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Improving oxidative stability of omega-3 enriched pork meat by addition of food grade sugars and sensory characterization of cooked and re-warmed pork meat patties by free choice profiling

by

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DEDICATION

This thesis is dedicated to my wife, Abitha and my son, Rohan

ABSTRACT

Meat patties (15% fat) were made using pork trim obtained from pigs fed a control diet or a diet containing 10% flax for 6 weeks, with vitamin E added at either 40 mg/kg feed (control) or 400 mg/kg feed in a 2×2 factorial design. Food grade sugars sucrose, glucose, xylose or no sugar (control) were added at a 2% level to ground pork in a split plot design. Dietary flax supplementation increased *n*-3 fatty acid concentrations and reduced *n*-6: *n*-3 ratio. Addition of sugars (glucose and sucrose) improved the oxidative stability of all meat patties over refrigerated storage and provided oxidative stability to only the high vitamin E/No flax treatment after cooking as measured by thiobarbituric acid reactive substances assay. Sensory study using free choice profiling approach conducted on pork meat patties showed that addition of sugars produced distinct sensory characteristics irrespective of the source grind fatty acid or tocopherol composition.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVATIONS

a*	red–green spectral axis,
b*	yellow–blue spectral axis
ALA	alpha linolenic acid
ATP	adenosine tri-phosphate
BHA	butylated hydroxyanisol
BHT	butylated hydroxytoluene
CFIA	Canadian Food Inspection Agency
COX	cyclooxygenase
DDGS	dried distillers grains with soluble
DFD	dark, firm and dry
DHA	docosahexaenoic acid
DMB	deoxymyoglobin
DPA	docosapentaenoic acid
EDTA	ethylenediaminetetracetic acid
EPA	eicosapentaenoic acid
FAME	fatty acid methyl ester
FCP	free-Choice Profiling
FCR	feed conversion ratio
GPA	Generalised Procrustes analysis
HPLC	high performance liquid chromatography
L*	lightness
LA	linoleic acid
LCPUFA	long chain polyunsaturated fatty acids

LOX	lipooxygenase
LSMEANS	least square means
LT	leukotrienes
LT	M. longissimus thoracis
LX	lipoxins
MCOOL	Mandatory Country of Origin Labelling
MDA	malondialdehyde
MMB	metmyoglobin
MRP	maillard reaction products
MUFA	monounsaturated fatty acids
n-3	omega-3
n-6	omega-6
NADH	nicotinamide adenine dinucleotide
OMB	oxymyoglobin
PG	prostaglandins
PROP	6-thiopropyluracil
P:S	polyunsaturated : saturated
PUFA	polyunsaturated fatty acids
SD	standard deviation
TBA-MDA	thiobarbituric acid malonaldehyde complex
TBARS	thiobarbituric acid reacting substances assay
TBHQ	tertiary butylhydroquinone
TCA	trichloracetic acid
TX	thromboxanes
WOF	warmed over flavour

CHAPTER 1

LITERATURE REVIEW

1.1. Trends in meat consumption

Meat is a good source of protein, minerals, vitamins, fats and fatty acids. Trends in meat consumption and how it relates to human health, as well as consumer behaviour and attitude towards meat consumption have been a matter of research in recent times (Delgado, 2003; Grunert, 2006). Nutritionally, meat is important for its high quality protein, which contains all essential amino acids and has highly bio-available minerals and vitamins (Williams, 2007). While meat consumption has been relatively static in the developed world, annual per capita consumption of meat has doubled since 1980 in developing countries and the world meat production is expected to be doubled by 2050 (FAO, 2009). Delgado (2003) stated that total meat consumption in the developing countries has increased considerably and predicted that 63% of the total meat consumption will be from developing countries by year 2020. Average meat consumption in North America is 90 kg/year (Bruinsma, 2003) and growing population and incomes, along with changing food preferences are increasing the demand for livestock products.

Despite this, in recent years, health concerns have increased regarding the consumption of meat, red meat in particular. Studies have shown that increased consumption of saturated fat is linked to the development of various diseases like cancer (Cross et al., 2007; Lam et al., 2009; Thiébaut et al., 2009) and heart attack (Katan et al., 2010). Growing health concerns led to significant change in the amount and type of fat consumed in the world over the last 20 years with increased emphasis on avoiding saturated fat (Higgs, 2000). This has primarily led to a shift in the consumption of specific fatty foods to reduced fat options (Lunn & Buttriss, 2008). Because meat and meat products are an integral part of the Western diet, considerable emphasis has also been given to reduce the fat content of meat carcasses. This has mainly been achieved through adopting changes in

animal breeding, husbandry and butchery techniques (Foster & Lunn, 2007). Peterson et al. (2001) studied consumers' perception about fat in meat and meat products and found that consumers tended to overestimate the amount of fat content in meat and meat products, with misperceptions of fat content greatest for pork and pork products. Increased demand for low fat foods encouraged food manufacturers to produce low fat versions of dairy and meat products along with other reduced-fat options that replaced foods traditionally associated with a high fat content such as baked foods, salad dressings and desserts (Foster & Lunn, 2007).

These changing dietary patterns have also led to a substantial modification of the fatty acid profile of the human diet as animal fats have been replaced by vegetable fats. Hard fat was replaced by oils and there has been an increase in the consumption of unsaturated fatty acids, which has led to an increased demand and intake of *n-6* (omega-6) polyunsaturated fatty acids (Lunn & Buttriss, 2008). The increase in *n*-6 fatty acid level intakes simultaneously led to reduced *n*-3 (omega-3) fatty acid levels (Lunn & Buttriss, 2008). This increased the dietary *n*-6:*n*-3 ratio, which has been linked to the pathogenesis of many diseases, including cardiovascular disease, cancer, inflammatory and autoimmune diseases (Simopoulos, 2008). It has also been linked to the predisposition of people to many chronic diseases like diabetes mellitus (Raheja et al., 1993), asthma (Broughton et al., 1997); and osteoporosis (Weiss et al., 2005). Today there is increased incidence of diet specific occurrence of diseases and this has been mainly attributed to the change in dietary habits away from what our ancestors evolved to eat (Eaton et al., 1988). But very little change has occurred to the human genetic makeup since, which may have limited the human ability to accommodate these dietary changes (Cordain et al., 2005). The current Western diet is very high in *n*-6 fatty acids with an *n*-6:*n*-3 ratio of 20 to 30:1 because of the indiscriminate use of *n*-6 fatty acids to substitute saturated fats (Simopoulos, 1999). It is globally accepted that the ratio of *n*:6 to *n*-3 had to be decreased from what it is currently to the desirable level of 2:1 (Simopoulos, 2008). Modifying

fatty acid profiles associated with meat could be a possible method to increase the dietary intake of *n*-3 fatty acids and decrease the *n*-6:*n*-3 ratio.

1.2. The Pork Industry in Canada

Canadian pork has an international reputation for high quality and excellent standards of food safety. Alberta and the other Prairie Provinces have a natural advantage for swine production with plentiful supplies of barley, wheat and canola for feeding pigs. The changing human dietary patterns that were aimed to reduce the consumption of fats could be one reason why people started to avoid eating pork and pork products as pork is considered to be rich in fat (Peterson et al., 2001). Apart from this, outbreaks of diseases like H1N1 that have been associated with swine have resulted in a decline in the production and export of pork. These events caused a significant change in the consumer perception in eating pork as found by Peterson et al. (2001) and may have led to a decline over the last decade in the per capita consumption of pork in Canada whereas no such decline has been observed for chicken (Statistics Canada, 2010). Steps to increase domestic consumption of pork have included putting 'Canadian pork' labels on pork packages as a way of catering to consumers who wish to buy local products. Other steps to diversify pork production aside from identifying Canadian product can also help the industry. Omega-3 enrichment of pork meat is an example of this kind of diversification and is gaining popularity due to its potential health benefits.

1.3. Fatty acids, their natural occurrence and role

Fats and oils have always been a part of the human diet and are essential for various chemical and biochemical processes inside the human body (Marcinčák et al., 2009). Lipids have an important impact on the sensory perception of foods as well due to their influence on food odour and flavour. Triglycerides are comprised of fatty acids and glycerol and the composition and nature of the fatty acids of the triglycerides in meat vary with animal diet. Fatty acids are comprised of long chains of carbon and hydrogen atoms with a carboxylic acid moiety (–COOH) at one end. Unsaturated fatty acids differ from saturated fatty acids structurally since they have double bond between the carbon

atoms. A double bond causes a kink in the hydrocarbon chain and thereby molecules with unsaturated fatty acids cannot pack together as tightly as those with saturated fatty acids, and their interactions with each other are therefore weakened. As a result, less thermal energy is required to disorder these poorly ordered arrays of unsaturated fatty acids, so fats with large proportions of unsaturated fatty acids have lower melting points than saturated fatty acids of the same chain length. Saturated fatty acids that contain fewer than eight carbon atoms are usually liquid at room temperature, whereas those containing more than ten are usually solid. Unsaturated fatty acids can be further subdivided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) based on the number of double bonds. MUFA's have only one double bond in their carbon chain, while PUFA's have two or more double bonds. The double bonds in the unsaturated fatty acids make it more susceptible to oxidation (Lunn & Buttriss, 2008).

Figure .1.3. a. Structure of α- linolenic acid (C18:3n-3)

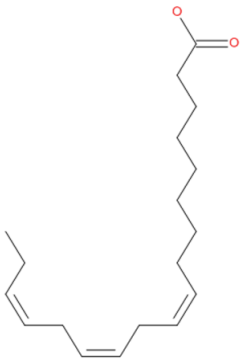


Figure 1.3. b. Structure of eicosapentaenoic acid (C20:5n-3)

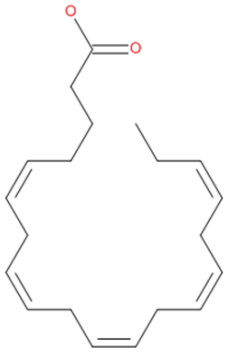
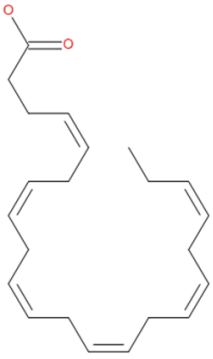


Figure 1. 3. c. Structure of docosahexaenoic acid (C22:6n-3)



1.3.1. Classification of Polyunsaturated fatty acids

Polyunsaturated fatty acids are mainly from two different categories: *n*-3 (omega-3) and *n*-6 (omega-6) fatty acids. The term omega designates the location of the double bond relative to the carbon atom that is located farthest from the functional carboxylic acid group ie (–COOH). Thus omega-3 indicates that the position of the first double bond is at the third carbon atom from the omega carbon atom. Linoleic acid (LA) is the major *n*-6 fatty acid whereas α-linolenic acid (ALA) is the major *n*-3 fatty acid. LA and ALA are essential fatty acids and have to be supplied in the diet as humans cannot synthesize them because they lack the Δ^{12} and Δ^{15} (delta 12 ,15) desaturase enzymes (Lunn & Buttriss, 2008; Roynette et al., 2004). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the important long chain polyunsaturated fatty acids (LCPUFA) and are synthesized from α-linolenic acid by addition of carbon atoms (elongation) and desaturation, which is the addition of a double bond to replace a single bond among neighboring carbon atoms (Komprda et al., 2005). In human beings, ALA is converted to EPA to some extent whereas there is only very little conversion of EPA to DHA (Burdge et al., 2002). Principal sources of ALA are dark green leafy plants, flax seeds, walnuts and canola whereas EPA and DHA are predominantly present in fatty fish such as tuna, salmon, and herring; however there are consumer dislikes over eating fish (Roynette et al., 2004). Marine algae are the primary sources of LCPUFA's and fish obtain LCPUFA's by consuming algae (Lunn & Buttriss, 2008). Also meat and eggs could be enriched with omega-3 fatty acids by adopting appropriate feeding strategies in animals and thereby making them as a good source of these fatty acids.

1.3.2. Omega -6 vs Omega -3 fatty acids

Omega-6 and omega-3 fatty acids are essential fatty acids that must be supplied in the diet for the human body to utilize them. Normally the human diet contains both of these types of fatty acids, but a balanced ratio between *n*-6 and *n*-3 is required to maintain a proper healthy condition. LA is the major *n*-6 or omega-6 fatty acid and is found in vegetable oils such as soybean oil, safflower oil, corn oil and cottonseed oil (Simopoulos, 2001). LA is the precursor for the synthesis of arachidonic acid (AA) which is the principal *n*-6

PUFA found in human beings. AA is the main precursor for the synthesis of eicosanoids. Eicosanoids are potent immuno-modulatory regulators that are synthesized from the 20C PUFA's; AA and EPA. The immuno-modulatory action exhibited by the eicosanoids generated from AA and EPA varies significantly (Roynette et al., 2004). Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and lipoxins (LX) (Kantarci & Van Dyke, 2003). Within these eicosanoid families there are different series of compounds and each produce a different inflammatory response. Eicosanoids generated from AA including the 2-series from the PG's and TX's and the 4-series of LT are pro-inflammatory. Eicosanoids generated from EPA that are of the 3- series of PG's and TX's and the 5-series of LT are anti-inflammatory (Roynette et al., 2004).

1.3.3. Metabolic pathways of *n*-6 and *n*-3 fatty acids

The metabolic processes of *n*-6 and *n*-3 fatty acids have common pathways (Roynette et al., 2004). Different enzymes like desaturase, elongase, cyclooxygenase (COX) and lipooxygenase (LOX) play an important role in the metabolism of these fatty acids. All these enzymes have strong affinity for *n*-3 fatty acids and thus *n*-6 fatty acids will be competitively inhibited whenever there are increased levels of *n*-3 fatty acids (Roynette et al., 2004). Hence whenever there is an increased level of *n*-3 fatty acids in the diet, they will be preferentially metabolized compared to *n*-6 fatty acids and lead to the production of eicosanoid classes which are anti-inflammatory. Both the *n*-3 and *n*-6 fatty acids are derived from the diet and exist primarily in the form of triglycerides. These fatty acids are digested in the small intestine and absorbed into the blood stream and transported to various tissues like the brain, heart and retina. Following digestion and absorption into the tissues, these fatty acids can either undergo beta oxidation to provide energy in the form of adenosine tri-phosphate (ATP) or they can undergo esterification to form triglycerides, phospholipids and cholesterol esters (Lewin, 2005). Phospholipids form part of the cell membrane. Fatty acids stored as triglycerides and cholesterol esters can be further metabolized to provide energy. Fatty acids derived from linoleic acid (LA) and α- linolenic acid (ALA) can be converted to fatty acyl CoA, which can undergo further desaturation and elongation to form LCPUFA's in the liver.

The beneficial effect of *n*-3 fatty acid consumption is attributed more to the LCPUFA's EPA and DHA than to ALA (Lunn & Buttriss, 2008; Roynette et al., 2004; Simopoulos, 2008). The extent of formation of LCPUFA's in human beings from α-linolenic acid also has been investigated (Burdge et al., 2002; 2006; Burdge & Calder, 2005; Mantzioris et al., 1994; Wallace et al., 2003). The formation of fatty acids takes place in the endoplasmic reticulum with the exception of docosahexaenoic acid (Burdge & Calder, 2005). The *n*-3 and *n*-6 fatty acids are metabolized by the same elongase and desaturase enzymes and these enzymes have more affinity towards *n*-3 fatty acids (Burdge & Calder, 2005; Hagve & Christophersen, 1984). The initial conversion of α-linolenic acid to 18:4n-3 by Δ^6desaturase enzyme is the rate limiting step (Sprecher, 2000) and Δ^6desaturase enzyme has more affinity for *n*-3 fatty acids compared to *n*-6 fatty acids. The metabolic pathways of *n*-6 and *n*-3 fatty acids are illustrated in Figure 1.11.1. The preferential conversion of these fatty acids depends on their individual concentration in the diet. Whenever there is a high concentration of *n*-6 fatty acids in the diet, the rate of *n*-3 fatty acid metabolism decreases and vice versa (Burdge & Calder, 2005).

Human beings cannot convert *n*-6 fatty acids to *n*-3 fatty acids because they lack *n*-3 desaturase, which is essential for this conversion (Simopoulos, 2008). In human beings, synthesis of the LCPUFA's (EPA and DHA) from ALA is dependent on different factors like sex, physiological conditions and disease conditions (Burdge, 2006; Carlson et al., 1986; Simopoulos, 2008). Burdge (2006) studied the metabolic conversion of α- linolenic acid in males and females and found that there is a greater conversion in females than males, which has been attributed to a regulatory effect produced by oestrogen. Age also can have an effect because infants have very little capacity to synthesize LCPUFA from ALA (Plourde & Cunnane, 2007). In disease conditions affecting the liver such as fatty liver diseases (Araya et al., 2004), there is reduced synthesis of LCPUFA from ALA. Emken et al. (1994) found that there was 15% conversion in adult males, whereas Pawlosky et al. (2001) noted a conversion rate of 0.2%. Hussein et al. (2005) found that a 0.1% conversion of ALA to LCPUFA's. Additionally, all these studies found that

the conversion of ALA to EPA dominated and that the synthesis of DHA was very limited.

1.3.4. Beneficial effects of omega-3 fatty acids

The potential beneficial effects of omega-3 fatty acid consumption are multidimensional and are essential for brain development and maintenance. These fatty acids helps in the prevention of cardiovascular diseases, atherosclerosis, hypertension, cancer, inflammatory, autoimmune and neurological disorders (Lee et al., 2008; Roynette et al., 2004; Simopoulos, 2008; Yashodhara et al., 2009). The cardio-protective effect of omega-3 fatty acids was first noticed in the Canadian Inuit population, which had a reduced incidence of heart disease. Further investigations indicated that the Canadian Inuit had a high proportion of fish in their diet and the long chain omega-3 fatty acids (EPA & DHA) present in fish had a direct cardio-protective effect (Dyerberg et al., 1975). The mechanisms by which the protection is offered have also been extensively studied (Dallongeville et al., 2003; Fleischhauer et al., 1993; Gapinski et al., 1993; Geelen et al., 2005; Griffin et al., 2006; Herrmann & Biermann, 1995; Toft et al., 1995). These studies revealed that omega-3 fatty acids were also related to reduced arrhythmias, platelet aggregation, plasma triglycerides, blood pressure; vasodilation of coronary blood vessels and increased high density lipoprotein and particle size of low density lipoprotein.

The beneficial effect of *n*-3 fatty acids in atherosclerosis and hypertension situations has been related to its blood pressure lowering effects (Dallongeville et al., 2003; Toft et al., 1995) and improvement in endothelial functions (Morgan et al., 2006). Whether *n*-3 fatty acids have a protective effect against cancer is still the subject of debate. Rose & Connolly (1999) studied the chemoprevention aspects of *n*-3 fatty acid consumption and found that *n*-3 fatty acids suppressed neoplastic transformation and enhanced apoptosis and these effects were associated with the inhibition of *n*-6 associated eicosanoid precursors. Review studies conducted by Hooper et al. (2006) and MacLean et al. (2006), however, stated that *n*-3 consumption did not give protection against cancer whereas Berquin et al. (2008) and Theodoratou et al. (2007) found that *n*-3 fatty acid consumption significantly reduced prostate and colorectal cancers.

Studies showed that present total *n*-3 fatty acid consumption in the human diet is very low and needs to be enhanced for health benefits and this led to a change in the recommendations for the intake of LCPUFA (Simopoulos, 2000). The International Society for the Study of Fatty Acids and Lipids recommended a daily intake of 2,220 mg of LNA and 650 mg of EPA + DHA, with a minimum of 220 mg of both EPA and DHA for adults (Simopoulos et al., 1999) while the Society also recommended that during pregnancy and lactation a minimum supplementation of 300mg/day of EPA and DHA was required. The United Kingdom Department of Health recommended a total LCPUFA intake of 200 mg/day for adults whereas the American Heart Association and European society of Cardiology recommended 100 mg/day (Kris-Etherton et al., 2003; Van de Werf et al., 2003). Even though it is known that the beneficial effects are more attributed to the consumption of LCPUFA's, the extent to which ALA, which is the most common form of *n*-3 fatty acid, is converted to EPA and DHA in humans is unknown and requires further study to understand the extent to which the high intake of *n*-6 fatty acids compromises benefits of *n*-3 fatty acid consumption. This increased understanding could then lead to a generalized approach towards the dietary requirement of these fatty acids.

1.4. Feeding flax to pigs as a source to enrich omega-3 fatty acids in pork meat

Flaxseed *(Linum usitatissiumum)*, which is also known as linseed, is one of the oldest crops cultivated in Canada and is valued for its fibre and oil (Jhala & Hall, 2010). The fibre, obtained from the stalks of the plant, is used to make fine linen and paper while the oil is used primarily for industrial purposes (Irvine et al., 2010). The by-product remaining after oil extraction is called flaxseed meal or linseed meal and is a good source of protein in livestock feeds. The seed has medicinal properties and is used as a laxative and for improving skin and hair quality. Flaxseed contains between 42 to 46% fat, 28% dietary fibre, 21% protein, 4% ash, and 6% carbohydrates (Flax Council, 2008). The fatty acid profile of flaxseed oil shows on average 9% saturated fat (SF), 18% monounsaturated fat (MUFA), and 73% polyunsaturated fatty acids (PUFA) (Morris, 2007). PUFA comprise approximately 16% of *n*-6 fatty acids, and are mainly LA, and 57% of *n*-3

fatty acids, and are mainly ALA (Flax Council, 2008). Because endogenous synthesis of *n*-3 fatty acids is limited in humans and there are no naturally occurring terrestrial plant sources of the long-chain *n*-3 fatty acids, manipulation of animal derived foods to provide *n*-3 fatty acids to humans has emerged as an option for increasing their dietary availability. This is mainly accomplished by feeding *n*-3 enriched diets to animals so that they deposit these fatty acids directly into their tissues for subsequent human consumption. As a result, there has been a renewed interest in using flaxseed and flax oil in animal rations to alter the fatty acid composition of egg and meat products and thereby provide potential health benefits for the consumer (Cherian & Hayat, 2009; Huang et al., 2008; Zhan et al., 2008). The fatty acid profile of meat and fat in pigs is directly affected by source of fat in the diet because they are monogastric and so little modification occurs to fats following digestion (Wood & Enser, 1997). The fatty acid profile of pork and its ratio of *n*-6 to *n*-3 fatty acids could therefore be altered by feeding pigs rich sources of *n*-3 fatty acids such as flax oil or flaxseed.

