1	0.0278	0.0278		
Group. Batch stratum					
Antioxidant	2	0.1067	0.0534	0.48	0.677
Fishmeal. Antioxidant	2	0.1461	0.0730	0.65	0.604
Vitamin E. Antioxidant	2	0.2099	0.1050	0.94	0.515
Residual	2	0.2232	0.1116		
Total	11	0.8593			

Table 6.218: Analysis of variance of the sensory evaluation of wieners after 27 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1473	0.1473		
Vitamin E	1	0.3164	0.3164		
Fishmeal. Vitamin E	1	0.5829	0.5829		
Group. Batch stratum					
Antioxidant	2	1.3009	0.6504	4.78	0.173
Fishmeal. Antioxidant	2	0.0494	0.0247	0.18	0.846
Vitamin E. Antioxidant	2	0.1043	0.0522	0.38	0.723
Residual	2	0.2724	0.1362		
Total	11	2.7735			

Table 6.219: Analysis of variance of the sensory evaluation of wieners after 31 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.1064	0.1064		
Vitamin E	1	0.0026	0.0026		
Fishmeal. Vitamin E	1	0.0260	0.0260		
Group. Batch stratum					
Antioxidant	2	1.7964	0.8982	5.96	0.144
Fishmeal. Antioxidant	2	0.0319	0.0160	0.11	0.904
Vitamin E. Antioxidant	2	0.1481	0.0740	0.49	0.671
Residual	2	0.3015	0.1507		
Total	11	2.4130			

Table 6.220: Analysis of variance of the sensory evaluation of wieners after 36 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.0062	0.0062		
Vitamin E	1	0.4241	0.4241		
Fishmeal. Vitamin E	1	0.0915	0.0915		
Group. Batch stratum					
Antioxidant	2	0.0709	0.0354	0.13	0.882
Fishmeal. Antioxidant	2	0.1161	0.0581	0.22	0.820
Vitamin E. Antioxidant	2	0.0962	0.0481	0.18	0.846
Residual	2	0.5305	0.2652		
Total	11	1.3355			

Table 6.221: Analysis of variance of the sensory evaluation of wieners after 40 weeks storage at –20°C.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.10069	0.10069		
Vitamin E	1	0.05677	0.05677		
Fishmeal. Vitamin E	1	0.00376	0.00376		
Group. Batch stratum					
Antioxidant	2	0.84134	0.42067	15.18	0.062
Fishmeal. Antioxidant	2	0.67388	0.33694	12.16	0.076
Vitamin E. Antioxidant	2	0.16931	0.08466	3.06	0.247
Residual	2	0.05542	0.02771		
Total	11	1.90116			

Table 6.222: Analysis of variance of the sensory evaluation of wieners, average sensory scores of all weeks.

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.09467	0.09467		
Vitamin E	1	0.02322	0.02322		
Fishmeal. Vitamin E	1	0.00002	0.00002		
Group. Batch stratum					
Antioxidant	2	0.50572	0.25286	6.18	0.139
Fishmeal. Antioxidant	2	0.03171	0.01585	0.39	0.721
Vitamin E. Antioxidant	2	0.03839	0.01919	0.47	0.681
Residual	2	0.08188	0.04094		
Total	11	0.77560			

Table 6.223: Analysis of variance of the sensory evaluation of wieners, sensory score slope (slope of linear regression line through the given data points (sensory score data of all weeks – week number)).

Source of variation	d.f.	s.s.	m.s.	F	p-value
Group stratum					
Fishmeal	1	0.032×10^{-4}	0.032×10^{-4}		
Vitamin E	1	0.184×10^{-3}	0.184×10^{-3}		
Fishmeal. Vitamin E	1	0.305×10^{-3}	0.305×10^{-3}		
Group. Batch stratum					
Antioxidant	2	0.127×10^{-3}	0.636×10^{-4}	0.22	0.820
Fishmeal. Antioxidant	2	0.501×10^{-3}	0.251×10^{-3}	0.86	0.537
Vitamin E. Antioxidant	2	0.680×10^{-4}	0.340×10^{-4}	0.12	0.895
Residual	2	0.581×10^{-3}	0.291×10^{-3}		
Total	11	0.177×10^{-2}			

6.6.2 Summary of results

Table 6.224: The effect of dietary treatments on formation of TBARS in wieners during storage at –20°C (n = 3), (mean values in mg malonaldehyde /kg sample ± SEM).

Sample	TBARS Week 0	TBARS Week 4	TBARS Week 8	TBARS Week 12	TBARS Week 16	TBARS Week 19	TBARS Week 23	TBARS Week 27	TBARS Week 31	TBARS Week 36	TBARS Week 40
Control	0.116 ± 0.0029	0.074 ± 0.0027	0.109 ± 0.0066	0.150 ± 0.0036	0.163 ± 0.0008	0.164 ± 0.0005	0.166 ± 0.0014	0.165 ± 0.0012	0.164 ± 0.0013	0.167 ± 0.0015	0.165 ± 0.0017
Vitamin E supplemented diet	0.083 ± 0.0083	0.064 ± 0.0044	0.118 ± 0.0026	0.146 ± 0.0033	0.154 ± 0.0005	0.154 ± 0.0014	0.154 ± 0.0008	0.156 ± 0.0010	0.157 ± 0.0019	0.156 ± 0.0013	0.158 ± 0.0011
Fishmeal supplemented diet	0.104 ± 0.0032	0.068 ± 0.0027	0.102 ± 0.0001	0.152 ± 0.0021	0.163 ± 0.0021	0.162 ± 0.0018	0.161 ± 0.0032	0.164 ± 0.0025	0.163 ± 0.0031	0.163 ± 0.0015	0.163 ± 0.0019
Vitamin E + fishmeal supplemented diet	0.139 ± 0.0072	0.071 ± 0.0026	0.110 ± 0.0029	0.152 ± 0.0012	0.155 ± 0.0007	0.154 ± 0.0017	0.155 ± 0.0007	0.156 ± 0.0003	0.159 ± 0.0013	0.159 ± 0.0019	0.161 ± 0.0022

Table 6.225: The effect of antioxidants on formation of TBARS in wieners during storage at –20°C (n = 3), (mean values in mg malonaldehyde/kg sample).

Sample	TBARS Week 0	TBARS Week 4	TBARS Week 8	TBARS Week 12	TBARS Week 16	TBARS Week 19	TBARS Week 23	TBARS Week 27	TBARS Week 31	TBARS Week 36	TBARS Week 40
No antioxidants	0.10	0.07	0.11	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16
0.03% rosemary extract	0.11	0.07	0.11	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16
2.5% whey powder	0.12	0.07	0.11	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16

Table 6.226: The effect of antioxidants on fluorescence shift (dF) in wieners during storage at –20°C (n = 3), (mean values).

Sample	dF Week 0	dF Week 4	dF Week 8	dF Week 12	dF Week 16	dF Week 19	dF Week 23	dF Week 27	dF Week 31	dF Week 36	dF Week 40
No antioxidants	10.96	12.28	12.06	13.00	13.62	13.70	13.73	13.76	13.75	14.01	14.46
0.03% rosemary extract	12.09	13.39	13.56	14.18	15.19	15.27	15.31	15.37	15.21	15.34	15.38
2.5% whey powder	10.58	10.81	10.18	11.25	12.07	12.16	12.20	12.22	12.16	12.29	12.45