Flax meal can be included at all stages of swine production (Jansman et al., 2007; Matthews et al., 2000; Thacker et al., 2004) up to a level of 3% in starter rations and 10% in grower and finisher rations without adverse effects on growth and feed intake. Research has shown that pork can be enriched with *n*-3 fatty acids by feeding flax seeds to pigs. Romans et al. (1995a) examined the effect of feeding graded levels of flax to pigs 25 days prior to slaughter. Feed containing 0, 5, 10, and 15% ground flax increased the ALA content from 10 mg/g to 23, 37 and 53 mg/g of inner back fat, respectively. EPA concentration increased from 0.09 mg/g to 0.20, 0.28 and 0.38 mg/g of back fat when fed 0, 5, 10 and 15% ground flaxseed respectively, clearly indicating the potential to increase the *n*-3 fatty acid content of pork products by feeding flaxseed in the finishing phase. Specht-Overholt et al. (1997) also observed significant improvements in ALA concentrations in back fat, liver and M. *longissimus thoracis* muscle of swine fed 15% flaxseed. After 28 days, ALA concentration increased from 1.1% to 8.8% while those fed 15% flax for 42 days increased from 1.3% to 12%. Romans et al. (1995a) examined the impact of duration of feeding flax on *n*-3 enrichment and found that flax fed for a minimum of 21 days prior to slaughter produced significant levels of *n*-3

deposition in M. *longissimus thoracis* compared to control. Fontanillas et al. (1998) fed 4% flax oil for 60 days and determined *n*-3 content of back fat by biopsies. They observed that ALA in the back fat increased from an initial 1.14% to 4.94, 7.40, and 7.89% after 17, 31, and 60 days feeding respectively and also found that 70% of the maximum ALA enrichment was achieved after feeding flaxseed for 30 days while 95% enrichment was achieved after 60 days. Juárez et al. (2010) found that feeding high levels (10 to 15%) of co-extruded flaxseed for short periods was more efficient at increasing *n*-3 fatty acids in pig back fat than feeding low levels (5%) for long periods even though the most consistent enrichments were found with feeding low levels for long durations. Feed conversion efficiency was increased with high flax feeding whereas average daily gain was decreased. These research results clearly indicated that feeding flax to pigs increased the *n*-3 content of pork.

1.5. Addition of Vitamin E to pork diet as an antioxidant

Polyunsaturated omega-3 fatty acids tend to oxidize rapidly and can lead to the generation of potent off-flavours that are termed as rancid, fishy, stale and cardboardy and these off-flavours are collectively called as 'warmed over flavour'. Off-flavours increase consumer dissatisfaction (Byrne et al., 2001). Lipid oxidation reactions can be reduced by the presence of anti-oxidants such as vitamin E in meat (Morrissey et al., 1994). Vitamin E is a common anti-oxidant that is lipid soluble and is available in eight different forms, namely $\alpha, \beta, \gamma,$ and δ tocopherols and $\alpha, \beta, \gamma,$ and δ tocotrienols (Lauridsen et al., 2002). Vitamin E is usually added into animal diets as α-tocopheryl acetate and is de-esterified in the gastrointestinal tract to produce its antioxidant effect (Buckley et al., 1995). Vitamin E acts as a free radical scavenger on the cell membrane and thereby inhibits the lipid oxidation (Buckley et al., 1995). Dietary vitamin E is readily incorporated into porcine tissues and the accumulation of vitamin E in the different organs of pig varies depending on their metabolic activities, with α-tocopherol concentrations varying from least to greatest in the kidney, lung, heart and liver following dietary supplementation (Asghar et al., 1991).

Studies have been conducted to investigate the levels of α-tocopherol deposition in porcine tissues and found that they depend on the duration of supplementation (Morrissey et al., 1994). Vitamin E supplementation could

also stabilize meat colour by preventing the free radical aided conversion of oxymyoglobin to metmyoglobin (Jambrenghi et al., 2005). Phillips et al. (2001) in their study fed 170 mg/kg of α -tocopheryl acetate to pigs for 6 weeks before slaughter. The animals were supplemented with a basal diet and the purpose of the study was to see whether dietary supplementation of vitamin E had an effect on meat colour. Even though lipid oxidation was significantly improved by vitamin E supplementation, there was no effect on meat colour. Studies conducted by Houben et al. (1998); Jensen et al. (1997) and Monahan et al. (1994) also found that dietary supplementation of vitamin E did not improve meat colour even though lipid oxidation was significantly reduced in all these studies. Houben et al. (1998) and Monahan et al. (1994) each used 200 mg of α-tocopheryl acetate /kg of feed whereas Jensen et al. (1997) used 700 mg α-tocopheryl acetate /kg feed and obtained comparable results. The results of these studies suggest that feeding α-tocopherol acetate at levels above 170 mg/kg feed will reduce lipid oxidation without affecting pork colour. Optimum levels of vitamin E supplementation needed to offer sufficient protection against lipid oxidation are unclear as results have varied according to study. Boler et al. (2009) found that dietary supplementation of 40mg/kg in pigs produced optimum protection against lipid oxidation in pork chops and ground pork, but did not provide additional protection against lipid oxidation at levels of vitamin E higher than this. In comparison, Houben et al. (1998) and Monahan et al. (1994) found that dietary levels of 200mg/kg offered optimum protection against lipid oxidation. Different levels of α-tocopherol supplementation ranging from 100 to 400mg/kg of diet increased the *α*-tocopherol deposition in meat and reduced lipid oxidation (Asghar et al., 1991; Monahan et al., 1994). However studies indicate that the effects of dietary α-tocopherol supplementation in pork meat colour development are yielding inconsistent results.

The use of naturally occurring α-tocopheryl acetate in animal feeds appears to be most effective at preventing lipid oxidation of pork. Boler et al. (2009) compared the inclusion of synthetic and natural α-tocopheryl acetate in swine diets containing dried distillers grains with solubles (DDGS) on subsequent pork oxidation. DDGS contain high levels of polyunsaturated fatty acids and can contribute to an increase in pork fat oxidation (Shurson et al.,

2004). Synthetic vitamin E was not found to be effective in preventing lipid oxidation of the pork as compared to the naturally occurring α-tocopheryl acetate. Also in this study, neither synthetic nor natural vitamin E improved meat colour.

He et al. (2010) compared the effects of β carotene supplementation and α-tocopheryl acetate supplementation on the lipid oxidative stability when the pigs were fed with a high linseed oil diet. β carotene did not appear to provide antioxidative protection when compared with α-tocopheryl acetate.

1.6. Omega-3 enrichment of pork meat

Meat is an integral part of the western diet and *n*-3 enrichment of meat may be an effective way of providing humans with adequate dietary levels of *n*-3 fatty acids (Lunn & Buttriss, 2008). Among the different livestock species, pork is considered a suitable species for *n*-3 enrichment because pork has a high fat content and, being a single stomached animal, the pig has the natural advantage of depositing dietary fats directly into its tissues. Cattle do not offer the same advantage because they are ruminants and the bacteria in the rumen tend to saturate the dietary unsaturated fats; therefore, enriching beef with *n*-3 fatty acids by dietary *n*-3 fatty acid supplementation is difficult (Bourre, 2005; Lunn & Buttriss, 2008). Dietary fat modulation by *n*-3 fatty acid supplementation causes significant changes to the triglyceride lipid fraction in pigs whereas there is little change to the phospholipids that are found associated with the cell membranes (Bourre, 2005).

Different approaches are used to enrich pork meat with *n*-3 fatty acids. Feeding of *n*-3 rich fatty acid sources like fish oil, flax seed, and linseed are common strategies (Bryhni et al., 2002; Kouba et al., 2003; Nuernberg et al., 2005). Dietary fish oil supplementation has been used to increase the LCPUFA's (EPA and DHA) in meat (Bryhni et al., 2002) but feeding fish oils to pigs can impart 'fishy' odours or flavours to pork (Wood et al., 2008). Also global fish stocks are declining and fear of mercury poisoning exists among people (Lunn & Buttriss, 2008). As a result, *n*-3 fatty acid enrichment of pork has been accomplished by feeding other diet ingredients that are rich in ALA such as flax to pigs and these have increased levels of α–linolenic acid and even LCPUFA's (Matthews et al., 2000; Nuernberg et al., 2005; Romans et al., 1995a; Romans et al., 1995b). Omega-3 fatty acid enrichment of pork also

appears to have no deleterious effect on animal growth, carcass and meat quality (Kouba et al., 2003; Nuernberg et al., 2005; Romans et al., 1995a); however increased levels of *n*-3 fatty acid content of pork has produced off-flavours that have affected the sensory characteristics of the meat (Nuernberg et al., 2005; Romans et al., 1995b).

Apart from enriching meat with *n*-3 fatty acids, the ratio of *n*-6:*n*-3 is also important. A balanced ratio of *n*-6 and *n*-3 fatty acids is required for normal human growth and development and may be helpful in reducing cardiovascular disease and other chronic illnesses along with improving mental health (Simopoulos, 2000; Wijendran & Hayes, 2004). Consumption of excess *n*-6 fatty acids lead to increased eicosanoid production from arachidonic acid, which is pro-inflammatory and harmful to human health as they have been linked to the onset of disease (Simopoulos, 1991). Increased *n*-6:*n*-3 ratio causes increased platelet aggregation (Freese et al., 1994), non-insulin diabetes mellitus (Raheja et al., 1993), rheumatoid arthritis (James & Cleland, 1997), asthma (Broughton et al., 1997), cancer (Maillard et al., 2002) and depression (Maes et al., 1996). Consequently, there is a need to reduce *n*-6:*n*-3 ratio by increasing *n*-3 fatty acid consumption. The ideal *n*-6: *n*-3 ratio in adults is 2:1 (Simopoulos, 2008).

1.6.1. Functional food

The concept of 'functional food' was first developed in Japan in the 1980's and the idea behind the concept was to characterize specific foods that were believed to provide protection against particular chronic diseases. These foods were purported to have medicinal properties other than simply supplying nutrients to the body. Later, the concept was globally accepted and most countries began instituting laws and definitions thereby regulating functional foods (Arai et al., 2001). According to Health Canada (1998), "Canadians have been taking greater control over their health, exploring alternative or traditional medicines, complementary therapies and natural health products. There has been a growing interest in the role that nutrition plays in our state of well being." Health Canada further states "As public knowledge of this field has evolved, manufacturers have sought to fulfill a consumer appetite for products derived from foods that could be used to promote good health. This resulted in the development and marketing of a growing spectrum of products

called nutraceuticals and functional foods." A *functional food* is defined as "food consumed as part of a usual diet, which has been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, such as it contains a bioactive compound" (Health Canada, 1998). Foods enriched with *n*-3 fatty acids such as *n*-3 enriched pork have attained functional food status as they provide the *n*-3 enriched food claim labeling requirement of 300 mg of *n*-3 fatty acids per 100 g of serving (Health Canada, 1998).

1.7. Defining pork quality

The term "meat quality" according to Andersen et al. (2005) is defined as "a complex and multivariate property of meat which is influenced by multiple interacting factors including the conditions under which the meat is produced". Because of this sweeping definition, there are many factors that affect meat quality, including animal management system, breed, genotype, type of feed and method of feeding, pre-slaughter handling and stunning, slaughter method, carcass chilling and storage conditions. The main attributes that are related to meat quality are safety, price, ethics, nutritional value, flavour, texture, water holding capacity, colour, lipid content, lipid composition, oxidative stability and uniformity (Andersen et al., 2005). There are differences in opinion regarding the most important factors that determine consumer acceptability as different researchers have conducted studies on consumer perception that have identified different meat quality parameters. According to Bredahl et al. (1998) overall appearance, colour, flavour, texture/tenderness, and juiciness are all considered to have a crucial impact on consumer acceptability. Appearance of meat is the most crucial factor in the buying situation and consumers relate colour of the meat to good quality and take it as a mark of freshness (Tikk et al., 2008). Bryhni et al. (2002) performed consumer perception studies in Denmark, Norway and Sweden and found that flavour and tenderness were often ranked as the most important sensory attributes by consumers, and that the presence of off-flavours and toughness was considered to be the most important factors causing consumer dissatisfaction. Overall, these studies assessing the consumer perception of meat quality parameters clearly indicate that colour, flavour and tenderness/texture have a direct impact on consumer acceptance for a

particular meat product. Manipulation of pig diet may affect the technological characteristics of meat by changing the fatty acid profile, which may affect the firmness, texture and taste of the raw and cooked product.

Altering the fatty acid composition of pork through diet offers an avenue for Canadian pig producers to differentiate their product not only domestically but in the world pork market. In order to construct a pig diet that alters the fatty acid composition of pork without compromising pork flavour, how fatty acids and other muscle components affect meat quality characteristics like aroma, colour, texture and flavour and how the fatty acid profile and meat quality attributes could be changed by adopting changes in animal diet must be understood.

1.7.1. Pork Colour

The colour of pork is influenced mainly by the pigment myoglobin and its concentration and its chemical form as well as the meat protein structure (Lindahl et al., 2001). The pigment content varies among different pig breeds (Warriss et al., 1990) and also between the different muscle types (Beecher et al., 1969). Red oxidative muscle fibres have more myoglobin than white glycolytic muscles (Lindahl et al., 2001). Myoglobin exists in different states or chemical forms, with each imparting a different colour to the meat (Figure 1.11.2). Deoxymyoglobin, which is deoxygenated myoglobin is purple red, while oxymyoglobin, which is oxygenated myoglobin is bright red. Metmyoglobin is the third myoglobin state and it is brown. Initially myoglobin will be in the deoxymyoglobin state as it is not exposed to high oxygen levels and hence the meat surface will be purple. Once meat is exposed to air such as when it is removed from a vacuum package or when the cut surface of meat is exposed to air for some time, oxygenation will takes place and deoxymyoglobin is converted to oxymyoglobin and meat surface colour becomes bright red and is referred to as “bloom” (MacDougall, 1982). As the length of oxygen exposure time increases, the oxymyoglobin steadily oxidizes to metmyoglobin and then the meat colour becomes brown (Shikama, 1998). Metmyoglobin formation is dependent on different factors like oxygen partial pressure, temperature, pH, the inherent reducing activity of the meat and also microbial growth (Mancini & Hunt, 2005). Another mechanism that has an effect on meat colour is the enzymatic reduction of metmyoglobin to

oxymyoglobin by different enzymes present in muscle such as superoxide dismutase, catalase and glutathione peroxidise which are dependent on the nicotinamide adenine dinucleotide (NADH) pool of the muscle (Mancini & Hunt, 2005) which is depleted as time *post mortem* progresses. The rate of discoloration of meat therefore depends on both oxidative processes and enzymatic process (Faustman & Cassens, 1990). The effect of *n*-3 enrichment on meat colour in pork has been extensively studied (Corino et al., 2008; Haak et al., 2008; Musella et al., 2009; Raes et al., 2004; Valencia et al., 2006) and has shown that *n*-3 enrichment does not affect meat colour. Haak et al. (2008) in their study found that feeding linseed or fish oil did not produce any measurable effect on meat colour, pH and drip loss. Leskanich et al. (1997) conducted studies in pigs by feeding rapeseed oil + fish oil and soyabean oil + tallow to alter fatty acid composition in pork meat and fat and found that the dietary treatments did not have any effect on meat colour.

1.7.2. Meat Flavour

Flavour is considered a very important component of the eating quality of meat and there had been much research done to understand the chemistry of meat flavour and the influence of various production and processing factors on it (Mottram, 1998). The development of aroma and flavour during the cooking of meat is a very complex process in which different components react to produce chemical intermediates or final flavour volatiles while raw meat has only a blood-like taste with little or no aroma (Mottram, 1998).

Pork meat flavour is generated through the cooking process, during which the Maillard reaction and thermally induced lipid oxidation are major pathways in generating flavour components (Meinert et al., 2009). Fatty acids present in the meat are major precursors of meat flavour (Mottram, 1998). Apart from fatty acids, other important meat flavour precursors are reducing monosaccharides, reducing phosphorylated monosaccharides, cysteine, ribonucleotides and thiamine (Mottram, 1998). The presence of these flavour precursors are influenced by factors such as diet, post mortem ageing (Koutsidis et al., 2008) and pre-slaughter stress (Wiklund et al., 1996).

Pork flavour intensity is reduced and detectable abnormal flavours increase as the concentrations of polyunsaturated fatty acids increase in meat

(Bryhni et al., 2002; Hertzman & Leif, 1988; Meadus et al., 2010; Musella et al., 2009; Nuernberg et al., 2005; Santos et al., 2008). Meadus et al. (2010) produced docosahexaenoic acid (DHA) enriched bacon by feeding microalgae to pigs. While carcass quality was unaffected in the study conducted by Meadus et al. (2010), off odours and off-flavours were detected by the sensory panel, despite 100 IU of α-tocopheryl acetate/kg of diet being used as an antioxidant. This increased oxidation of the polyunsaturated fatty acids was detected by increased thiobarbituric acid reacting substances assay (TBARS) values. Similar results were obtained by Santos et al. (2008), who detected off-odours and off-flavours that were associated with lowered sensory acceptability of hams from pigs fed a diet containing linseed. Most studies have shown that attempts to increase unsaturated fatty acid levels in pork lead to poor sensory characteristics as perceived by sensory panellists, which appear to be due to the increased levels of lipid oxidation associated with the increased unsaturation of the fatty acids.

1.7.3. Maillard reaction, Lipid Oxidation and Meat Flavour

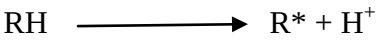
The Maillard reaction is a complex non-enzymatic reaction that occurs between amino compounds and reducing sugars and it is one of the most important reactions producing flavour and browning in cooked foods (Mottram, 1998; Nursten, 2005). The Maillard reaction produces large numbers of heterocyclic compounds that are responsible for savoury, roast and boiled flavours found in the volatiles of cooked meat (Mottram, 1998; Nursten, 2005). Figure 1.11.3 shows the schematic representation of the Maillard reaction. The Maillard reaction starts with the reaction between a reducing sugar and aminoacid leading to the formation of an N- substituted glycosylamine. This compound is unstable and undergoes the "Amadori rearrangement" to form "1-amino-1-deoxy-2-ketoses" (known as "ketosamines") which can undergo subsequent dehydration, fission and polymerization reactions. Amadori rearrangement is the isomerization of glycosylamine to corresponding aminodeoxy sugars. The 1-amino-1-deoxy-2-ketoses can then react according to three pathways. There could be formation of Schiff's bases by the furfural pathway. (A schiff's base is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not hydrogen). This

involves the loss of 3 water molecules, then a reaction with amino acids and water. These also undergo aldol condensation and could polymerise further into melanoidins (Calkins & Hodgen, 2007).

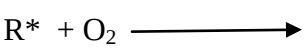
A second pathway is by dehydration through the loss of two water molecules leading to the formation of reductones & dehydro reductones. A third pathway leads to the production of short chain hydrolytic fission products such as acetol, pyruvaldehyde etc. These could further undergo "Strecker degradation" with amino acids to form aldehydes and by condensation to aldols. All these products react further with amino acids to form the brown pigments and flavour active compounds collectively called "melanoidins" (Fay & Brevard, 2005; Mottram, 1998).

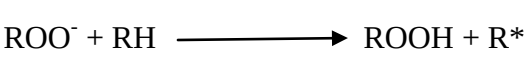
Lipids in meat undergo oxidation and produce volatile odour compounds which contribute to both desirable and undesirable flavours (Mottram, 1998). Polyunsaturated fatty acids tend to oxidize faster than saturated fatty acids owing to the reduced amount of energy required to break the double bond. However the most predominant sites of free radical attack in lipid oxidation reactions are the weak C-H bonds. The position of the weak C-H bonds varies among different fatty acids as for example in oleic acid it is C-8 and C-11 whereas in linolenic acid it is C-11 and C-14. The basic mechanism of lipid oxidation can be classified in three steps: initiation, propogation and termination. The initiation reactions give rise to small numbers of highly reactive fatty acid molecules that have unpaired electrons, free radicals. Free radicals are very short-lived and highly reactive, as they seek a partner for their unpaired electron. In the propagation reactions, atmospheric oxygen reacts with these to generate peroxy radicals, (ROO-). These are also highly reactive and go on to react with other unsaturated fatty acids, generating hydroperoxides and another free radical. This free radical can then go round and repeat the process, forming a chain reaction. The result is that ever increasing numbers of free radicals accumulate in the fat, which absorbs considerable quantities of oxygen from the air. Eventually the concentration of free radicals reaches a point when they start to react with each other to produce stable end-products. These are the termination reactions.

Initiation

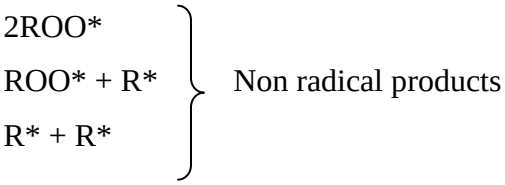


Propogation





Termination



Auto-oxidation of lipids occurs at room temperature and even at refrigeration and frozen temperatures and gives rise to rancid flavours in raw meat or 'warmed-over flavour' in stored cooked meat when reheated (Tikk et al., 2007). Thermal oxidation of lipids also occurs during cooking and follows very similar pathways to auto-oxidation but give slightly different products. These reactions contribute to the desirable flavour formed during cooking and volatile compounds formed are determined by the level of heat applied during cooking and the contribution of Maillard browning (Tikk et al., 2007). Apart from this, thermal oxidation reaction products could also enter into the Maillard reaction pathways and could produce additional volatile compounds, which makes the flavour formation a complex phenomenon to explain (Mottram, 1998). Lipids also act as a solvent for several aroma compounds that are formed during cooking and processing of meat (Elmore et al., 2002). The oxidation of lipids gives a wide range of compounds that includes saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, acids and esters as well as some cyclic compounds such as furans, lactones and cyclic ketones. All of these compounds possess intense odours and contribute to the overall aromas of many different kinds of foods (Mottram, 1998).

Lipid oxidation and the Maillard reaction do not occur separately and there are interactions between these two pathways, which causes a wide range of effects on the volatile aroma compounds produced (Farmer & Mottram,

1990). The presence of amino acids and sugars could reduce the amounts of the lipid oxidation reaction products, and the presence of lipids can reduce the quantities of some Maillard products. The interaction of these two reactions gives a new range of aroma compounds (Farmer & Mottram, 1990; Mottram, 1998). Alteration of one precursor could affect the products of several reaction pathways. The level of unsaturation of fatty acids and the structure and composition of fatty acids in phospholipids (for example *n*-6: *n*-3) will affect the products of lipid oxidation and the way these components affect the Maillard reaction (Farmer & Mottram, 1992).

1.7.4. Warmed over flavour

Warmed over flavour (WOF) is defined as the undesirable flavour that develops when cooked foods are reheated after refrigeration and it is one of the primary causes of quality deterioration in cooked, refrigerated and pre-cooked, frozen meat products (Tims & Watts, 1958). It includes both the development of undesirable flavours as well as concurrent loss of desirable meat flavour characteristics (Spanier et al., 1988). WOF is usually associated with reheated meats which have been refrigerated for 48 hours or less; however, WOF can develop in pre-cooked frozen meats after a few days or weeks of storage. Sensory panellists often describe WOF by using terms such as "stale", "cardboard-like", "painty", ”fishy” or "rancid" (Brewer & Vega, 1995).

Lipid oxidation marks the beginning of reactions that lead to WOF development (Willemot et al., 1985). Unsaturated fatty acids tend to oxidize faster than saturated fatty acids and polyunsaturated fatty acids oxidize more rapidly than mono-unsaturated fatty acids. Based on the relative polyunsaturated fatty acid content in different species, the rate of off odour development because of lipid oxidation and subsequent development of WOF could be in the order: fish > poultry > pork > beef > lamb (Cross et al., 1987; Rhee et al., 1996). Hydrogen atoms within a PUFA that are attached to the carbon atom located near to the double bond are more unstable and can detach. When hydrogen atoms detach from PUFAs, they become free radicals which are extremely reactive and trigger further oxidation. Subsequently fatty acids are broken into small molecules such as pentanal, hexanal, and 2,4-decadienal, which are responsible for the production of off-flavours that are recognized as

"warmed over flavour" (WOF) (Brewer & Vega, 1995). These highly volatile compounds are perceptible in very low concentrations (parts per billion) (Brewer & Vega, 1995) and are easily detected by consumers, which eventually lead to consumer dissatisfaction.

Apart from lipid oxidation reactions, proteins coagulate during cooking and lose their functional capabilities to hold water. This causes shrinkage and denaturation of proteins and the cessation of enzymatic activity. These structural changes lead to the release of iron from hemoglobin and myoglobin. Free iron which is in its reduced (Fe2+) state is readily converted into its oxidized (Fe3+) form and acts as a catalyst in the lipid oxidation reactions thus increasing WOF generation (Igene et al., 1979).

Other conditions that have an effect on lipid oxidation and subsequent development of warmed over flavour are cooking method (Satyanarayan & Honikel, 1992), time (Schricker & Miller, 1983) and temperature of cooking (Einerson & Reineccius, 1977). Cooking meat to 70 to 80°C causes disruption of the muscle membrane structure thereby exposing the unsaturated fatty acids to oxidation and subsequent generation of free radicals (Pearson et al., 1977). Free radical formation and oxidative process will be further catalyzed by the presence of metal ions like iron released from hemoglobin and myoglobin and leads to the propagation of a series of reactions that generate warmed over flavour (Igene et al., 1979). However when meat is heated to high temperatures above 100°C, the warmed over flavour formation is reduced due to the antioxidant properties of the Maillard reaction products especially melanoidins, which are produced at temperatures above 100°C (Bailey, 1988). Oxidation is a chain reaction and once initiated, it continues as free radicals are formed and further catalyzes additional free radical-generating reactions and subsequent oxidations (Frankel, 1984).

WOF generation could be minimised with the use of antioxidants, which reduce lipid oxidation reactions. There are a wide variety of antioxidants that are in use nowadays that are capable of preventing oxidation of fats. The most commonly and widely used is α-tocopheryl acetate (Morrissey et al., 1994). Compounds such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG) are also antioxidants (Barbut et al., 1985). Antioxidants

protect the PUFA from oxidation by sacrificing themselves to oxidation and thereby delaying the onset of oxidation (Jadhav & Madhavi, 1996). Another class of compounds that can prevent lipid oxidation are chelators, which are compounds that form complexes with metal ions and thereby prevent the catalytic action of metal ions that promotes oxidation (Shahidi et al., 1986). Commonly used chelators are citric acid, ethylenediaminetetracetic acid (EDTA), and some phosphate salts like sodium tripolyphosphate, pyrophosphate or hexametaphosphate. Ascorbic acid and erythorbic acid, added to cured meat products to prevent nitrosamine formation, also act as chelators to prevent fatty acid oxidation (Tims & Watts, 1958). Apart from these substances, certain herbs and spices like rosemary, marjoram, sage, thyme, mace, allspice and clove also exhibit antioxidant properties (Bailey, 1988).

1.7.5. Tenderness and juiciness

Tenderness is considered to be an important meat quality trait and it is mainly affected by the carcass conditions post-mortem as proteolytic changes post-mortem have a profound impact on meat tenderness (Maltin et al., 2003). Huff-Lonergan et al. (2002) observed a correlation between tenderness and flavour in which panellists often rated the samples that were most tender and juicy as having good pork meaty flavour. High muscle pH is correlated with increased sensory tenderness and juiciness scores. Other factors that influence tenderness and juiciness include the extent of postmortem ageing and proteolysis (Wheeler et al., 2000), rate of pH decline (Gardner et al., 2005), muscle type (Melody et al., 2004), and breed (Lonergan et al., 2001) and level of connective tissue (Sylvestre et al., 2002).

1.8. Role of sugars in generation of pork meat flavour

Sugars play a role in the generation of pork meat flavour through involvement in the Maillard reaction (Mottram, 1998). The concentrations of glycogen and individual mono-saccharides vary with the type of feed given to pigs (Koutsidis et al., 2008). Glucose is stored as glycogen in the muscle and post-mortem it is liberated by glycogen phosphorylase and glycogen debranching enzymes. Pigs fed glucose increased glycogen concentration in their M. *longissimus dorsi* (Bowers et al., 1968) and so feeds that provide abundant glucose may increase muscle glycogen stores prior to slaughter.

Feeding high carbohydrate concentrations might not always lead to increased glycogen or sugar concentrations in the meat because carbohydrates might be used when pigs are fasted overnight prior to slaughter (Rosenvold & Andersen, 2003). However feeding high amounts of digestible carbohydrate (sugar) to pigs reduces the incidence of dark, firm and dry (DFD) meat (Rosenvold & Andersen, 2003) suggesting some retention of carbohydrates even with fasting. Because the role of sugars in Maillard reaction is known, different researchers have used model systems to understand the effects of sugars in the generation of meat flavour (Campo et al., 2003; Elmore et al., 2002; Farmer et al., 1989; Farmer & Mottram, 1990; Farmer & Mottram, 1992; Salter et al., 1989).

Campo et al. (2003) produced meaty odours by cooking different mixtures of fatty acids with ribose and cysteine *in vitro.* Oleic, linoleic and α-linolenic acids, which are the main mono-, di- and tri-enoic fatty acids, respectively, in meat lipids were used individually and in mixtures and were cooked with ribose and cysteine. The role of these fatty acids in the production of odours after cooking was assessed and Campo et al. (2003) found that the presence or absence of an amino acid (cysteine) and a sugar (ribose) modified the odour perception of individual fatty acids by the panellists and could be attributed to its respective role in the Maillard reaction. Among the three fatty acids, α-linolenic acid produced clear aroma differences in triangle sensory tests when compared with either oleic or linoleic acids. When cysteine and ribose were present in the reaction mixture, all three fatty acids produced similar meaty aroma. In the absence of cysteine and ribose, however, fatty acids individually produced different odours; oleic was characterised as ‘oily’, linoleic scored highly for ‘cooking oil’ and linolenic produced ‘fishy’ and ‘linseed’ like odours. These notes were all reduced when cysteine and ribose were included.

Elmore et al. (2002) used model systems in which methyl linolenate and methyl linoleate were heated along with ribose and cysteine. There was an overall reduction in the generation of the volatile compounds as well as a shift in the type of volatile compounds produced following the addition of ribose and cysteine to the fatty acid methyl esters. Addition of ribose and cysteine led to the increased production of alcoholic and alkylfuran compounds and

lowering of saturated and unsaturated aldehydes. However there are chances of production of methanol by heating of methyl esters. Farmer & Mottram (1992) also observed increased production of alcohols and alkylfurans and lowering of saturated and unsaturated aldehydes when lecithin was heated with ribose and cysteine. The antioxidant nature of Maillard reaction products might have caused the modification of the lipid oxidation pathways (Bailey, 1988; Mottram & Whitfield, 1995). Because unsaturated and saturated aldehydes like 2-propenal, 2-butenal, -2-pentenal, -2,4-hexadienal, and -2,4-heptadienal are responsible for the formation of off-flavours produced during lipid oxidation in meat (Calkins & Hodgen, 2007), adding ribose to meat enriched with unsaturated fatty acids might have decreased the production of off-flavours .

It is widely accepted that sugars have the potential to influence flavour formation in meat during cooking due to its involvement in the Maillard reaction (Mottram, 1998) and hence sugars are considered to be an important flavour precursor in meat. Different sugars like ribose (Aliani & Farmer, 2005), glucose (Lauridsen et al., 2006), glucose-6-phosphate (Farmer et al., 1999) and xylose (Hudson & Loxley, 1983) are all considered to be flavour precursors. Meinert et al. (2009) added glucose, glucose-6-phosphate, ribose and mannose to minced pork and heated the mixtures to 160°C and found that glucose and glucose-6-phosphate were the major flavour precursors, followed by ribose and ribose-5-phosphate. Mannose was not found to have an important role in flavour generation when compared with the other sugars in this study.

Hudson & Loxley (1983) conducted studies to understand the properties of xylose in the formation of meat flavour through Maillard reaction and found that a 2% inclusion of xylose in comminuted mutton improved its acceptability to the sensory panel. Apart from improving the flavour, xylose addition also masked the potential off-flavours that otherwise were produced from cooked mutton. Young & Cummings (2008) found that the xylose derived Maillard reaction products had potent anti- oxidant activity, which may have contributed to minimizing off-flavours in the mutton due to oxidation.

Figure 1.8.a. Chemical structure of glucose

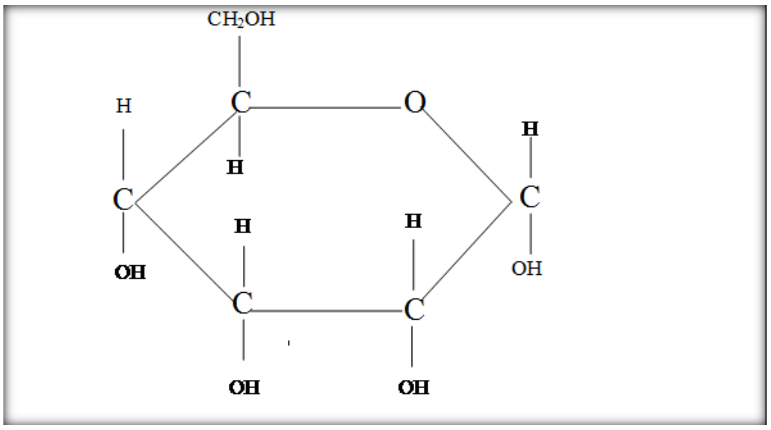


Figure .1. 8. b. Chemical structure of sucrose

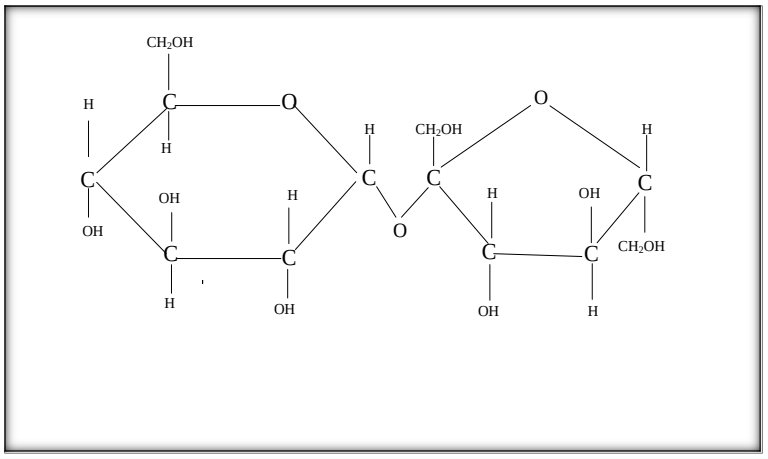
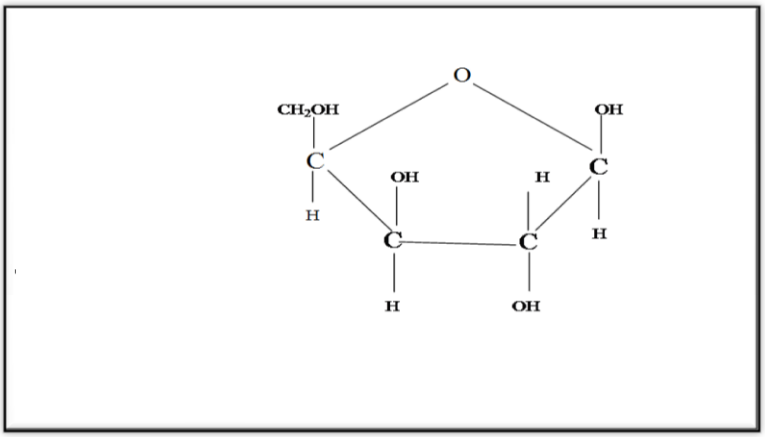


Figure.1.8.c. Chemical structure of xylose



1.9. Sensory approach to characterize pork meat quality traits

Meat is a consumer product and improvement of all meat quality traits is aimed at the consumer level as consumer acceptability is the key towards the success of any attributable change in meat quality. Even though different scenarios exist in technological advancement towards measurement of different meat quality traits, none of them could be considered as equivalent to that perceived by human sense organs. This makes meat science always a consumer science and hence sensory characterization of meat and meat products has a potential influence towards bringing a significant change in meat quality aspects.

Sensory characterization of meat products has been used as a common practice in meat science and descriptive sensory tests are the most sophisticated tools used for product characterization (Lawless & Heymann, 1999). This involves identifying and describing qualitative and quantitative sensory components of a consumer product by trained panels of judges (Meilgaard et al., 2007). Appearance, aroma, texture and flavour are some of the main qualitative attributes usually assessed in sensory studies and are quantified to characterize products objectively.

Descriptive sensory analyses allow quality control, product comparison, sensory mapping and product matching (Gacula, 1997). There are different methods of descriptive analysis used which include the Flavour Profile Method (Cairncross & Sjostrom, 1950), Texture Profile Method (Brandt et al., 1963), Quantitative Descriptive Analysis™ (Stone et al., 1974), Spectrum™ method (Meilgaard et al., 2007), Quantitative Flavour Profiling (Stampanoni, 1993) and Free-choice Profiling (Langron, 1983).

Free-Choice Profiling (FCP) is a sensory approach used to characterize meat and meat products without extensive training of the panellists. This approach was developed in the United Kingdom during the 1980s (Williams & Arnold, 1985). In this method panellists are free to have their own list of descriptors that describe the characteristics of the product in their own words, hence the name free choice profiling. FCP allows panellists to use any number of their own attributes to describe and quantify product attributes and is based on the principle that panellists do not differ in their

perceptions about a product but merely in the way in which they describe them (Oreskovich et al., 1991).

Free choice profiling has certain advantages over other sensory approaches. Panel training is not compulsory and hence it is not as expensive as trained panels, which require significant time to learn how to discern product characteristics (Beilken et al., 1991). Compared with a trained panel approach FCP only needs time for the sensory evaluation as there is little or no training required. The panellists also need not have had previous sensory experience to participate successfully. FCP has been successfully used in numerous studies with a variety of products like cheese (Jack et al., 1993) salmon (Morzel et al., 1999), meat (Beilken et al., 1991), alcoholic beverages (Gains & Thomson, 1990) and coffee (Williams & Arnold, 1985). The panel leader should interact with the panellists on a continuous basis to make sure the descriptors developed are properly interpreted by both the panel leader and the panellists themselves. FCP provides the opportunity for the panellists to generate a description of the product without the bias of training.

Data obtained in free choice profiling is usually analyzed by Generalised Procrustes analysis (Gower, 1975). In GPA, the data obtained from individual panellists is transformed by translation, rotation and scaling (Gower 1975). GPA allows individual variation in descriptor use by the panellists to be examined by correlating them with principal axes of the centroid of the assessor sample spaces so that differences in scale use are adjusted to a common scale for comparison (Jones et al., 1989). The principle of Generalized Procrustes analysis is similar to Principal Component analysis however in GPA the individual data is used for analysis and not the means.

1.10. Summary

Omega-3 enriched food materials are now distinct market items and their demand is driven by the link between *n*-3 fatty acid consumption and the prevention of many diseases including cardiovascular diseases, cancer and inflammatory diseases like rheumatoid arthritis. Considering the potential importance of *n*-3 fatty acid, it has been given 'functional food' status. Omega-3 fatty acids are essential fatty acids, (especially α- linolenic acid) and they must be obtained via diet. As meat and meat products form an integral part of the human diet, there is an opportunity to modulate the fatty acid

profile of meat by incorporating *n*-3 fatty acids into meat to make them regularly available to consumers. Among the different types of meat, pork is considered to be an ideal species for *n*-3 enrichment because pork contains fat and dietary modulation of fatty acid profile is easy due to the anatomical peculiarities of the digestive system in pork. Also *n*-3 enrichment of pork meat offers a potential for value addition of pork meat and meat products.

Fatty acid modulation of pork may be achieved easily by feeding pigs feedstuffs such as flax seeds and fish meal, which are rich in *n*-3 fatty acids. For meat to reach Health Canada lebel requirements for functional food status in Canada, it must contain 300 mg *n*-3 fatty acid /100 g of serving. There is mostly inferior sensory quality due to generation of off-flavours because of lipid oxidation. Lipid oxidation is a serious problem with unsaturated lipids and occurs even though there are antioxidants added in the feeding trials. This is an overall problem during *n*-3 enrichment and is relevant as observed in the literature reviewed. Although the oxidation mechanisms of unsaturated fatty acids are well-known, as are those of antioxidants, how sugars interact with fatty acids to mask or produce flavours in complex foods such as meat is not known. The literature suggests that the addition of sugars to meat will improve its oxidative stability due to the involvement of Maillard reaction products as antioxidants. Consequently, the addition of sugars to *n*-3 enriched pork meat may improve its sensory characteristics and reduce warmed-over flavour.

This thesis will describe a study conducted at AAFC Lacombe and the University of Alberta with the collaboration of Northern Alberta Institute of Technology (NAIT) that investigated the effects of the addition of food grade sugars viz. glucose, sucrose and xylose on the oxidative stability and sensory characteristics of pork meat patties enriched with *n*-3 fatty acids. In this work, dietary treatment was aimed to increase the levels of α-linolenic acid in pork. Throughout the thesis, the term '*n*-3 fatty acid' refers to the sum of all *n*-3 fatty acids, ie. all of the 18- 20- and 22-carbon chain length *n*-3 fatty acids given in Table 2.7.6.

1.11. Tables

Table.1.11.1. Fatty acid composition of meat of different species (m/m%) [Modified from Varnam & Sutherland (1995)]

Fatty acid	Common name	Mutton	Beef	Pork	Chicken
C14:0	Myristic acid	2.0	2.5	1.5	1.3
C14:1	Myristoleic acid	0.5	0.5	0.5	0.2
C15:0	Pentadecyclic acid	0.5	0.5	--	--
C16:0	Palmitic acid	21.0	24.5	24.0	23.2
C16:1	Palmitoleic acid	3.0	3.1	3.5	6.5
C17:0	Margaric acid	1.0	1.0	0.5	0.3
C18:0	Stearic acid	28.0	18.5	14.0	6.4
C18:1	Oleic acid	37.0	40.0	43.0	41.6
C18:2	Linoleic acid	4.0	5.0	9.5	18.9
C18:3	Linolenic acid	--	0.5	1.0	1.3
C20:0	Arachidonic acid	0.5	0.5	0.5	--
C20:1	Arachidic acid	0.5	0.5	1.0	--
Others		2.0	2.5	1.5	0.3
PUFA/SFA ratio		0.07	0.11	0.25	0.64

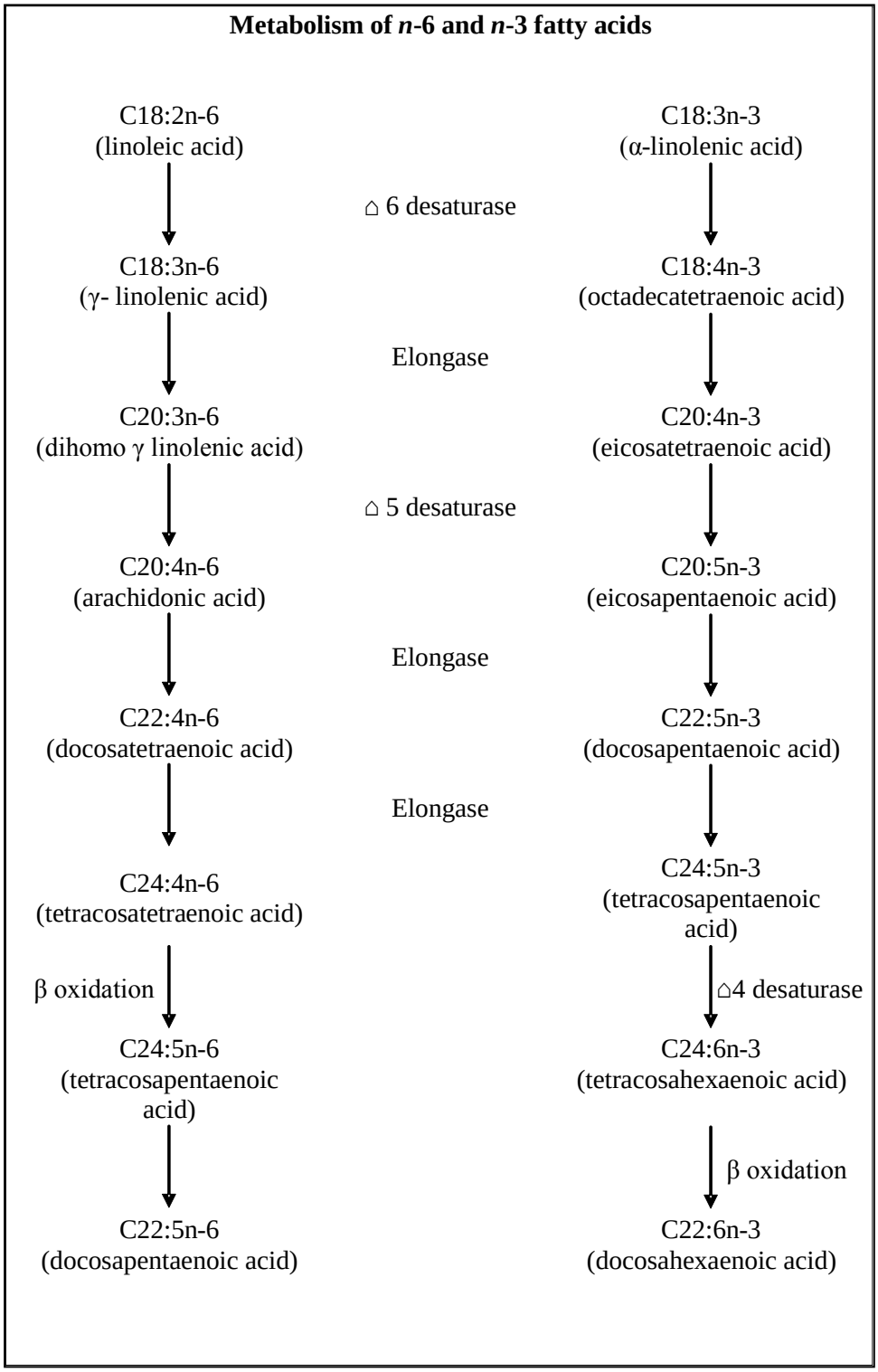
Table 1.11.2. Fatty acid ratios in meat of different species [Modified from Enser et al., (1996)]

Species	Tissue	P:S	*n*-6:*n*-3
Beef	Muscle	0.11	2.11
Beef	Adipose tissue	0.05	2.30
Pork	Muscle	0.15	1.32
Pork	Adipose tissue	0.09	1.37
Sheep	Muscle	0.58	7.22
Sheep	Adipose tissue	0.61	7.64

P = Polyunsaturated fatty acids; S= Saturated fatty acids

1.12. Figures

Figure 1.12.1. Metabolic pathways of *n*-3 and *n*-6 fatty acids



Adapted from Simopoulos, (2008)

Figure 1.12.2. Schematic representation of the various forms of myoglobin

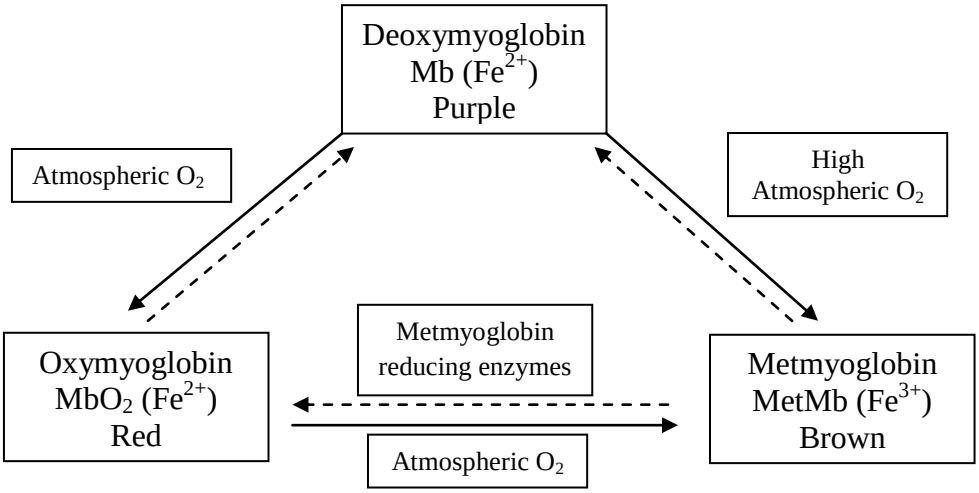
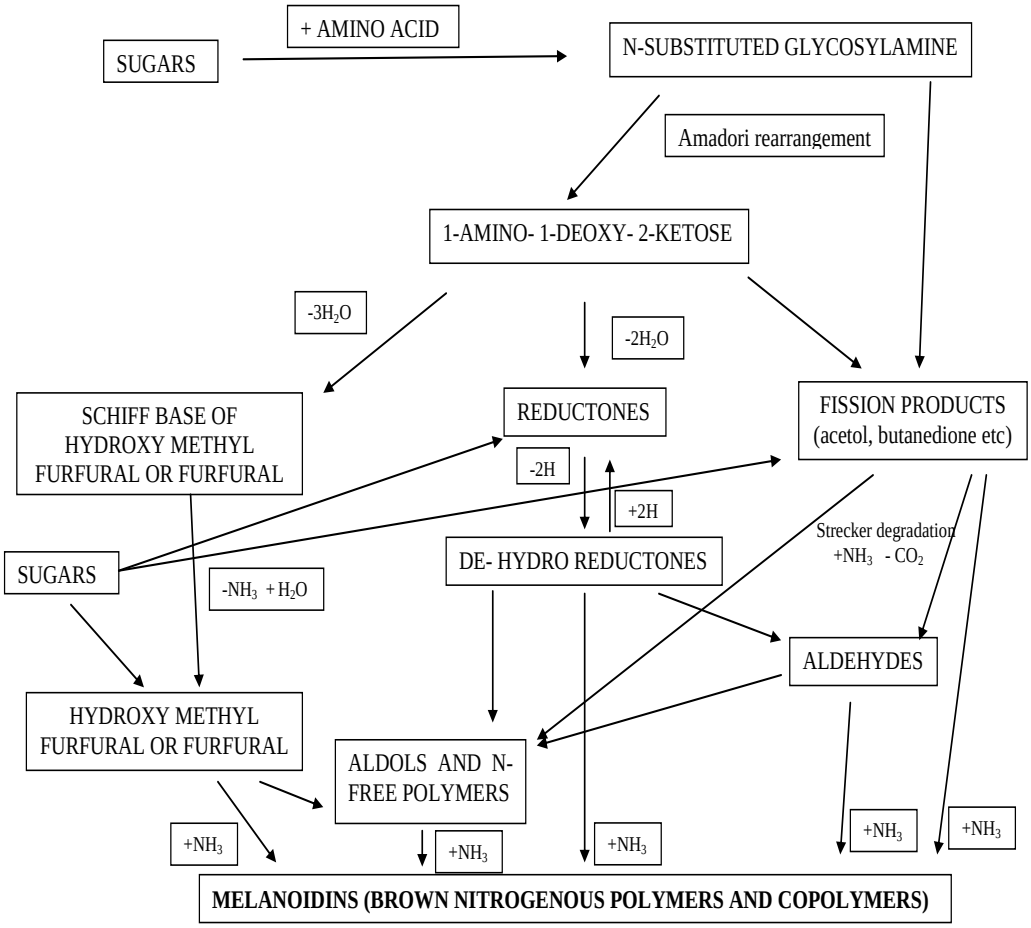


Figure 1.12.3. Schematic representation of Maillard reaction



1.13. Bibliography

Aliani, M., & Farmer, L. J. (2005). Precursors of chicken flavour. II. identification of key flavour precursors using sensory methods. *Journal of Agricultural and Food Chemistry, 53*(16), 6455-6462.

Andersen, H. J., Oksbjerg, N., Young, J. F., & Therkildsen, M. (2005). Feeding and meat quality-a future approach. *Meat Science, 70*(3), 543-554.

Arai, S., Osawa, T., Ohigashi, H., Yoshikawa, M., Kaminogawa, S., Watanabe, M., et al. (2001). A mainstay of functional food science in Japan—history, present status, and future outlook. *Bioscience, Biotechnology, and Biochemistry, 65*(1), 1-13.

Araya, J., Rodrigo, R., Videla, L. A., Thielemann, L., Orellana, M., Pettinelli, P., et al. (2004). Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clinical Science, 106*(6), 635-643.

Asghar, A., Gray, J. I., Miller, E. R., Ku, P. K., Booren, A. M., & Buckley, D. J. (1991). Influence of supranutritional vitamin E supplementation in the feed on swine growth performance and deposition in different tissues. *Journal of the Science of Food and Agriculture, 57*(1), 19-29.

Bailey, M. E. (1988). Inhibition of warmed-over flavour, with emphasis on maillard reaction products. *Food Technology, 42*(6), 123-126.

Barbut, S., Josephson, D. B., & Maurer, A. J. (1985). Antioxidant properties of rosemary oleoresin in turkey sausage. *Journal of Food Science, 50*(5), 1356-1359.

Beecher, G. R., Kastenschmidt, L. L., Hoekstra, W. G., Cassens, R. G., & Briskey, E. J. (1969). Energy metabolites in red and white striated muscles of the pig. *Journal of Agricultural and Food Chemistry, 17*, 29-33.

Beilken, S. L., Eadie, L. M., Griffiths, I., Jones, P. N., & Harris, P. V. (1991). Assessment of the sensory characteristics of meat patties. *Journal of Food Science, 56*(6), 1470-1475.

Berquin, I. M., Edwards, I. J., & Chen, Y. Q. (2008). Multi-targeted therapy of cancer by omega-3 fatty acids. *Cancer Letters, 269*(2), 363-377.

Boler, D. D., Gabriel, S. R., Yang, H., Balsbaugh, R., Mahan, D. C., Brewer, M. S., et al. (2009). Effect of different dietary levels of natural-source vitamin E in grow-finish pigs on pork quality and shelf life. *Meat Science, 83*(4), 723-730.

Bourre, J. M. (2005). Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: What is actually useful? *The Journal of Nutrition, Health & Aging, 9*(4), 232-242.

Bowers, J. A., Harrison, D. L., & Kropf, D. H. (1968). Browning and associated properties of porcine muscle. *Journal of Food Science, 33*(2), 147-149.

Brandt, M. A., Skinner, E. Z., & Coleman, J. A. (1963). Texture profile method. *Journal of Food Science, 28*(4), 404-409.

Bredahl, L., Grunert, K. G., & Fertin, C. (1998). Relating consumer perceptions of pork quality to physical product characteristics. *Food Quality and Preference, 9*(4), 273-281.

Brewer, M. S., & Vega, J. D. (1995). Detectable odor thresholds of selected lipid oxidation compounds in a meat model system. *Journal of Food Science, 60*(3), 592-595.

Broughton, K. S., Johnson, C. S., Pace, B. K., Liebman, M., & Kleppinger, K. M. (1997). Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *American Journal of Clinical Nutrition, 65*(4), 1011-1017.

Bruinsma, J. (2003). *World agriculture: Towards 2015/2030: An FAO perspective*. Earthscan Publications Ltd., London, UK.

Bryhni, E. A., Kjos, N. P., Ofstad, R., & Hunt, M. (2002). Polyunsaturated fat and fish oil in diets for growing-finishing pigs: Effects on fatty acid composition and meat, fat, and sausage quality. *Meat Science, 62*(1), 1-8.

Buckley, D. J., Morrissey, P. A., & Gray, J. I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science, 73*(10), 3122-3130.

Burdge, G. C. (2006). Metabolism of [alpha]-linolenic acid in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 75*(3), 161-168.

Burdge, G. C., Jones, A. E., & Wootton, S. A. (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. *British Journal of Nutrition, 88*(04), 355-363.

Burdge, G. C., & Calder, P. C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction, Nutrition, Development, 45*(5), 581-597.

Byrne, D. V., Bredie, W. L. P., Bak, L. S., Bertelsen, G., Martens, H., & Martens, M. (2001). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science, 59*(3), 229-249.

Cairncross, S. E., & Sjostrom, L. B. (1950). Flavour profiles - a new approach to flavour problems. *Food Technology, 4*(8), 308-311.

Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavour. *Meat Science, 77*(1), 63-80.

Campo, M. M., Nute, G. R., Wood, J. D., Elmore, S. J., Mottram, D. S., & Enser, M. (2003). Modelling the effect of fatty acids in odour development of cooked meat in vitro: Part I--sensory perception. *Meat Science, 63*(3), 367-375.

Carlson, S. E., Rhodes, P. G., & Ferguson, M. G. (1986). Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *American Journal of Clinical Nutrition, 44*(6), 798-804.

Cherian, G., & Hayat, Z. (2009). Long-term effects of feeding flaxseeds on hepatic lipid characteristics and histopathology of laying hens. *Poultry Science, 88*(12), 2555-2561.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., et al. (2005). Origins and evolution of the western diet: Health implications for the 21st century. *American Journal of Clinical Nutrition, 81*(2), 341-354.

Corino, C., Musella, M., & Mourot, J. (2008). Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of liveweight. *Journal of Animal Science, 86*(8), 1850-1860.

Cross, A. J., Leitzmann, M. F., Gail, M. H., Hollenbeck, A. R., Schatzkin, A., & Sinha, R. (2007). A prospective study of red and processed meat intake in relation to cancer risk. *Public Library of Science Medicine, 4*(12), 1973-1984.

Cross, H. R., Leu, R., & Miller, M. F. (1987). Scope of warmed-over flavour and its importance to the meat industry. *Warmed-Over Flavour of Meat,*1–18.

Dallongeville, J., Yarnell, J., Ducimetiere, P., Arveiler, D., Ferrieres, J., Montaye, M., et al. (2003). Fish consumption is associated with lower heart rates. *Circulation, 108*(7), 820-825.

Delgado, C. L. (2003). Rising consumption of meat and milk in developing countries has created a new food revolution. *Journal of Nutrition, 133*(11), 3907S-3910S.

Dyerberg, J., Bang, H. O., & Hjorne, N. (1975). Fatty acid composition of the plasma lipids in greenland eskimos. *American Journal of Clinical Nutrition, 28*(9), 958-966.

Eaton, S. B., Konner, M., & Shostak, M. (1988). Stone agers in the fast lane: Chronic degenerative diseases in evolutionary perspective. *The American Journal of Medicine, 84*(4), 739-749.

Einerson, M. A., & Reineccius, G. A. (1977). Inhibition of warmed-over flavour in retorted turkey by antioxidants formed during processing. *Journal of Food Processing and Preservation, 1*(4), 279-291.

Elmore, J. S., Campo, M. M., Enser, M., & Mottram, D. S. (2002). Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. *Journal of Agricultural and Food Chemistry, 50*(5), 1126-1132.

Emken, E. A., Adlof, R. O., & Gulley, R. M. (1994). Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochimica Et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1213*(3), 277-288.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J., & Wood, J. D. (1996). Fatty acid content and composition of english beef, lamb and pork at retail. *Meat Science, 42*(4), 443-456.

Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. *Journal of the Science of Food and Agriculture, 53*(4), 505-525.

Farmer, L. J., & Mottram, D. S. (1992). Effect of cysteine and ribose on the volatile thermal degradation products of a triglyceride and three phospholipids. *Journal of the Science of Food and Agriculture, 60*(4), 489-497.

Farmer, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maillard reactions involving cysteine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 49*(3), 347-368.

Farmer, L. J., Hagan, T. D. J., & Paraskevas, O. (1999). Role of selected precursors in meat flavour formation. *XiongYL, HoC-T, ShahidiF, Editors.Quality Attributes of Muscle Foods.New York: Kluwer Academic/Plenum Publishers,*159–172.

Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods, 1*(3), 217-243.

Fay, L. B., & Brevard, H. (2005). Contribution of mass spectrometry to the study of the maillard reaction in food. *Mass Spectrometry Reviews, 24*(4), 487-507.

Flax Council of Canada (2008) Retrieved from http://www.flaxcouncil.ca/english/pdf/Flax%20Feed%20Industry%20Guide%20Final.pdf, Date accessed : November `4, 2010

Fleischhauer, F. J., Yan, W. D., & Fischell, T. A. (1993). Fish oil improves endothelium-dependent coronary vasodilation in heart transplant recipients. *Journal of the American College of Cardiology, 21*(4), 982-989.

Fontanillas, R., Barroeta, A., Baucells, M. D., & Guardiola, F. (1998). Backfat fatty acid evolution in swine fed diets high in either cis-monounsaturated, trans, or (n-3) fats. *Journal of Animal Science, 76*(4), 1045-1055.

Food and Agricultural Organization (2009): Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/home.html, Date accessed: November `4, 2010

Foster, R., & Lunn, J. (2007). 40th anniversary briefing paper: Food availability and our changing diet. *Nutrition Bulletin, 32*(3), 187-249.

Frankel, E. N. (1984). Lipid oxidation: Mechanisms, products and biological significance. *Journal of the American Oil Chemists' Society, 61*(12), 1908-1917.

Freese, R., Mutanen, M., Valsta, L. M., & Salminen, I. (1994). Comparison of the effects of two diets rich in monounsaturated fatty acids differing in their

linoleic/alpha-linolenic acid ratio on platelet aggregation. *Thrombosis and Haemostasis, 71*(1), 73-77.

Gacula, M. C. (1997). *Descriptive sensory analysis in practice* Wiley-Blackwell.

Gains, N., & Thomson, D. M. H. (1990). Contextual evaluation of canned lagers using repertory grid method. *International Journal of Food Science & Technology, 25*(6), 699-705.

Gapinski, J. P., VanRuiswyk, J. V., Heudebert, G. R., & Schectman, G. S. (1993). Preventing restenosis with fish oils following coronary angioplasty: A meta-analysis. *Archives of Internal Medicine, 153*(13), 1595-1601.

Gardner, M. A., Huff-Lonergan, E., & Lonergan, S. M. (2005). Prediction of fresh pork quality using indicators of protein degradation and calpain activation. *Proceedings of the 51st International Congress of Meat Science and Technology,* 7–12.

Geelen, A., Brouwer, I. A., Schouten, E. G., Maan, A. C., Katan, M. B., & Zock, P. L. (2005). Effects of n-3 fatty acids from fish on premature ventricular complexes and heart rate in humans. *American Journal of Clinical Nutrition, 81*(2), 416-420.

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika, 40*(1), 33-51.

Griffin, M. D., Sanders, T. A. B., Davies, I. G., Morgan, L. M., Millward, D. J., Lewis, F., et al. (2006). Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: The OPTILIP study. *American Journal of Clinical Nutrition, 84*(6), 1290-1298.

Grunert, K. G. (2006). Future trends and consumer lifestyles with regard to meat consumption. *Meat Science, 74*(1), 149-160.

Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K., & Raes, K. (2008). Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *Journal of Animal Science, 86* (6), 1418-1425. doi:10.2527/jas.2007-0032

Hagve, T. A., & Christophersen, B. O. (1984). Effect of dietary fats on arachidonic acid and eicosapentaenoic acid biosynthesis and conversion to C22 fatty acids in isolated rat liver cells. *Biochimica Et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 796*(2), 205-217.

Health Canada. (1998).Policy paper-Nutraceuticals /functional foods and health claims on foods. Retrieved from http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/nutra-funct_foods-nutra-fonct_aliment-eng.php, Date accessed : November 14, 2010

He, Y., Wang, K., & Wang, L. (2010). Effect of α-tocopherol and β-carotene supplementation on meat quality and antioxidant capacity of pigs fed high-linseed oil diet. *The Journal of Animal & Plant Sciences, 20*(3), 180-188.

Herrmann, W., & Biermann, J. (1995). Comparison of effects of N-3 to N-6 fatty acids on serum level of lipoprotein (a) in patients with coronary artery disease. *The American Journal of Cardiology, 76*(7), 459-462.

Hertzman, C. & Leif, C. (1988). Influence of fishmeal, rape-seed, and rape-seed meal in feed on the fatty acid composition and storage stability of porcine body fat. *Meat Science, 23*(1), 37-53.

Higgs, J. D. (2000). The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science & Technology, 11*(3), 85-95.

Hooper, L., Thompson, R. L., Harrison, R. A., Summerbell, C. D., Ness, A. R., Moore, H. J., et al. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: Systematic review. *British Medical Journal, 332*(7544), 752-755.

Houben, J. H., Eikelenboom, G., & Hoving-Bolink, A. H. (1998). Effect of the dietary supplementation with vitamin E on colour stability and lipid oxidation in packaged, minced pork. *Meat Science, 48*(3-4), 265-273.

Huang, F. R., Zhan, Z. P., Luo, J., Liu, Z. X., & Peng, J. (2008). Duration of dietary linseed feeding affects the intramuscular fat, muscle mass and fatty acid composition in pig muscle. *Livestock Science, 118*(1-2), 132-139.

Hudson, J. E., & Loxley, R. A. (1983). The effect of pentose sugars on the aroma and flavour of mutton. *Food Technology in Australia, 83*, 174-175.

Huff-Lonergan, E., Baas, T. J., Malek, M., Dekkers, J. C., Prusa, K., & Rothschild, M. F. (2002). Correlations among selected pork quality traits. *Journal of Animal Science, 80*(3), 617-627.

Hussein, N., Ah-Sing, E., Wilkinson, P., Leach, C., Griffin, B. A., & Millward, D. J. (2005). Long-chain conversion of [13C] linoleic acid and {alpha}-linolenic acid in response to marked changes in their dietary intake in men. *The Journal of Lipid Research, 46*(2), 269-280.

Igene, J. O., King, J. A., Pearson, A. M., & Gray, J. I. (1979). Influence of heme pigments, nitrite, and nonheme iron on development of warmed-over flavour (WOF) in cooked meat. *Journal of Agricultural and Food Chemistry, 27*(4), 838-842.

Irvine, R. B., McConnell, J., Lafond, G. P., May, W. E., Huitgreen, G., Ulrich, A., et al. (2010). Impact of production practices on fiber yield of oilseed flax under canadian prairie conditions. *Canadian Journal of Plant Science, 90*(1), 61-70.

Jack, F. R., Piggott, J. R., & Paterson, A. (1993). Discrimination of texture and appearance in cheddar cheese using consumer free ‐ choice profiling. *Journal of Sensory Studies, 8*(2), 167-176.

Jadhav, S. J., Nimbalkar, S. S., Kulkarni, A. D., and Madhavi, D. L. (1995). Lipid Oxidation in Biological and Food System. In S. S. Deshpande, U. S. Deshpande, and D. K. Salunkhe (Eds.), Food Antioxidants: Technological,

Toxicological, and Health Perspectives (pp. 361-370). New York: Marcel Dekker, Inc.

Jambrenghi, A. C., Colonna, M. A., Giannico, F., Favia, R., Minuti, F., Scafizzari, M., et al. (2005). Dietary supplementation of garlic and rosemary: Effects on colour stability and lipid oxidation in lamb meat. *Italian Journal of Animal Science, 4*, 366-368.

James, M. J., & Cleland, L. G. (1997). Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Seminars in Arthritis and Rheumatism, 27*(2) 85-97.

Jansman, A. J. M., van Wikselaar, P., & Wagenaars, C. M. F. (2007). Effects of feeding linseed and linseed expeller meal to newly weaned piglets on growth performance and gut health and function. *Livestock Science, 108*(1-3), 171-174.

Jensen, C., Guider, J., Skovgaar, I. M., Staun, H., Skibsted, L. H., Jensen, S. K., et al. (1997). Effects of dietary [alpha]-tocopheryl acetate supplementation on [alpha]-tocopherol deposition in porcine m. psoas major and m. longissimus dorsi and on drip loss, colour stability and oxidative stability of pork meat. *Meat Science, 45*(4), 491-500.

Jhala, A. J., & Hall, L. M. (2010). Flax (linum usitatissimum L.): Current uses and future applications. *Australian Journal of Basic and Applied Sciences, 4*(9), 4304-4312.

Jones, P. N., Macfie, H. J. H., & Beilken, S. L. (1989). Use of preference mapping to relate consumer preference to the sensory properties of a processed meat product (tinned cat food). *Journal of the Science of Food and Agriculture, 47*(1), 113-123.

Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., et al. (2010). Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower-finisher pigs. *Meat Science, 84*(3), 578-584.

Kantarci, A., & Van Dyke, T. E. (2003). Lipoxins in chronic inflammation. *Critical Reviews in Oral Biology & Medicine, 14*(1), 4-12.

Katan, M. B., Brouwer, I. A., Clarke, R., Geleijnse, J. M., & Mensink, R. P. (2010). Saturated fat and heart disease. *American Journal of Clinical Nutrition, 92*(2), 459-460.

Komprda, T., Zelenka, J., Fajmonova, E., Fialova, M., & Kladrobas, D. (2005). Arachidonic acid and long-chain n− 3 polyunsaturated fatty acid contents in meat of selected poultry and fish species in relation to dietary fat sources. *Journal of Agricultural and Food Chemistry, 53*(17), 6804-6812.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., & Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science, 81*(8), 1967-1979.

Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. effect of diet and breed. *Meat Science, 79*(1), 124-130.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(2), e20-e31.

Lam, T. K., Cross, A. J., Consonni, D., Randi, G., Bagnardi, V., Bertazzi, P. A., et al. (2009). Intakes of red meat, processed meat, and meat mutagens increase lung cancer risk. *Cancer Research, 69*(3), 932-939.

Langron, S. P. (1983). The application of procrustes statistics to sensory profiling. *Sensory Quality in Food and Beverages: Definition, Measurement and Control*, 89-95.

Lauridsen, C., Engel, H., Craig, A. M., & Traber, M. G. (2002). Relative bioactivity of dietary RRR-and all-rac-alpha-tocopheryl acetates in swine assessed with deuterium-labeled vitamin E. *Journal of Animal Science, 80*(3), 702-707.

Lauridsen, L., Miklos, R., Schäfer, A., Aaslyng, M. D., & Bredie, W. L. P. (2006). Influence of added carbohydrates on the aroma profile of cooked pork. *Developments in Food Science, 43*, 355-358.

Lawless, H. T., & Heymann, H. (1999). *Sensory evaluation of food: Principles and practices* Aspen Publishers.

Lee, J. H., O'Keefe, J. H., Lavie, C. J., Marchioli, R., & Harris, W. S. (2008). Omega-3 fatty acids for cardioprotection. *Mayo Clinic Proceedings, 83*(3) 324-332.

Leskanich, C. O., Matthews, K. R., Warkup, C. C., Noble, R. C., & Hazzledine, M. (1997). The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. *Journal of Animal Science, 75*(3), 673-683.

Lewin, G. A. (2005). *Effects of omega-3 fatty acids on child and maternal health*. Evidence Report/Technology Assessment Number 118. Agency for Healthcare Research and Quality.

Lindahl, G., Lundström, K., & Tornberg, E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science, 59*(2), 141-151.

Lonergan, S. M., Huff-Lonergan, E., Rowe, L. J., Kuhlers, D. L., & Jungst, S. B. (2001). Selection for lean growth efficiency in duroc pigs influences pork quality. *Journal of Animal Science, 79*(8), 2075-2085.

Lunn, J., & Buttriss, J. L. (2008). Incorporating omega ‐ 3 in the food chain–why, where and how? *Nutrition Bulletin, 33*(3), 250-256.

MacDougall, D. B. (1982). Changes in the colour and opacity of meat. *Food Chemistry, 9*(1-2), 75-88.

MacLean, C. H., Newberry, S. J., Mojica, W. A., Khanna, P., Issa, A. M., Suttorp, M. J., et al. (2006). Effects of omega-3 fatty acids on cancer risk: A systematic review. *Journal of the American Medical Association, 295*(4), 403-415.

Maes, M., Smith, R., Christophe, A., Cosyns, P., Desnyder, R., & Meltzer, H. (1996). Fatty acid composition in major depression: Decreased [omega] 3 fractions in cholesteryl esters and increased C20: 4 [omega] 6/C20: 5 [omega] 3 ratio in cholesteryl esters and phospholipids. *Journal of Affective Disorders, 38*(1), 35-46.

Maillard, V., Bougnoux, P., Ferrari, P., Jourdan, M. L., Pinault, M., Lavillonnière, F., et al. (2002). N‐3 and N‐6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case‐control study in tours, france. *International Journal of Cancer, 98*(1), 78-83.

Maltin, C., Balcerzak, D., Tilley, R., & Delday, M. (2003). Determinants of meat quality: Tenderness. *Proceedings of the Nutrition Society, 62*(02), 337-347.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*(1), 100-121.

Mantzioris, E., James, M. J., Gibson, R. A., & Cleland, L. G. (1994). Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *American Journal of Clinical Nutrition, 59*(6), 1304-1309.

Marcinčák, S., Nemcová, R., Sokol, J., Popelka, P., Gancarčíková, S., & Švedová, M. (2009). Impact of feeding of flaxseed and probiotics on meat quality and lipid oxidation process in pork during storage. *Slovenian Veterinary Research,*13-18.

Matthews, K. R., Homer, D. B., Thies, F., & Calder, P. C. (2000). Effect of whole linseed (linum usitatissimum) in the diet of finishing pigs on growth performance and on the qualityand fatty acid composition of various tissues. *British Journal of Nutrition, 83*(06), 637-643.

Meadus, W. J., Duff, P., Uttaro, B., Aalhus, J. L., Rolland, D. C., Gibson, L. L., et al. (2010). Production of docosahexaenoic acid (DHA) enriched bacon. *Journal of Agricultural and Food Chemistry, 58*(1), 465-472.

Meilgaard, M., Civille, G. V., & Carr, B. T. (2007). *Sensory evaluation techniques* CRC Press Inc.

Meinert, L., Schäfer, A., Bjergegaard, C., Aaslyng, M. D., & Bredie, W. L. P. (2009). Comparison of glucose, glucose 6-phosphate, ribose, and mannose as flavour precursors in pork; the effect of monosaccharide addition on flavour generation. *Meat Science, 81*(3), 419-425.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science, 82*(4), 1195-1205.

Monahan, F. J., Asghar, A., Gray, J. I., Buckley, D. J., & Morrissey, P. A. (1994). Effect of oxidized dietary lipid and vitamin E on the colour stability of pork chops. *Meat Science, 37*(2), 205-215.

Morgan, D. R., Dixon, L. J., Hanratty, C. G., El-Sherbeeny, N., Hamilton, P. B., McGrath, L. T., et al. (2006). Effects of dietary omega-3 fatty acid supplementation on endothelium-dependent vasodilation in patients with chronic heart failure. *The American Journal of Cardiology, 97*(4), 547-551.

Morris, D. H. (2007). *Flax: A health and nutrition primer* Flax Council of Canada.

Morrissey, P. A., Buckley, D. J., Sheehy, P. J. A., & Monahan, F. J. (1994). Vitamin E and meat quality. *Proceedings of the Nutrition Society, 53*(02), 289-295.

Morzel, M., Sheehan, E. M., Delahunty, C. M., & Arendt, E. K. (1999). Sensory evaluation of lightly preserved salmon using free‐choice profiling. *International Journal of Food Science & Technology, 34*(2), 115-123.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S., & Whitfield, F. B. (1995). Maillard-lipid interactions in nonaqueous systems: Volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *Journal of Agricultural and Food Chemistry, 43*(5), 1302-1306.

Musella, M., Cannata, S., Rossi, R., Mourot, J., Baldini, P., & Corino, C. (2009). Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. *Journal of Animal Science, 87*(11), 3578-3588.

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G., et al. (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science, 70*(1), 63-74.

Nursten, H. E. (2005). *The Maillard reaction: Chemistry, biochemistry, and implications.* Royal Society of Chemistry, UK.

Oreskovich, D. C., Klein, B. P., & Sutherland, J. W. (1991). Procrustes analysis and its applications to free-choice and other sensory profiling. *LAWLESS, HT; KLEIN, BP Sensory Science Theory and Applications in Foods.New York, Marcel Dekker,* 353-394.

Pawlosky, R. J., Hibbeln, J. R., Novotny, J. A., & Salem, N. (2001). Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *Journal of Lipid Research, 42*(8), 1257-1265.

Pearson, A. M., Love, J. D., & Shorland, F. B. (1977). Warmed-over flavour in meat, poultry and fish. *Advances in Food Research, 23*(1), 1-72.

Peterson, E. B., Van Eenoo Jr, E., McGuirk, A., & Preckel, P. V. (2001). Perceptions of fat content in meat products. *Agribusiness, 17*(4), 437-453.

Phillips, A. L., Faustman, C., Lynch, M. P., Govoni, K. E., Hoagland, T. A., & Zinn, S. A. (2001). Effect of dietary [alpha]-tocopherol supplementation on color and lipid stability in pork. *Meat Science, 58*(4), 389-393.

Plourde, M., & Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: Implications for their dietary essentiality and use as supplements. *Applied Physiology, Nutrition, and Metabolism, 32*(4), 619-634.

Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Animal Feed Science and Technology, 113*(1-4), 199-221.

Raheja, B. S., Sadikot, S. M., Phatak, R. B., & Rao, M. B. (1993). Significance of the N‐6/N‐3 ratio for insulin action in diabetes. *Annals of the New York Academy of Sciences, 683*(1), 258-271.

Rhee, K. S., Anderson, L. M., & Sams, A. R. (1996). Lipid oxidation potential of beef, chicken, and pork. *Journal of Food Science, 61*(1), 8-12.

Romans, J. R., Johnson, R. C., Wulf, D. M., Libal, G. W., & Costello, W. J. (1995a). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: I. dietary level of flaxseed. *Journal of Animal Science, 73*(7), 1982-1986.

Romans, J. R., Wulf, D. M., Johnson, R. C., Libal, G. W., & Costello, W. J. (1995b). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II. duration of 15% dietary flaxseed. *Journal of Animal Science, 73*(7), 1987-1999.

Rose, D. P., & Connolly, J. M. (1999). Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacology & Therapeutics, 83*(3), 217-244.

Rosenvold, K., & Andersen, H. J. (2003). Factors of significance for pork quality--a review. *Meat Science, 64*(3), 219-237.

Roynette, C. E., Calder, P. C., Dupertuis, Y. M., & Pichard, C. (2004). n-3 polyunsaturated fatty acids and colon cancer prevention. *Clinical Nutrition, 23*(2), 139-151.

Salter, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maiilard reactions involving glycine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 46*(2), 227-242.

Santos, C., Hoz, L., Cambero, M. I., Cabeza, M. C., & Ordóñez, J. A. (2008). Enrichment of dry-cured ham with [alpha]-linolenic acid and [alpha]-tocopherol by the use of linseed oil and [alpha]-tocopheryl acetate in pig diets. *Meat Science, 80*(3), 668-674.

Satyanarayan, V. T., & Honikel, K. O. (1992). Effect of different cooking methods on warmed-over flavour development in pork. *Zeitschrift Für Lebensmitteluntersuchung Und-Forschung A, 194*(5), 422-425.

Schricker, B. R., & Miller, D. D. (1983). Effects of cooking and chemical treatment on heme and nonheme iron in meat. *Journal of Food Science, 48*(4), 1340-1343.

Shahidi, F., Rubin, L. J., Diosady, L. L., Kassam, N., Fong, J. C., & Wood, D. F. (1986). Effect of sequestering agents on lipid oxidation in cooked meats. *Food Chemistry, 21*(2), 145-152.

Shikama, K. (1998). The molecular mechanism of autoxidation for myoglobin and hemoglobin: A venerable puzzle. *Chemical Reviews, 98*(4), 1357-1374.

Shurson, G. C., Spiehs, M., & Whitney, M. (2004). The use of maize distiller's dried grains with solubles in pig diets. *Pig News and Information, 25*(2), 75N-83N.

Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poultry Science, 79*(7), 961-970.

Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition, 54*(3), 438-463.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition, 70*(3), 560S-569S.

Simopoulos, A. P. (2001). n− 3 fatty acids and human health: Defining strategies for public policy. *Lipids, 36*, 83-89.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine, 233*(6), 674-688.

Simopoulos, A. P., Leaf, A., & Salem Jr, N. (1999). Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Journal of the American College of Nutrition, 18*(5), 487-489.

Spanier, A. M., Edwards, J. V., & Dupuy, H. P. (1988). The warmed-over flavour process in beef. A study of meat proteins and peptides. *Food Technology, 42*(6), 110-118.

Specht-Overholt, S., Romans, J. R., Marchello, M. J., Izard, R. S., Crews, M. G., Simon, D. M., et al. (1997). Fatty acid composition of commercially manufactured omega-3 enriched pork products, haddock, and mackerel. *Journal of Animal Science, 75*(9), 2335-2343.

Sprecher, H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica Et Biophysica Acta, 1486*(2-3), 219-231.

Stampanoni, C. R. (1993). Quantitative flavour profiling: An effective tool in flavour perception. *International Journal of Food Marketing Technology, 2*, 4-8.

Statistics Canada, 2010. http://www.statcan.gc.ca/daily-quotidien/100527/dq100527d-eng.htm Retrieved on 27.01.2011

Stone, H., Sidel, J., Oliver, S., Woolsey, A., & Singleton, R. C. (1974). Sensory evaluation by quantitative descriptive analysis. *Food Technology, 28*(11), 24-34.

Sylvestre, M. N., Balcerzak, D., Feidt, C., Baracos, V. E., & Bellut, J. B. (2002). Elevated rate of collagen solubilization and postmortem degradation inmuscles of lambs with high growth rates: Possible relationship with activity of matrix metalloproteinases. *Journal of Animal Science, 80*(7), 1871-1878.

Thacker, P. A., Racz, V. J., & Soita, H. W. (2004). Performance and carcass characteristics of growing-finishing pigs fed barley-based diets supplemented with linpro (extruded whole flaxseed and peas) or soybean meal. *Canadian Journal of Animal Science, 84*(4), 681-688.

Theodoratou, E., McNeill, G., Cetnarskyj, R., Farrington, S. M., Tenesa, A., Barnetson, R., et al. (2007). Dietary fatty acids and colorectal cancer: A case-control study. *American Journal of Epidemiology, 166*(2), 181-195.

Thiébaut, A., Jiao, L., Silverman, D. T., Cross, A. J., Thompson, F. E., Subar, A. F., et al. (2009). Dietary fatty acids and pancreatic cancer in the NIH-AARP diet and health study. *Journal of the National Cancer Institute, 101*(14), 1001-1011.

Tikk, K., Lindahl, G., Karlsson, A. H., & Andersen, H. J. (2008). The significance of diet, slaughter weight and aging time on pork colour and colour stability. *Meat Science, 79*(4), 806-816.

Tikk, K., Tikk, M., Aaslyng, M. D., Karlsson, A. H., Lindahl, G., & Andersen, H. J. (2007). Significance of fat supplemented diets on pork quality-connections between specific fatty acids and sensory attributes of pork. *Meat Science, 77*(2), 275-286.

Tims, M. J., & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technology, 12*(5), 240-243.

Toft, I., Bonaa, K. H., Ingebretsen, O. C., Nordoy, A., & Jenssen, T. (1995). Effects of n-3 polyunsaturated fatty acids on glucose homeostasis and blood pressure in essential hypertension. *Annals of Internal Medicine, 123*(12), 911-918.

Valencia, I., Ansorena, D., & Astiasarán, I. (2006). Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. *Meat Science, 72*(4), 727-733.

Van de Werf, F., Ardissino, D., Betriu, A., Cokkinos, D. V., Falk, E., Fox, K. A. A., et al. (2003). Management of acute myocardial infarction in patients presenting with ST-segment elevation. *European Heart Journal, 24*(1), 28-66.

Varnam, A. H., & Sutherland, J. P. (1995). *Meat and meat products: Technology, chemistry, and microbiology* Aspen Publishers.

Wallace, F. A., Miles, E. A., & Calder, P. C. (2003). Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. *British Journal of Nutrition, 89*(05), 679-689.

Warriss, P. D., Kestin, S. C., Rolph, T. P., & Brown, S. N. (1990). The effects of the beta-adrenergic agonist salbutamol on meat quality in pigs. *Journal of Animal Science, 68*(1), 128-136.

Weiss, L. A., Barrett-Connor, E., & von Muhlen, D. (2005). Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: The rancho bernardo study. *American Journal of Clinical Nutrition, 81*(4), 934-938.

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *Journal of Animal Science, 78*(4), 958-965.

Wijendran, V., & Hayes, K. C. (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Nutrition, 24*(1), 597-615.

Wiklund, E., Andersson, A., Malmfors, G., & Lundström, K. (1996). Muscle glycogen levels and blood metabolites in reindeer (rangifer tarandus tarandus L.) after transport and lairage. *Meat Science, 42*(2), 133-144.

Willemot, C., Poste, L. M., Salvador, J., Wood, D. F., & Butler, G. (1985). Lipid degradation in pork during warmed-over flavour development= dégradation des lipides dans de la viande de porc au cours du développement de la flaveur de réchauffé. *Canadian Institute of Food Science and Technology Journal, 18*(4), 316-322.

Williams, A. A., & Arnold, G. M. (1985). A comparison of the aromas of six coffees characterised by conventional profiling, free‐choice profiling and similarity scaling methods. *Journal of the Science of Food and Agriculture, 36*(3), 204-214.

Williams, P. (2007). Nutritional composition of red meat. *Nutrition and Dietetics, 64*(4), 234-240.

Wood, J. D., & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition, 78*(01), 49-60.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78*(4), 343-358.

Yashodhara, B. M., Umakanth, S., Pappachan, J. M., Bhat, S. K., Kamath, R., & Choo, B. H. (2009). Omega-3 fatty acids: A comprehensive review of their role in health and disease. *Postgraduate Medical Journal, 85*(1000), 84-90.

Young, O. A., & Cummings, T. L. (2008). Effect of xylose on sheepmeat flavours in Casserole‐Style cooking. *Journal of Food Science, 73*(6), S308-S313.

Zhan, Z. P., Huang, F. R., Luo, J., Dai, J. J., Yan, X. H., & Peng, J. (2008). Duration of feeding linseed diet influences expression of inflammation related genes and growth performance of growing-finishing barrows. *Journal of Animal Science, 87* (2), 603-611.doi:10.2527/jas.2007-0177

Chapter 2

Growth performance, carcass characteristics and chemical composition of pork from pigs fed an omega-3 fatty acid enriched diet

2.1. Introduction

Canada has a tradition of a sustainable pork industry over the past several decades even though there were some severe market adjustments in recent years. Pork has been a popular choice of meat in the past, but present statistics show that there has been a reduction in swine herd numbers, overall pork production and consumption and also a decrease in pork export volume (Statistics Canada, 2010). Many factors have contributed to this reduction in pork business, including the initiation of Mandatory Country of Origin Labelling (MCOOL) by the United States of America (US), high grain prices, parity of Canadian dollar with US dollar and disruptions to international and domestic trade due to the outbreak of the H1N1 influenza.

Consumer perception of pork is based on its main attributes, namely leanness, healthiness, taste and tenderness (Verbeke et al., 1999). Nutritionally, pork meat contains high quality proteins as well as essential B vitamins and minerals like iron and zinc (Verbeke et al., 1999). The apprehensions about eating pork stem primarily from concerns about its fat content and consumers refrain from eating pork due to the belief that pork also contains high cholesterol (Verbeke et al., 1999). The cholesterol content in raw pork subcutaneous fat is estimated to be 55 mg cholesterol/100 g fat, while that of raw lean pork is estimated to be 60 mg cholesterol/100 g of lean, both of which are much lower than that of butter, which contains 240 mg of cholesterol/ 100g of butter (Honikel & Arneth, 1996). The fatty acid profile of pork can be easily manipulated by dietary changes (Bou et al., 2010) and the level of unsaturated fatty acids could be increased thereby modifying the polyunsaturated fatty acid to saturated fatty acids ratio (P:S) to a level that is beneficial to human health. Furthermore, there is potential for converting pork meat to a source of omega-3 fatty acids (*n*-3 fatty acids) so that it could serve as a functional food.

Consumption of *n*-3 fatty acids is considered to be beneficial to human health and, as these fatty acids cannot be synthesised by human body, they must be supplied in the diet. There are *n*-3 tablets and other supplements available that can directly supply *n*-3 fatty acids, but this is a therapeutic measure rather than continuous dietary source of *n*-3 fatty acids. Hence the most acceptable way of ensuring that there is sufficient intake of these fatty acids in the human diet is by incorporating them into routinely eaten foods. Recent trends in the food industry have given *n*-3 enriched foods a functional food status due to their potential health benefits to consumers and food producers have been encouraged to re-consider supplying products to this niche market (Giner, 2009).

Enrichment of pork with *n*-3 fatty acids may add value to fresh pork and assist with differentiating Canadian pork on the global market. Previous research aimed at increasing *n*-3 fatty acid content in pork meat has shown that *n*-3 fatty acid enrichment may be more easily accomplished in pigs than in other livestock species. Feeding pigs flax, fish meal, and algae, which are good sources of *n*-3 fatty acids, has been shown to enrich pork with *n*-3 fatty acids (Bryhni et al., 2002; He et al., 2002; Nuernberg et al., 2005). Dietary supplementation of *n*-3 enriched feed to pigs usually also necessitates dietary antioxidant supplementation to reduce oxidation of the *n*-3 fatty acids. There are different antioxidants fed to pigs, but the most prevalent is Vitamin E (α-tocopherol). Different levels of α-tocopherol supplementation ranging from 100 to 400 mg/kg of diet can increase deposition of *α*-tocopherol in meat and reduce lipid oxidation (Asghar et al., 1991; Monahan et al., 1994).

Dietary changes can modify carcass and muscle composition. Kouba et al. (2003) fed pigs a diet containing 6% crushed linseed along with 150 mg vitamin E and found dietary treatments did not produced any significant change on the animal live weight, carcass weight, weight gain, fat thickness and muscle pH. Matthews et al. (2000) fed 100 g/kg of linseed along with 100 mg of vitamin E to pigs and also found that there were no negative effects of feeding linseed on feed conversion ratio, weight gain, live and carcass weights as well as on fat hardness measurements. Nuernberg et al. (2005) found that supplementation of 5% linseed oil did not produce any negative effects on

animal growth or carcass characteristics. Similar results were obtained in the study conducted by Corino et al. (2008) in which pigs were fed a diet that contained 5% whole extruded linseed along with 170 mg of vitamin E and 250 µg of selenium and had no effects on feed intake, weight gain as well on the live and carcass weights and dressing percentage. Guillevic et al. (2009) fed 4.2% of extruded linseed along with 40 mg vitamin E and 0.25 mg selenium to pigs and there were no deleterious effects on animal performance and carcass characteristics. Realini et al. (2010) also had similar results with no effects on animal performance and carcass characteristics when different swine diet fat sources including tallow, sunflower oil and linseed oil were compared.

Considering that *n*-3 enrichment of pork can be accomplished easily by manipulation of pig diet feed ingredients, it is also important to understand how these dietary changes may affect pig growth performance, carcass characteristics and overall cooked meat quality characteristics when fed at levels that produce CFIA labelling standards of 300mg/ 100g of product serving. The present study examined the animal growth, carcass quality and muscle fatty acid composition of pork when fed high levels of flax (10%) and vitamin E (400mg/kg) for 6 weeks. It tested the hypothesis that supplementation of flax and vitamin E at these high levels will not produce any deleterious effects on the animal performance and carcass characteristics.

2.2. Materials and methods

2.2.1. Animal selection and experimental design

Animals used in this study were cared according to the guidelines set out by the Canadian Council of Animal Care. The animals were part of a different experiment at AAFC Lacombe Unit with three durations of 10% flax treatments (0, 3 and 6 weeks) and three levels of vitamin E (40mg/kg, 200mg/kg and 400mg/kg) and the data was taken from only those animals representing the highest and lowest of the dietary treatments. Thirty-six Manor Hybrid x Duroc crossbred barrows were stratified from highest to lowest by genetics and weight at weaning and assigned to a pen with three pigs per pen. Dietary treatments were allocated in a balanced manner to pens (n=3 for diet replication) and were blocked by room. Three research rooms were used for a total of 12 study pens. The study was conducted at the Swine Unit at

Agriculture and Agri-Food Canada, Lacombe as a 2 × 2 factorial feeding trial with two dietary flax treatments of 10% flax fed for either 0 (no flax-control) or 6 weeks and two levels of Vitamin E (50% DL-alpha tocopheryl acetate, Long Wing International, Inc. Oakville ON), either 40 (control) or 400 mg Vitamin E/kg of feed. Experimental flax diets contained LinPro® (O & T Farms, Regina, Saskatchewan) as a commercial source of flax, which contained 50:50 mixture of co-extruded flaxseed and field peas processed using single screw extrusion technology at 400 psi without water and a barrel temperature of 135 °C (Htoo et al., 2008).

Pigs were fed once daily and feed weight was recorded. Pigs were weighed weekly and all the weekly feed weigh backs were also recorded. Feed and animal weights, health events, treatment records and experimental deviations were noted according to the Standard Operating Procedures developed by the AAFC-Lacombe Swine Unit. Diets were adjusted as required to meet the changing nutritional needs of the growing and finishing pigs (Table 2.7.1). Pigs received the grower diet when they attained a body weight of 34.6 ± 3.8 kg (mean ± standard deviation (SD)) and were switched to finisher diet when they reached a body weight of 62.6 ± 4.4 kg. Pigs were fed the experimental diet when they attained a body weight of 78.6 ± 4.6 kg and were fed the diet for 6 weeks before slaughter. Feed conversion ratio (FCR) during the study was calculated by dividing total feed intake by total weight gain. Animals were slaughtered on three slaughter dates, with one complete room (4 pens; 1 from each dietary treatment, n=12) slaughtered in a single day. On the morning of a slaughter, a final live weight and feed remaining were recorded before shipping. Pigs were shipped to the AAFC-Lacombe research abattoir where they were held overnight without feed but were given free access to water until slaughter.

2.2.2. Slaughter

At the time of slaughter final live weights were recorded and animals were electrically stunned, killed by exsanguination and the carcass dressed in a manner similar to that of commercial processing. During the course of dressing, weights of kidneys, leaf lard, front feet and head were recorded for calculating commercial hot weight. After splitting the carcass, hot side weights were recorded. At 45-min post-mortem, an estimated lean yield was

determined on the left side between 3rd and 4th anterior rib approximately 7 cm from the midline using an Anitech PG 100 Grading Probe (Anitech Information Systems Inc., Markham ON). A 45-min pH and temperature was recorded for the left *longissimus thoracis* (LT) between the 11th and 12th vertebrae using a Hanna H19025C pH meter (Hanna Instruments, Mississauga ON) equipped with an Orion Ingold electrode (Udorf, Switzerland).

2.2.3. Carcass characteristics

After 24 h post-mortem, left and right carcass sides were weighed to determine cooler shrink loss. Muscle pH and temperature readings were recorded on the left LT between the 10th and 11th vertebrae using a Fisher Scientific Accumet AP72 pH meter (Fisher Scientific, Mississauga ON) equipped with an Orion Ingold electrode. Fat hardness readings (0 to 100 durometer units) were determined on the left sides of the carcasses at the 1st and 2nd thoracic vertebrae on the second subcutaneous fat layer using a durometer (Model LG2400, Rex Gauge Company, Buffalo Grove, IL) in accordance with the specification standard D-2240 (ASTM 2008).

2.2.4. Preparation of meat grinds & patties

Spinalis dorsi and *Gluteus medius* muscles and subcutaneous fat from the loin primal were removed from both sides of each carcass and were labelled, weighed, packaged under vacuum (Multivac AGW, Multivac Inc., Kansas City, MO) and frozen at –35°C until further processing. Following the collection of tissues from all three replicates, frozen lean and subcutaneous fat collected from three animals receiving the same treatment were pooled across pens and tempered at -2°C until they were sufficiently thawed to use in grind production. Three replicates of 10 kg lean grind were produced by combining 8.5 kg of lean and 1.5 kg of subcutaneous fat tissue to produce a nominal 15% fat to lean grind. The meat and fat were passed once through a commercial grinder equipped with a coarse grind plate (0.95cm) (Hollymatic 175 Mixer Grinder, Hollymatic Corp., Countryside, IL). About 250 g of the grinds were collected from the pooled coarse grind for pH, proximate analyses, fatty acid analyses and α-tocopherol estimation.

2.2.5. Proximate analysis

Approximately 100 g of pork grind was weighed into a stainless steel beaker and dried at 102°C for 24 h in a gravity convection-drying oven (VWR

Scientific Model 1370FM; Mississauga, ON). After 24 h, the beakers were removed from the oven and were allowed to cool to room temperature for 10 min before final weights were recorded. Dried samples were crushed using a grinder (Grindomix Model GM200, Retsch Inc., Newton, PA). About 2 g of the crushed samples were weighed into a thimble in duplicate and analyzed for crude fat content (AOAC, 1995; Official Method 991.36) by petroleum ether extraction (Foss Soxtec System Model 2050, Foss Analytical AB, Hoganas, Sweden). Nitrogen content was determined on fat-free samples with a Nitrogen/Protein Determinator CNS2000 (Leco Corp., St. Joseph, MI) and crude protein content was determined (AOAC, 1997; Official Method 981.10) from the nitrogen content by the formula $N_2 \times 6.25$ to complete the proximate analysis.

2.2.6. Tocopherol analysis

Approximately 30 g of the coarse meat grind were homogenized in a food processor (Robot Couple Blixir BX3; Robot Coupe USA Inc., Ridgeland MS) and the homogenate collected into Whirlpak™ bags (Nasco, Salida, CA) and kept frozen at -80°C until analysis. Homogenates were removed from the freezer and thawed overnight at 4°C prior to analysis. Tocopherol content was estimated by high performance liquid chromatography (HPLC; Alliance Waters, Separation Module e2695 equipped with a multi-wavelength fluorescence detector 2475, Waters Corporation, Milford, MA) according to the method outlined by Hewavitharana et al. (2004) which is followed as the standard operating procedure for tocopherol estimation at AAFC, Lacombe. Approximately 1 g of each sample was weighed in duplicate into 50 mL polyethylene screw top centrifuge tubes and capped to avoid oxidation of the samples during the process. To each tube 300 µL of internal standard (0.2 mg α-tocopherol acetate /mL hexane) was added. The capped tubes were placed in ice and 4 mL of absolute ethanol (HPLC grade) added and the mixture homogenized (Polytron Model 3100, Kinematica AG, Switzerland) for 30 sec at 14,000 rpm. A 5 mL aliquot of reverse osmosis water was added to the tube and the contents homogenised for 15 sec. Then a 4 mL aliquot of hexane-butylated hydroxytoluene (hexane-BHT) was added and the contents homogenized for a further 15 sec. Samples were subjected to centrifugation (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) at 400 x *g* for 10

min. Following centrifugation, the top liquid layer of each sample was transferred into an amber HPLC vial containing a 300 µL glass insert. From each sample, 50 µL were analyzed for α-tocopherol content under fluorescence using an excitation wavelength at 295 nm and an emission wavelength at 330 nm.

2.2.7. Fatty acid analysis

Lipid extraction was performed using 2:1 chloroform: methanol and with the same solvent to sample ratio as reported by Folch et al. (1957) which is followed as the standard operating procedure at AAFC, Lacombe. Approximately 20 g of the coarse meat from loin sub-primal were homogenized using a food processor (Robot Coupe Blixer BX3, Robot Coupe USA Inc., Ridgeland MS), collected into Whirlpak bags and kept frozen at -80°C until analysis. Prior to analysis, samples were thawed overnight at 4°C. From thawed samples, 1 g in duplicate was weighed into 20 × 150 mm Pyrex screw capped culture tubes with Teflon-lined caps. To each tube, 6.65 mL methanol were added and mixed by vortex (Thermolyne M37615 Maximix II [Barnstead Thermolyne, USA]). To this, 1 mL of internal standard (15 mg 19:0;(nonadecanoate) methyl ester/ml chloroform) was added and homogenized (VirTis Cyclone, VirTis Company, Gardiner, NY) using a 10 mm × 195 mm generator for 30 sec at 15,000 rpm. To this mixture, 12.3 mL chloroform were added, mixed by vortex and homogenised for 30 sec at 15,000 rpm. The entire mixture was filtered through a coarse sintered glass filter funnel and then poured into a pre-weighed 50 mL graduated conical glass centrifuge tube.

Following filtration, 3.35 mL of methanol and 6.65 mL of chloroform were added to the original 20×150 mm glass culture tube, mixed by vortex and homogenized for 30 sec at 15000 rpm. The contents were then filtered through the sintered glass filter funnel under vacuum and combined with the original filtrate in the 50 mL graduated conical glass centrifuge tube. To form a biphasic system (lipids in lower phase), 6.75 mL of 0.88% potassium chloride solution were added and mixed well by shaking and vortex. The tube was then subjected to centrifugation (Heraeus Omnifuge RT 3842 [Heraeus Christ GmbH, Osterode, Germany]) at 1000 x *g* for 10 min.

Following centrifugation, the upper phase was aspirated without disturbing the lower phase and then 10 mL of 1:1 methanol: water added to the lower phase, following which the upper layer was again aspirated without disturbing the lower phase. The lower phase containing lipid was dried by rotary evaporation (Multivapor P-12 with Buchi vacuum controller –V-800, Buchi, Switzerland) at 40°C for 20 min. The samples were then dried at ambient temperature under high vacuum over night in a vacuum oven (VWR 1400E, Sheldon Manufacturing Inc. Comelius, Oregon, USA) with an attached cold trap (Labonco, Centrivap 78110-00D, Kansas City, Missouri, USA) and vacuum pump (E-LAB 2, Edwards High Vacuum International, West Sussex, UK). After drying, the head spaces of the sample tubes were flushed with nitrogen and the weights of the tubes + lipids were recorded and the lipids were then dissolved in 10 mL chloroform, transferred into 20 mL glass scintillation vials with Teflon lined caps and stored at -20 C until methylation.
Lipids were methylated using 1.5 N methanolic hydrochloric acid as described by Kramer et al. (1997) which is followed as the standard operating procedure at AAFC, Lacombe. Lipid extract containing 10 mg lipid was transferred using a calibrated pipette to a 16 × 125 mm screw top glass culture tube with a Teflon lined cap. The samples were dried under nitrogen using an analytical evaporator (Organomation N-EVAP 111, Organomation Associates, Inc., Berlin, MA, USA). To solubilize the lipid, 1 mL of toluene was added to the sample and mixed by vortex. To this, 3.0 mL of 1.5 N methanolic hydrochloric acid were added, mixed by vortex, the headspace flushed with nitrogen and then capped. Samples were placed in a heating block (VWR 949031, Henry Troemner LLC, Thorofare, NJ USA) set at 80°C for 1 h. A reagent blank was also prepared in a 16 × 125 mm screw top culture tube containing 1 mL of toluene and placed in the heating block along with the samples. During heating, samples were mixed well using a vortex mixer at 5 and 30 min interval and caps were tightened. After 1 h the samples were removed from the heating block and cooled to room temperature. To sample tubes, 1 mL of deionized water was added followed by 3 mL of hexane and the tubes capped again and mixed well with a vortex mixer. The samples were then subjected to centrifugation (Heraeus Omnifuge RT 3842, Heraeus Christ GmbH, Osterode, Germany) at 1000 x *g* for 5 min. Following centrifugation, the upper organic

layer was transferred to a 4 mL vial and dried over 100 mg of sodium sulphate. Fatty acid methyl ester (FAME) thus obtained were re-solubilized with 1 mg/mL hexane and FAMEs identified by gas chromatography. One µL of the hexane solution was injected into a Varian CP-3800 GC (Varian Chromatography Systems, Walnut Creek, CA USA) using a Model 1079 injector at 250°C. FAMEs were detected at 250°C using flame ionization after separation on a Varian CP-Sil88 – 100m column. Column pressure was a constant 25 psi and hydrogen was used as the carrier gas. 20:1 split injection. Column temperature throughout FAME separation was 45° C for the first 4 min and then increased to 175°C at 13 °C/min, which was maintained for 27 min and then increased to 215°C at 4°C/min and held for 35 min.

The fatty acid content was determined as following:

Quantity of fatty acid (mg) in the methylated sample = area of fatty acid peak / area internal standard peak * relative response factor.

The relative response factors (RRF) are calculated from chromatograms of the Nu-Chek-Prep 603 fatty acid methyl ester standard (Nu-Chek-Prep Inc., Elysian, MN). RRF = (% fatty acid in standard / fatty acid peak area in standard chromatogram)*(internal standard peak area in standard chromatogram / % internal standard in standard).

Total fatty acid weight in sample= mg of fatty acid in methylated sample * 15 ml total chloroform / 1 ml chloroform used for methylation.

The mg fatty acid / g tissue = total fatty acid weight / tissue weight extracted

2.3. Statistical analysis

Data were analyzed as a 2 x 2 factorial design using PROC MIXED (SAS Version 9.2, SAS Institute Inc., Cary, NC) with sources of variation including levels of vitamin E, periods of flax treatments and their interactions. Meat grind pool was included as a random effect. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. For significant main or interaction effects ($P>0.05$), differences between treatments or their interaction means were computed using least square means

(LSMEANS) and separated using *t*-tests with the PDIFF option. The model used to fit the data was:

$$Y_{ij} = \mu + V_i + F_j + VF_{ij} + e$$

Where Y =response variable corresponding to the i^{th} level of vitamin and j^{th} level of flax treatment

μ= Overall mean;

V_i =Effect of i^{th} level of vitamin E (i=40, 400mg/kg Vitamin E);

F_j =Effect of j^{th} level of flax (j=0 weeks, 6 weeks);

VF_{ij}=effect of interaction of i^{th} vitamin and j^{th} flax levels;

e = Experimental error.

2.4. Results

2.4.1. Animal performance

Results for animal performance are presented in Table 2.7.2. One animal was removed from the study as it was discovered to be a gilt. As a result, there were only eight animals in the control group (40/0) and so the total number of animals used in the study was thirty five. There was no interaction between dietary vitamin E and the duration of flax administration on animal growth performance (P>0.05). Dietary vitamin E administration alone also had no effect on the growth performance parameters of pigs in either the growing or finishing phases (P>0.05). Flax feeding for 6 weeks resulted in an increase in mean feed conversion ratio of pigs in the finishing phase from 3.00 to 3.20 (0 versus 6 weeks flax; P =0.0258) and in the whole experiment phase from 2.77 to 2.92 (0 versus 6 weeks flax; P = 0.0413). Pig mean weight gain and mean average daily gain were unaffected by treatments (P=0.87 and 0.90, respectively).

2.4.2. Carcass data

Results for carcass characteristics are presented in Table 2.7.3. There were no interactions between vitamin E and duration of flax administration for the pig carcass characteristics (P>0.05). Dietary vitamin E administration of 400 mg/kg feed reduced mean dressing percentage from 83.35% to 82.65%, (40 versus 400 mg Vitamin E/kg feed; P=0.0331). Dietary supplementation of 10% flax for 6 weeks resulted in softening of subcutaneous fat from 59.38 to

52.60 durometer units (0 versus 6 weeks flax; P=0.0170). The 45 min post-mortem pH and temperature as well as 24 h post-mortem pH and temperature were unaffected by dietary vitamin E and flax supplementation. There were no effects of vitamin E administration or duration of flax supplementation on lean yield or cooler shrinkage (P <0.05).

2.4.3. pH and proximate analysis

Results for pH and proximate analysis are presented in Table 2.7.4. Vitamin E or flax administration or their interaction did not have significant effects on meat pH (P>0.05). Supplementation of 10% flax for 6 weeks resulted in an increase in meat grind mean fat content from 18.5% to 24.2% (0 versus 6 weeks flax; P =0.0446) as well as a reduction in meat grind mean moisture content from 62.09% to 56.30% (0 versus 6 weeks flax; P = 0.0106). There were no effects of vitamin E or flax or their interaction on the protein content of the meat grinds (P>0.05).

2.4.4. Vitamin E analysis

Results of the Vitamin E analysis are presented in Table 2.7.5. Dietary supplementation of Vitamin E increased levels of Vitamin E detected in the meat grinds (P=0.0001). Pigs that were fed 400 mg of vitamin E /kg of feed had a mean of 12.92 µg vitamin E /g of meat grind while control animals had a mean of only 5.90 µg vitamin E/g of meat grind.

2.4.5. Fatty acid analysis

Results of fatty acid analysis are presented in Table 2.7.6. Dietary administration of 10% flax for 6 weeks significantly increased the concentration of polyunsaturated fatty acids in the meat grind compared to from control pig carcasses (39.49 versus 22.19 mg/g of meat, supplemented versus control; P=0.0001). Saturated and monounsaturated fatty acid levels were unaffected by dietary flax treatment (P>0.05), but there was a significant reduction in the *n*-6:*n*-3 ratio with dietary flax supplementation (1.25 versus 7.40, supplemented versus control; P=0.0001). There was a slight reduction in the concentrations of arachidonic (C20:4n-6) (0.57 versus 0.76 mg/g, supplemented versus control; P=0.0010) and docosatetraenoic (C22:4n-6) (0.14 versus 0.19 mg/g; supplemented versus control, P=0.0265) acids with dietary flax supplementation. There was no statistical difference in the total *n*-6 fatty acids when compared with the control although there was a trend

(P=0.07) towards the total *n*-6 fatty acids increasing with 10% flax supplementation. There were significant increases in the levels of α-linolenic acid (1.87 versus 14.78 mg/g meat grind; control versus supplemented, P=0.0001), docosapentaenoic acid (0.23 versus 0.48 mg/g meat grind; control versus supplemented, P=0.0001) and eicosatrienoic acid (0.35 versus 1.86 mg/g meat grind; control vs supplemented, P=0.0001) when flax was supplemented for 6 weeks. There were slight reductions in the levels of cis-11-octadecenoic acid (c11-18:1) (6.10 versus 5.20 mg/g meat grind; control versus supplemented, P=0.0033), cis-9-hexadecenoic acid (c9-16:1) (4.87 versus 3.97 mg/g meat grind; control versus supplemented, P=0.0103) and cis-11-eicosenoic acid (c11-20:1) (1.66 versus 1.18 mg/g meat grind; control versus supplemented, P=0.0001) following the supplementation of flax for 6 weeks. Supplementation of vitamin E did not have any effect on the fatty acid profile (P>0.05) except for decreasing the mean cis-11-eicosenoic acid concentration (1.51 versus 1.32 mg/g meat grind; control versus supplemented P=0.0129). There was a significant interaction between dietary vitamin E and flax supplementation that increased meat grind mean eicosapentaenoic acid concentration (P=0.0257) eventhough the levels of eicosapentaenoic acid was small (0.3mg/g of meat) and the total mean *n*-3 fatty acid content (P=0.0001). Means of these interactions are illustrated in Figures 2.8.1 and 2.8.2 respectively.

2.5. Discussion

The present study indicated that feeding 10% flax to pigs for 6 weeks increased finisher and total feed conversion ratio. These results conformed to those of Corino et al. (2008), Juárez et al. (2010) and Kouba et al. (2003) who fed 5% extruded flax, 5%, 10% and 15% co-extruded flaxseed for either 4, 8 or 12 weeks and 60g whole crushed linseed /kg feed to pigs for 60 days, respectively. However in the study conducted by Matthews et al. (2000), FCR was unaffected by feed that contained 0, 50 g and 100 g linseed /kg of feed fed for 65 days. Increased FCR indicates that feed efficiency is reduced in pigs fed a diet high in *n*-3 fatty acids, which may reduce returns to producers. The economics of production could be improved if increased returns from the sale of value added *n*-3 enriched meat were realized. Final live weights, average daily gain and total weight gain were unaffected by feeding 10% flax for 6

weeks, and this result also was in agreement with previous studies (Corino et al., 2008; Juárez et al., 2010; Kouba et al., 2003; Matthews et al., 2000).

Dietary supplementation of flax caused softening of carcass subcutaneous fat as observed by the fat hardness score but there were no problems encountered with the softening while making pork meat patties. Meat grind pH was unaffected by the dietary treatments and agreed with the results of Corino et al. (2002) and Myer et al. (1992) who also did not observe changes in meat pH when different unsaturated fatty acid sources were fed to pigs. Dietary supplementation of flax for 6 weeks resulted in an obvious increase in the fat content of the pork grinds and was at the expense of the moisture content whereas protein content was unaffected. Similar trends of elevated lipid content were observed in the studies conducted by Corino et al. (2008), Juárez et al. (2010) and Kouba et al. (2003) in which pigs were fed flax. The increased fat content observed in the proximate analysis of the flax patties suggests that the fat content added to make meat patties could be reduced; however in the present study 15% fat was added to ensure that the patties within the study were similar.

Dietary flax supplementation resulted in an increase in total mean *n*-3 fatty acid concentrations, especially of ALA and DPA, and there was a reduction in the *n*-6: *n*-3 ratio, as well. These results agree with results from previous studies (Bečková & Václavková, 2010; Corino et al., 2008; Enser et al., 2000; Musella et al., 2009). Dietary supplementation of flax resulted in an eight-fold increase in the total *n*-3 fatty acid content, which could be attributed to the long duration of flax feeding. However in the present study, grinds obtained from animals that received 10% flax supplementation for 6 weeks and 400 mg/kg vitamin E showed an increase in the concentration of eicosapentaenoic acid (EPA) as well as the total *n*-3 fatty acid content.

The levels of arachidonic acid were reduced following the feeding of flax, even though there was no difference in the linoleic acid (LA) content or the total *n*-6 fatty acid levels of meat grinds. Flax feeding has resulted in reduced mean muscle arachidonic acid levels in some studies (Bečková & Václavková, 2010; Juárez et al., 2010). Lowering of arachidonic acid, dihomo gamma linolenic acid, docosatetraenoic acid in this study could be attributed to the fact that the enzymatic system (desaturases and elongases), which plays

an important role in the metabolism of *n*-3 and *n*-6 fatty acids, has a preference for the *n*-3 fatty acids over *n*-6 fatty acids particularly when there are higher concentrations of *n*-3 fatty acids than *n*-6; thus arachidonic acid synthesis might have lowered due to this competitive inhibition (Bečková & Václavková, 2010; Raes et al., 2004). However there was no difference in the total *n*-6 fatty acid content and this was in agreement with some previous studies (Bečková & Václavková, 2010; Corino et al., 2008). Dietary linseed supplementation has caused a reduction in the total *n*-6 fatty acid content in other studies (Enser et al., 2000; Musella et al., 2009).

Feeding of linseed caused an increase in total *n*-3 fatty acid content particularly α-linolenic acid in all studies reviewed; however the extent of increase was variable and depended on factors like age, genetics and gender of the animal, duration and amount of flax feeding, type of flax (oil versus meal) and so generalisation of its effects is difficult (Nguyen et al., 2003; Raes et al., 2004). In the present study feeding of 10% flax for 6 weeks caused a direct increase in total omega-3 fatty acid concentration from 263 mg/100 g to 1751 mg/100 g pork grinds (15% fat added). In order to achieve the required functional food claim legislated by CFIA & Health Canada, (2009) there should be 300 mg of omega-3 fatty acids per 100g of serving of the product. Dietary supplementation of 10% flax for 6 weeks caused an approximately four fold increase in the total *n*-3 fatty acid content in the pork grinds. This could be controlled by lowering the level of flax or the duration of feeding could be reduced so that levels of *n*-3 fatty acids could be reduced to the desired functional food claim level. Lowering the level of flax or the duration of feeding will have a direct impact on reducing the cost of animal feeding and also help to minimise meat quality problems such as 'soft fat', which can be associated with increased levels of *n*-3 fatty acid levels in tissues (Matthews et al., 2000). This will in turn have a direct impact in improving sensory characteristics, consumer acceptability and shelf life.

Dietary supplementation of 400mg/kg vitamin E caused a reduction in the dressing percentage in this study; however effects of vitamin E supplementation on the dressing percentage in pigs yielded inconsistent results in other studies. Corino et al. (1999) found that mean dressing percentage was increased at dietary vitamin E levels of 200 and 300 mg/kg when compared

with a control (25mg/kg) level. Asghar et al. (1991) in their study fed pigs with 100 mg/kg and 200 mg/kg vitamin E did not affected dressing percentage compared with a control (10mg/kg). The exact reason for this difference needs further investigation. Dietary supplementation of vitamin E increased the level of vitamin E in the pork grind in this study and, even though there was a 10-fold increase in the dietary supplementation compared with the control, there was not a corresponding 10-fold increase in the tissue levels. The levels of vitamin E in the pork grind of the present study were similar to the levels noted in the pork from studies conducted by Asghar et al. (1991); Buckley et al. (1995) implying that vitamin E levels in tissue are limited by either absorption or metabolism of vitamin E, but this was not explored further in the present study.

The deposition of vitamin E in the tissues following dietary supplementation is dependent on the metabolic activity with the greatest deposition in the tissues with greatest oxidative capacity (Jensen et al., 1998). In most of the studies, the M. *longissimus dorsi* was the muscle of choice for estimating the levels of vitamin E following dietary supplementation (Buckley et al., 1989; Dirinck et al., 1996; Monahan et al., 1992; Monahan et al., 1994) most likely because it is a large muscle with high retail market value. Dietary supplementation of 200 mg α-tocopherol acetate/ kg feed for two weeks prior to slaughter produced a deposition of 7 µg α-tocopherol /g of M. *longissimus dorsi* whereas 30 mg/kg dietary supplementation resulted in 3.2 µg α-tocopherol /g of M. *longissimus dorsi* in the study conducted by Monahan et al., (1990). However in another study a tissue level of 4.1 µg α-tocopherol/g was attained following dietary supplementation with 200 mg α-tocopherol acetate /kg feed starting from weaning (average 7 kg) until pigs are slaughtered at an average weight of 84 kg (Monahan et al., 1992). Jensen et al. (1997) fed pigs with 100, 200 and 700 mg α-tocopherol acetate /kg feed when pigs reached an average weight of 50 kg until they attained 90 kg body weight at slaughter and estimated the levels of α-tocopherol in the M. *longissimus dorsi* and M. *psoas major* muscles. The mean levels of α-tocopherol in the M. *longissimus dorsi* for each of the diets were 5.2 µg/g, 7.9 and 11.1µg/g, respectively, while the mean levels of α-tocopherol deposited in the M. *psoas major* muscles for each of the diets were 5.9µg/g, 11.7 and 15.7 µg/g

respectively. The increased deposition in the M. *psoas major* could be attributed to it having a higher oxidative capacity than the M. *longissimus dorsi* (Yamauchi et al., 1984). The deposition of α-tocopherol also increased linearly with dietary supplementation levels (Jensen et al. 1997). In the present study, pigs that were fed 400 mg of α- tocopherol acetate/kg of feed had a mean of 12.92 µg vitamin E /g of meat grind while control animals (40 mg vitamin E/kg feed) had a mean of 5.90 µg vitamin E/g of meat grind. The levels of vitamin E attained in the meat grinds following dietary supplementation were found to be comparable with those obtained in the previous research cited; however a generalisation over the levels of vitamin E deposited in the tissues following dietary supplementation might not yield accurate results owing to the fact that there are other underlying mechanisms such as metabolic activity of the muscles that could affect the tissue deposition.

2.6. Conclusion

Feeding of flax caused a direct increase of total omega-3 fatty acids in a 15% fat pork patty product, attaining 1751 mg omega-3 fatty acids/100 g pork grind. Feeding pigs 10% flax for 6 weeks provided sufficient *n*-3 content in the pork lean and subcutaneous fat to achieve and exceed the required source claim of 300 mg of *n*-3 fatty acids per 100 g of serving of product as legislated by CFIA and Health Canada (2009). This study also showed that pig producers can include 10% flax in pig diets for 6 weeks without adverse effects on animal performance, carcass characteristics or meat grinds up to 15% fat.

2.7. Tables

Table 2.7.1 Feed composition

	Grower	Finisher	
Ingredient (kg.ton)	Control	Control	Flax diet
Wheat	285	471.87	100
Barley	464.01	275	527.09
soybean meal (47.5%)	110	70	37.5
pork tallow (no other sources of oil)	5	46.5	2.5
Limestone	14.5	15	14
Linpro (50:50 peas flax)[1]			200
Field peas	100	100	100
dicalcium phosphate (21%)	8	8.5	8
sodium bicarbonate	2	2	2
copper sulfate (25%)	0.14	0.14	0.14
tylan 40	1.25	1.25	1.25
vit and mineral premix[2]	1.175	1.175	1.175
ADE vitamin premix[3]	0.3	0.3	0.3
millrun flush	1	1	1
lysine hydrochloride	3.2	2.85	1.675
L-threonine	0.825	0.775	
choline chloride (60%)	0.48	0.78	1.02

alimet-L-methionine (83%)	0.6	0.3	0.1
selenium (100mg.kg)	0.3	0.3	0.3
Salt	2.22	2.26	1.95
Vitamin E 50% dl-alpha tocopheryl acetate[4]	0.08	0.08	0.8
DE (Kcal/Kg)[5]	2960	3200	3200
Lysine (%)	1	0.85	0.85
Threonine (%)	0.62	0.527	0.527
Methionine (%)	0.3	0.255	0.255
Tryptophan (%)	0.2	0.17	0.17
Calculated nutrient composition			
Moisture	8.26	8.53	8.51
Dry Matter	91.7	91.5	91.5
Crude Protein %	17.7	15.6	15.8
Crude Fibre %	3.63	3.39	4.88
Fat%	2.55	6.15	5.81
Ash%	4.29	4.14	4.57
Ca%	0.80	0.85	0.83
P%	0.53	0.52	0.53
Mg%	0.16	0.14	0.16
K%	0.65	0.59	0.66
Cu%	74.0	60.9	57.0
Na%	0.21	0.20	0.19

NaCl%	0.52	0.51	0.48
Zn%	0.02	0.02	0.01
Mn%	0.01	0.01	0.01
Fe%	0.03	0.02	0.02
Total Digestible Nutrients %	75.8	75.9	74.1
Digestible Energy kcal/kg	3556	3665	3512
Non Fibre Carbohydrates %	63.6	62.3	60.5
Gross Energy kcal/kg	4020	4185	4151
Metabolizable Energy kcal/kg	3423	3546	3395

[1] Co-extruded 50% flaxseed and 50% field pea (Linpro; O&T Farms, Regina, Saskatchewan, Canada). Fatty acid composition (% total fatty acids): 16:0 (6.05%), 18:0 (3.32%), 18:1n-9 (18.6%), 18:2n-6 (20.6%), 18:3n-3 (50.6%).

[2]Provided per kilogram of diet: Manadione 4,500 mg, Biotin 1,000 mg, Folic acid 3,250 mg, Niacin 75,000 mg, Pantothenic acid 45,000 mg, Pyridoxine 9,000 mg, Riboflavin 17,000 mg, Thiamin 15,000 mg, Cobalt 1,000 mg, Copper 20,000 mg, Iron 85,000 mg, Iodine 1,000 mg, Manganese 80,000 mg, Zinc 125,000 mg.

[3]Vitamin premix (vit A 30,000,000 IU.kg; vit D_3 3,000,000 IU.kg; vit E 100,000 IU.kg)

[4] Vitamin E 50% dl-alpha tocopheryl acetate from Long Wing International, Inc. Oakville ON

[5] Digestible energy

Table 2.7.2. Effects of Vitamin E and period of 10% flax dietary treatments on animal performance data

Variable	Vitamin E (mg/kg)		10% Flax (weeks)			V×F[1]
	40	400	0	6	SEM[2]	
N	6	6	6	6		
Start Feed trial w (kg)	78.78	78.61	78.19	78.19	0.95	NS
3 week weight (kg)	102.82	103.76	102.72	103.86	1.08	NS
Final weight (kg)	125.64	126.78	125.75	126.67	1.18	NS
Grower gain (kg)	27.88	28.22	27.66	28.44	2.73	NS
Finisher gain (kg)	62.86	64.31	63.86	63.31	1.09	NS
Total gain (kg)	90.74	92.52	91.52	91.74	2.22	NS
ADG[3](Grower) (kg d^{-1})	1.08	1.10	1.07	1.11	0.02	NS
ADG (Finisher) (kg d^{-1})	1.14	1.17	1.16	1.15	0.02	NS
ADG (Total) (kg d^{-1})	1.12	1.15	1.13	1.14	0.02	NS
Grower feed intake (kg)	2.49	2.50	2.45	2.54	0.05	NS
Finisher feed intake (kg)	3.53	3.61	**3.48[b]**	**3.67[a]**	0.05	NS
Total feed intake (kg)	3.20	3.26	**3.15[b]**	**3.31[a]**	0.04	NS
FCE[4](Grower)	2.31	2.28	2.29	2.29	0.02	NS
FCE (Finisher)	3.11	3.10	**3.00[b]**	**3.21[a]**	0.05	NS
FCE (Total)	2.86	2.85	**2.78[b]**	**2.92[a]**	0.03	NS

[1]Vitamin E and 10% flax duration interaction; [2] Standard Error of mean; [3]Average daily gain; [4] Feed conversion efficiency
[a,b] LSMEANS within the same row and source of variation in bold and with different superscript letters are significantly different at (P>0.05), NS- Not significant

Table 2.7.3. Effects of Vitamin E and period of 10% flax treatments on carcass characteristics

Variable	Vitamin E(mg/kg)		10% Flax (weeks)			V×F[1]
	40	400	0	6	SEM[2]	
N	6	6	6	6		
pH (45 min)[3]	6.15	6.20	6.22	6.15	0.02	NS
Temp °C (45min)[4]	40.64	40.84	40.60	40.88	0.10	NS
pH (24 h)[5]	5.58	5.65	5.61	5.60	0.02	NS
Temp °C (24 h)[6]	0.811	0.94	0.85	0.90	0.08	NS
Hot Carcass	102.02	102.44	102.27	102.19	1.09	NS
Cooler shrinkage	2.48	2.45	2.47	2.47	0.03	NS
Dressing %	**83.36[a]**	**82.65[b]**	83.17	82.84	0.42	NS
Fat hardness[7]	56.77	55.44	**59.61[a]**	**52.60[b]**	1.64	NS
Lean yield (%)	58.63	58.63	58.21	59.05	0.40	NS

[1]Vitamin E and 10% flax duration interaction.
[2]Standard Error of mean
[3]Post-mortem pH after 45 minutes.
[4]Post-mortem temperature after 45 minutes.
[5]Post-mortem pH after 24 hour.
[6]Post-mortem temperature after 24 hour.
[7]Fat hardness measured in durometer readings (0 to 100).
[a,b] LSMEANS within the same row within treatment and with different superscript letters are significantly different at (P>0.05).
NS- Not significant

Table 2.7.4. Effects of Vitamin E and 10% flax treatments on pork grind pH and proximate analysis

	Vitamin E(mg/kg)		10% Flax (weeks)			V x F[1]
	40	400	0	6	SEM[2]	
n	6	6	6	6		NS
pH	5.91	5.93	5.90	5.95	0.03	NS
Moisture(%)	58.79	59.60	**62.09[a]**	**56.30[b]**	1.23	NS
Protein (%)	19.54	18.00	18.77	18.77	0.53	NS
Fat (%)	20.90	21.72	**18.5[b]**	**24.2[a]**	1.70	NS

[1]Vitamin E and 10% flax duration interaction
[2]Standard error of mean
[a,b] LSMEANS within the same row in bold with different superscript letters are significantly different at (P>0.05)
NS- Not significant

Table 2.7.5. Effects of dietary vitamin E, duration of 10% flax and on meat grinds vitamin E levels

Vitamin E (µg /g of meat grind)

Fatty acid	Dietary Vitamin E (mg/kg)		10% flax (weeks)			V×F[1]
	40	400	0	6	SEM[2]	
n	6	6	6	6		
α-tocopherol	**5.90[b]**	**12.92[a]**	9.68	9.14	0.30	NS

[1]Vitamin E and 10% flax duration interaction
[2]Standard error of mean
NS- Not significant

Table 2.7.6. Fatty acid profile (mg fatty acids/g of meat) in meat patties

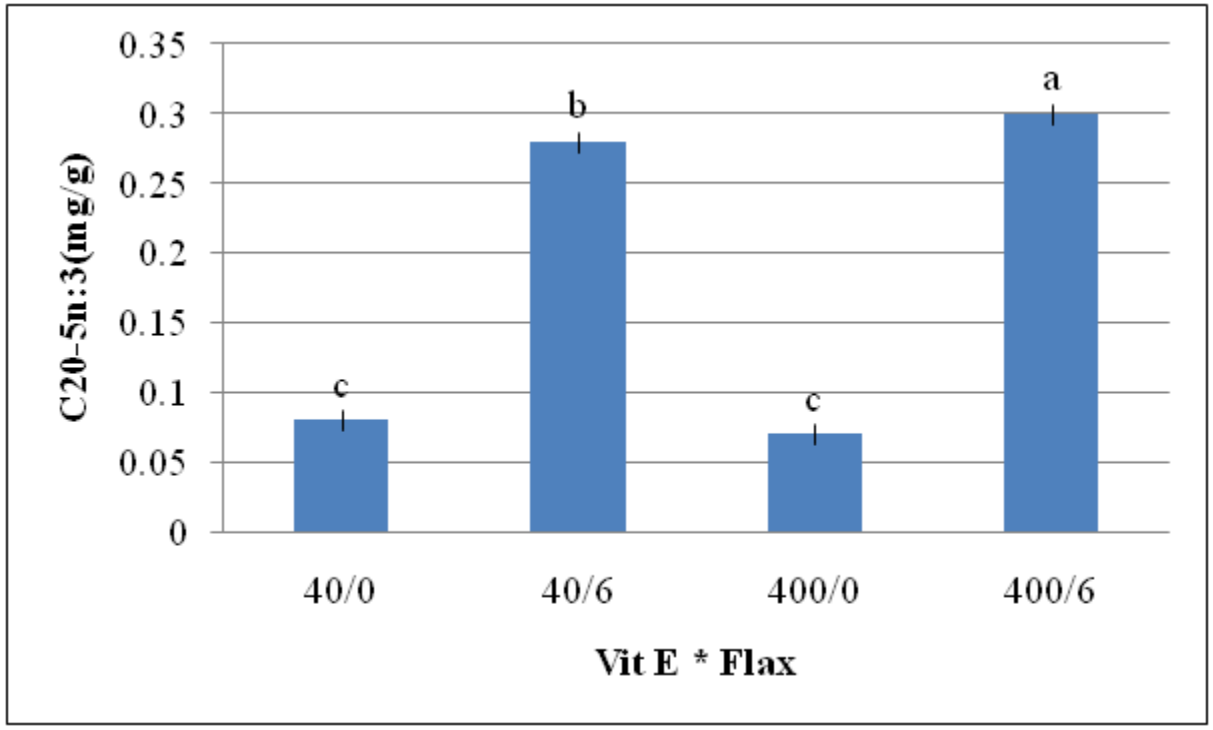
Fatty acid	Vitamin E (mg/kg)		10% flax (weeks)			V×F[2]
	40	400	0	6	SEM[1]	
n	6	6	6	6		
C14:0	2.59	2.40	2.44	2.55	0.08	NS[3]
C16:0	48.01	45.51	46.49	47.03	1.31	NS
c7-16:1	0.62	0.55	0.64	0.54	0.04	NS
c9-16:1	4.58	4.27	**4.87[a]**	**3.97[b]**	0.19	NS
C17:0	0.71	0.69	0.63	0.77	0.03	NS
C18:0	25.91	24.56	24.56	25.91	0.83	NS
c9-18:1	80.60	76.25	78.83	78.03	2.48	NS
c11-18:1	5.87	5.43	**6.10[a]**	**5.20[b]**	0.15	NS
C18:2n-6	19.24	18.33	17.49	20.08	0.79	NS
C20:0	0.40	0.41	0.39	0.41	0.02	NS
c11-20:1	**1.51[a]**	**1.32[b]**	**1.66[a]**	**1.18[b]**	0.04	NS
C18:3n-3	8.28	8.38	**1.87[b]**	**14.78[a]**	0.44	NS
C20:2n-6	0.78	0.73	0.71	0.81	0.04	NS
C20:3n-6	0.20	0.20	0.21	0.19	0.01	NS
C20:3n-3	1.10	1.11	**0.35[b]**	**1.86[a]**	0.05	NS
C20:4n-6	0.67	0.66	**0.76[a]**	**0.57[b]**	0.03	NS
C20:5n-3	0.18	0.19	0.08	0.29	0.004	**

C22:4n-6	0.18	0.15	**0.19[a]**	**0.14[b]**	0.01	NS
C22:5n-3	0.34	0.36	**0.23[b]**	**0.48[a]**	0.02	NS
C22:6n-3	0.10	0.11	0.11	0.10	0.02	NS
TOTAL	203.83	193.45	190.51	206.77	5.87	NS
SAT[4]	78.04	73.98	74.94	77.09	2.16	NS
MONO[5]	94.20	88.74	93.06	89.88	2.80	NS
POLY[6]	31.26	30.42	**22.19[b]**	**39.49[a]**	1.22	NS
n-6[7]	21.07	20.07	19.36	21.78	0.83	NS
n-3[8]	9.99	10.14	**2.63[b]**	**17.51[a]**	1.5	**
n-6:n-3[9]	4.40	4.24	**7.40[a]**	**1.25[b]**	0.23	NS

[1]Standard error of mean
[2] Vitamin E and 10% flax duration interaction
[3] NS – Not significant
[4]Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7]Omega 6 fatty acid
[8]Omega 3 fatty acid
[9]Omega6:Omega3 ratio
** Significant at P<0.05
LSMEANS within column which are bold and with different superscript letters are significantly different at P <0.05

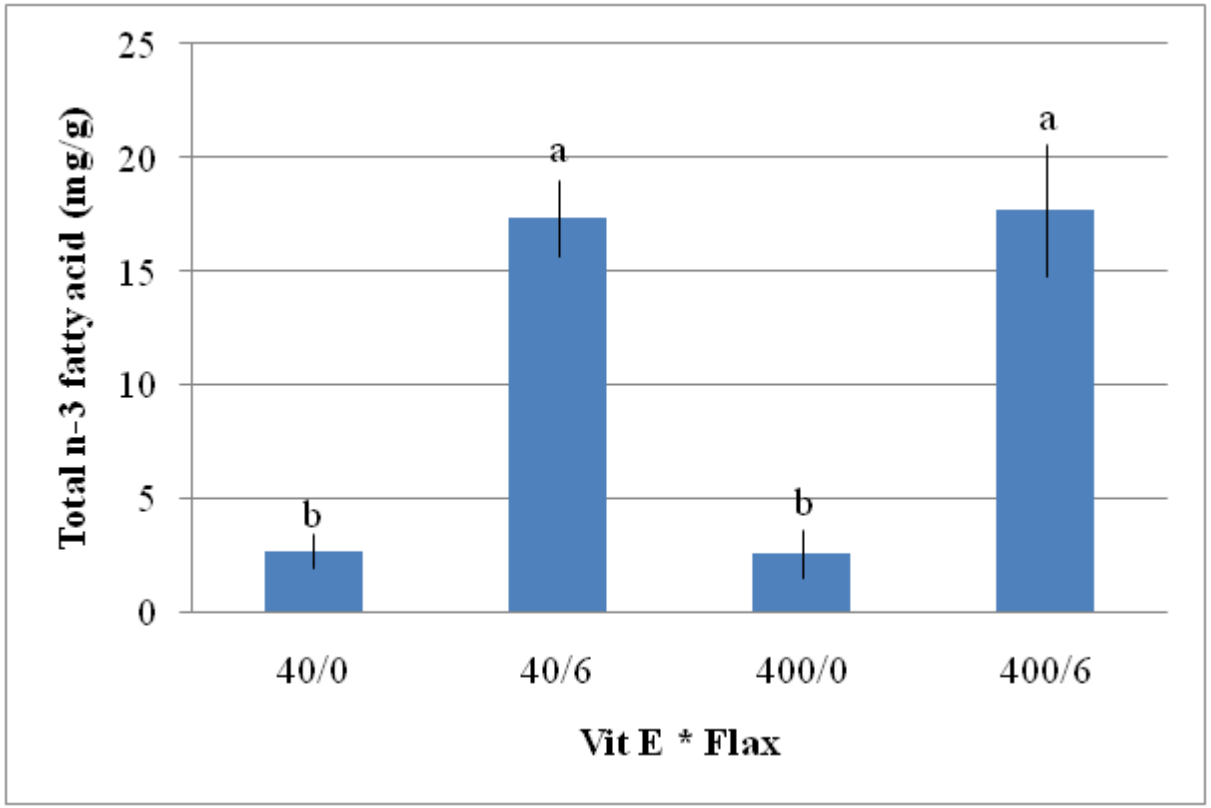
2.8. Figures

2.8.1. Effects of Vitamin E and flax interaction on the levels of C20-5n-3 in mg/g of meat grind



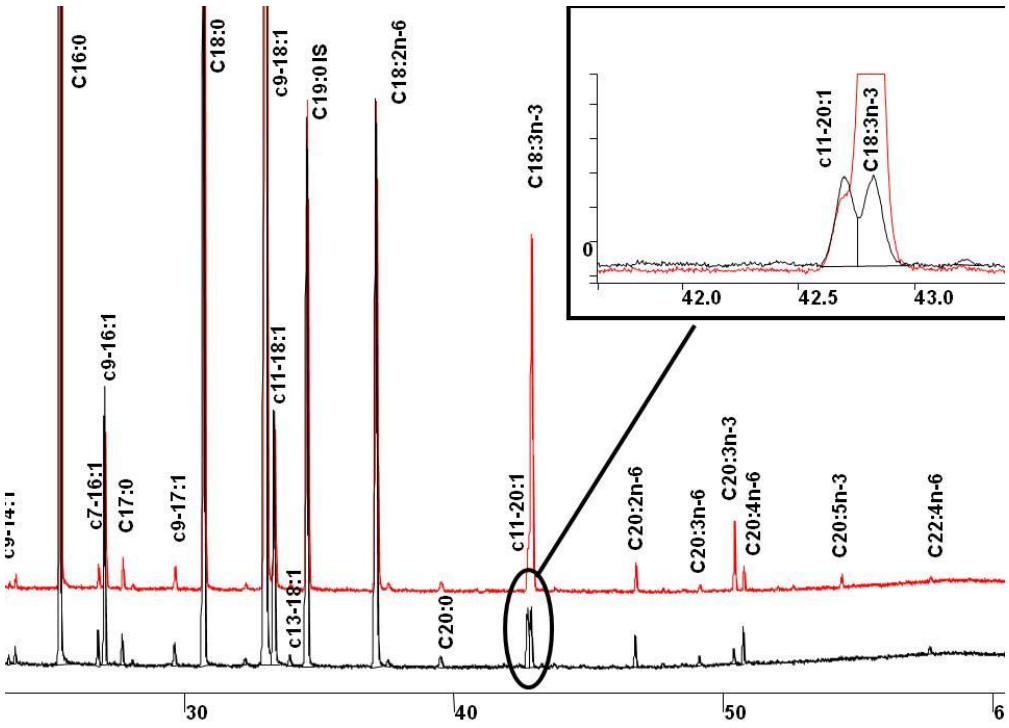
Columns with different letters are significantly different ($P < 0.05$) according to *t test* with SEM indicated by error bars. 40/0 – Interaction between 40mg/kg vitamin E and no flax; 40/6 – Interaction between 40mg/kg vitamin E and 10% flax for 6 weeks; 400/0 – Interaction between 400mg/kg vitamin E and no flax; 400/0 – Interaction between 400mg/kg vitamin E and 10% flax for 6 weeks.

2.8.2. Effects of Vitamin E and flax interaction on the levels of total *n*-3 fatty acid in mg/g of meat grind



Columns with different letters are significantly different ($P < 0.05$) according to *t test* with SEM indicated by error bars.
40/0 – Interaction between 40mg/kg vitamin E and no flax; 40/6 – Interaction between 40mg/kg vitamin E and 10% flax for 6 weeks; 400/0 – Interaction between 400mg/kg vitamin E and no flax; 400/0 – Interaction between 400mg/kg vitamin E and 10% flax for 6 weeks.

Figure 2.8.3. Typical chromatogram obtained from the meat grind from animals fed with 10% flax for 6 weeks



2.9. Bibliography

AOAC. (1995) AOAC Official Method 960.39. Fat (crude) or ether extract in meat. Final Action, AOAC Official Methods of Analysis 1995, Association of Official Analytical Chemists, Arlington, VA.

AOAC. (1997) AOAC Official Method 992.15. Crude Protein in Meat and Meat Products Including Pet Foods. Combustion Method. First Action 1992., AOAC Official Methods of Analysis 1995. Supplement March 1997, Association of Official Analytical Chemists, Arlington, VA.

Asghar, A., Gray, J. I., Booren, A. M., Gomaa, E. A., Abouzied, M. M., Miller, E. R., et al. (1991). Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. *Journal of the Science of Food and Agriculture, 57*(1), 31-41.

ASTM Standards. (2008) D2240 – 05 Test methods for Rubber Property\Durometer hardness, In ASTM Volume 9.01 Rubber, Natural and Synthetic – General Test Methods, ASTM International, West Conshohocken, PA.

Bečková, R., & Václavková, E. (2010). The effect of linseed diet on carcass value traits and fatty acid composition in muscle and fat tissue of fattening pigs. *Czech Journal of Animal Science, 55*(8), 313-320.

Bou, R., Guardiola, F., & Codony, R. (2010). Enrichment of meats and eggs in n-3 PUFA through dietary manipulation: Quality and safety drawbacks. http://www.foodsciencecentral.com/fsc/ixid16014

Bryhni, E. A., Kjos, N. P., Ofstad, R., & Hunt, M. (2002). Polyunsaturated fat and fish oil in diets for growing-finishing pigs: Effects on fatty acid composition and meat, fat, and sausage quality. *Meat Science, 62*(1), 1-8.

Buckley, D. J., Gray, J. I., Asghar, A., Price, J. F., Crackel, R. L., Booren, A. M., et al. (1989). Effects of dietary antioxidants and oxidized oil on membranal lipid stability and pork product quality. *Journal of Food Science, 54*(5), 1193-1197.

Buckley, D. J., Morrissey, P. A., & Gray, J. I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science, 73*(10), 3122-3130.

CFIA & Health-Canada (2009). Guide to Food Labelling and Advertising. Canadian Food Inspection Agency, http://www.inspection.gc.ca/english/fssa/labeti/guide/ch7be.shtml. Retrieved on 8 December 2010.

Corino, C., Magni, S., Pagliarini, E., Rossi, R., Pastorelli, G., & Chiesa, L. M. (2002). Effects of dietary fats on meat quality and sensory characteristics of heavy pig loins. *Meat Science, 60*(1), 1-8.

Corino, C., Musella, M., & Mourot, J. (2008). Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one

hundred ten and one hundred sixty kilograms of liveweight. *Journal of Animal Science, 86*(8), 1850-1860.

Corino, C., Oriani, G., Pantaleo, L., Pastorelli, G., & Salvatori, G. (1999). Influence of dietary vitamin E supplementation on" heavy" pig carcass characteristics, meat quality, and vitamin E status. *Journal of Animal Science, 77*(7), 1755-1761.

Dirinck, P., De Winne, A., Casteels, M., & Friggs, M. (1996). Studies on vitamin E and meat quality. 1. effect of feeding high vitamin E levels on time-related pork quality. *Journal of Agricultural and Food Chemistry, 44*(1), 65-68.

Enser, M., Richardson, R. I., Wood, J. D., Gill, B. P., & Sheard, P. R. (2000). Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science, 55*(2), 201-212.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*(1), 497–509.

Giner, C. (2009). New Avenues of Value Creation in the Agro-Food Sector. *OECD Food, Agriculture and Fisheries Working Papers, No. 13*, OECD Publishing. doi: 10.1787/221300175226

Guillevic, M., Kouba, M., & Mourot, J. (2009). Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of french fresh and cooked pork meats. *Meat Science, 81*(4), 612-618.

He, M. L., Hollwich, W., & Rambeck, W. A. (2002). Supplementation of algae to the diet of pigs: A new possibility to improve the iodine content in the meat. *Journal of Animal Physiology and Animal Nutrition, 86*(3-4), 97-104.

Hewavitharana, A. K., Lanari, M. C., & Becu, C. (2004). Simultaneous determination of vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. *Journal of Chromatography A, 1025*(2), 313-317.

Honikel, K. O., & Arneth, W. (1996). Cholesterol content of various meat species and its relation to fat content. *Proceedings of the 42nd International Congress on Meat Science Technology*, 214–215.

Htoo, J. K., Meng, X., Patience, J. F., Dugan, M. E. R., & Zijlstra, R. T. (2008). Effects of co-extrusion of flaxseed and field pea on the digestibility of energy, ether extract, fatty acids, protein, and amino acids in grower-finisher pigs. *Journal of Animal Science.86* (11), 2942-2951. doi:10.2527/jas.2007-0313

Jensen, C., Guider, J., Skovgaar, I. M., Staun, H., Skibsted, L. H., Jensen, S. K., et al. (1997). Effects of dietary [alpha]-tocopheryl acetate supplementation on [alpha]-tocopherol deposition in porcine m. psoas major and m. longissimus dorsi and on drip loss, colour stability and oxidative stability of pork meat. *Meat Science, 45*(4), 491-500.

Jensen, C., Lauridsen, C., & Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science & Technology, 9*(2), 62-72.

Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., et al. (2010). Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower-finisher pigs. *Meat Science, 84*(3), 578-584.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., & Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science, 81*(8), 1967-1979.

Kramer, J. K. G., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossoba, M. M., & Yurawecz, M. P. (1997). Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids, 32*(11), 1219-1228.

Matthews, K. R., Homer, D. B., Thies, F., & Calder, P. C. (2000). Effect of whole linseed (linum usitatissimum) in the diet of finishing pigs on growth performance and on the qualityand fatty acid composition of various tissues. *British Journal of Nutrition, 83*(06), 637-643.

Monahan, F. J., Gray, J. I., Booren, A. M., Miller, E. R., Buckley, D. J., Morrissey, P. A., et al. (1992). Influence of dietary treatment on lipid and cholesterol oxidation in pork. *Journal of Agricultural and Food Chemistry, 40*(8), 1310-1315.

Monahan, F. J., Asghar, A., Gray, J. I., Buckley, D. J., & Morrissey, P. A. (1994). Effect of oxidized dietary lipid and vitamin E on the colour stability of pork chops. *Meat Science, 37*(2), 205-215.

Monahan, F. J., Buckley, D. J., Gray, J. I., Morrissey, P. A., Asghar, A., Hanrahan, T. J., et al. (1990). Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science, 27*(2), 99-108.

Musella, M., Cannata, S., Rossi, R., Mourot, J., Baldini, P., & Corino, C. (2009). Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. *Journal of Animal Science, 87*(11), 3578-3588.

Myer, R. O., Lamkey, J. W., Walker, W. R., Brendemuhl, J. H., & Combs, G. E. (1992). Performance and carcass characteristics of swine when fed diets containing canola oil and added copper to alter the unsaturated: Saturated ratio of pork fat. *Journal of Animal Science, 70*(5), 1417-1423.

Nguyen, L. Q., Nuijens, M., Everts, H., Salden, N., & Beynen, A. C. (2003). Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. *Meat Science, 65*(4), 1399-1406.

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G., et al. (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science, 70*(1), 63-74.

Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Animal Feed Science and Technology, 113*(1-4), 199-221.

Realini, C. E., Duran-Montgé, P., Lizardo, R., Gispert, M., Oliver, M. A., & Esteve-Garcia, E. (2010). Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. *Meat Science*, 85(4), 606-612.

Roynette, C. E., Calder, P. C., Dupertuis, Y. M., & Pichard, C. (2004). n-3 polyunsaturated fatty acids and colon cancer prevention. *Clinical Nutrition, 23*(2), 139-151.

SAS Institute Inc.2009.SAS User's guide:Statistics.SAS for Windows,Version 9.2 SAS Institute,Inc.,Cary,NC.

Statistics Canada, (2010) Retrieved from http://www.statcan.gc.ca/pub/23-010-x/2010001/part-partie1-eng.htm on December 6.2010

Verbeke, W., Van Oeckel, M. J., Warnants, N., Viaene, J., & Boucque, C. V. (1999). Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. *Meat Science, 53*(2), 77-100.

Yamauchi, K., Yada, K., Ohashi, T., & Pearson, A. M. (1984). The interrelationship between polyunsaturated fatty acids, a-tocopherol and glutathione peroxidase in chicken and porcine skeletal muscles. *Agricultural and Biological Chemistry, 48*(11), 2831-2832.

Chapter -3

Effects of the addition of sugars to high omega-3 pork on its oxidative stability and sensory characteristics

3.1. Introduction

There has been considerable interest in increasing the *n*-3 fatty acid content in pork due to the health benefits associated with the consumption of *n*-3 fatty acids (Bousquet et al., 2008; Yashodhara et al., 2009). Pork has been considered as a good vehicle for *n*-3 enrichment because the fatty acid profile in pork can be easily changed by modification of the pig diet (D'Souza et al., 2005). Different approaches may be used for *n*-3 enrichment of pork and feeding pigs *n*-3 enriched feedstuffs is the most common and widely accepted strategy. Flax, fish meal, and algae are good sources of *n*-3 fatty acids and may be fed to pigs so their tissues will be enriched with *n*-3 fatty acids (Bryhni et al., 2002; He et al., 2002; Kouba et al., 2003; Nuernberg et al., 2005). Recently, the Canadian Food Inspection Agency (CFIA) gave '*n*-3 enriched foods' source claim due to their potential health benefits to the consumers with a food considered enriched if the *n*-3 concentration equalled or exceeded 300 mg/100 g of food product (Health Canada, 1998).

The greatest challenge presented by *n*-3 enrichment of pork meat is the reduced oxidative stability of *n*-3 fatty acids, which may lead to the generation of off-flavours during cooking (Meadus et al., 2010; Musella et al., 2009; Santos et al., 2008). Off-flavours may increase consumer eating dissatisfaction and reduce product retail shelf life. As a result, addition of antioxidants like vitamin E to the feed of pigs has been adopted as a standard method of limiting lipid oxidation reactions in *n*-3 enriched pork (Morrissey et al., 1994). Different levels of vitamin E ranging from 100 mg/kg of feed to 400 mg/kg have been used to improve lipid oxidative stability (Guo et al., 2006); however, as to the level of vitamin E that needs to be in tissues to offer sufficient protection against lipid oxidation has yet to be determined.

Maillard and lipid oxidation reactions also contribute to the generation of meat flavour during cooking (Meinert et al., 2009). The Maillard reaction is a complex series of non-enzymatic reactions that occur between amino compounds and reducing sugars and is one of the most important flavour reactions. It is the reaction that produces browning of meat cooked at high temperatures and imparts a characteristic flavour to cooked foods (Mottram, 1998). In light of this, the addition of sugars to meat products may improve the flavour of high omega-3 pork. Sugars like ribose (Aliani & Farmer, 2005), glucose (Lauridsen et al., 2006), glucose-6-phosphate (Farmer et al., 1999) and xylose (Hudson & Loxley, 1983) may be used to improve meat product flavour and reduce oxidation off-flavours as they are all considered to be important cooked meat flavour precursors (Meinert et al., 2009). Some Maillard reaction products also act as antioxidants and could increase the oxidative stability of lipids in cooked pork products (Bailey, 1988; Mottram & Whitfield, 1995). The antioxidant activity of the Maillard reaction products has been characterized in model systems (Campo et al., 2003; Elmore et al., 2002) as well as in meat systems (Meinert et al., 2009); therefore, addition of sugars to meat could modulate the Maillard reaction pathways and subsequently generate Maillard reaction products that specifically act as antioxidants and reduce lipid oxidation.

Off odours and off-flavours generated by lipid oxidation are typically detected by sensory characterization of meat products and descriptive sensory tests are the most sophisticated tools used for product characterization (Lawless & Heymann, 1999). There are different approaches used for sensory characterization of meat and meat products and Free Choice Profiling (FCP) is one of the approaches that has been used in the sensory science to study the perception of different sensory attributes (Williams & Langron, 1984). The thiobarbituric acid reacting substances (TBARS) assay is another common analytical method of determining the extent of lipid oxidation in meat and studies to assess lipid oxidation in pork have shown that lipid oxidative off-flavours were detected by sensory panellists when TBARS were above 0.50 mg malondialdehyde (MDA) equivalents (Lanari et al., 1995). However it should be noted in this regard that the magnitude of threshold detection levels

of thiobarbituric acid reacting substances in the TBARS assay and the sensory perception threshold of the volatile compounds is quite different.

Lipid oxidation proceeds as a chain reaction and during the reaction there is formation of a compound called, malonaldehyde (MA) which is a secondary lipid oxidation product. In TBARS assay, malonaldehyde is reacted with thiobarbituric acid (TBA) to form a pink MA-TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm. The extent of oxidation is reported as the TBA value and is expressed as milligrams of MA equivalents per kilogram sample or as micromoles of MA equivalents per gram of sample. It must, however, be noted that other lipid oxidation products such as alkenals and alkadienals also react with the TBA reagent and produce a pink color. Thus, the term thiobarbituric acid reactive substances (TBARS) are now used instead of malonaldehyde.

The present study was conducted to examine the effects of addition of food grade sugars (xylose, glucose and sucrose) at 2% levels to pork meat obtained from pigs fed with high levels of flax (10%) for 6 weeks and vitamin E (400mg/kg) for 6 weeks on oxidative stability and sensory characteristics. It is hypothesised that addition of food grade sugars will improve pork oxidative stability and reduce the incidence of off-flavours in fresh and re-warmed pork.

3.2. Materials and methods

3.2.1. Experimental design and animal management

A 2 × 2 factorial feeding trial was conducted on thirty-six Manor Hybrid x Duroc crossbred barrows at the Swine Unit at Agriculture and Agri-Food Canada, Lacombe. Dietary treatments included 10% flax (LinPro®; O & T Farms, Regina, Saskatchewan) fed for either 0 (no flax-control) or 6 weeks and two levels of Vitamin E (50% DL-alpha tocopheryl acetate, Long Wing International, Inc. Oakville ON), either 40 (control) or 400 mg Vitamin E/kg of feed. Pigs were allocated to pens with three pigs per pen. Dietary treatments were allocated in a balanced manner to pens (n=3 for diet replication) and were blocked by room. Three research rooms were used for study with four pens in each room. Detailed description of experimental design and animal management is given in section 2.2.1.

3.2.2. Slaughter and sample collection

Animals were slaughtered at the AAFC – Lacombe research abattoir on three slaughter dates, with one complete room (4 pens; 1 from each dietary treatment, n=12) slaughtered in a single day. One animal was removed from the study as it was discovered to be a gilt. As a result, there were only eight animals in the control group (40/0) and so the total number of animals used in the study was thirty five. On the morning of slaughter date a final live weight and feed weigh back were recorded before shipping. Pigs were shipped to the AAFC-Lacombe research abattoir and were held without feed but were given free access to water until slaughter. Animals were stunned electrically, killed by exsanguination and the carcasses were dressed in a manner similar to that of commercial processing. After 24 h post mortem, *Spinalis dorsi* and *Gluteus medius* muscles and subcutaneous fat from the loin primal were removed from both sides of each carcass and were labelled, weighed, packaged under vacuum (Multivac AGW, Multivac Inc.,Kansas City, MO) and frozen at –35°C until further processing.

3.2.3. Preparation of meat grinds and patties

Frozen lean and subcutaneous fat obtained from three animals which received the same treatment were pooled across pens and tempered at -2°C until they were sufficiently thawed to use in grind production. Three replicates of 10 kg lean grind were produced by combining 8.5 kg of lean and 1.5 kg of subcutaneous fat tissue and thus a 15% fat to lean grind was obtained. The meat and fat were ground by passing once through a commercial grinder equipped with a coarse grind plate (0.95cm) (Hollymatic 175 Mixer Grinder, Hollymatic Corp., Countryside, IL).

Each 10 kg portion of coarse ground pork was then divided into four batches (approximately 2.5 kg each) to which one of the following four sugar (xylose, glucose, sucrose, no sugar treatments were applied at 20 g sugar per kg meat for a total of 50 g sugar in each 2.5 kg batch) to attain a concentration of 2% of sugars in the grind. Sugars were sprinkled on each replicate grind, hand mixed for approximately 3 min, then homogenized by passing through a commercial mixer (Koch 80H, Koch equipment LLC, Kansas City MO) equipped with a (0.3 cm) grind plate for 2 min. Approximately 150 g of each grind was retained for subsequent thiobarbituric acid reactive substances assay

(TBARS) as a 'fresh' sample. Meat grinds were subsequently stuffed into plastic casings (Trespade, Torino, Italy) and frozen at – 23°C. The frozen meat tubes were then cut into meat patties approximately 10 cm diameter (~120 g each) and 13 mm thick using a band saw (Hobart 5801, Hobart, Ontario, Canada). Immediately after they were cut, meat patties were packaged under vacuum in bags (VC999 Vacuum Sealer, Herisau, Switzerland) and stored at -20°C until further analysis.

3.2.4. Cooking and re-warming of meat patties

Meat patties were thawed overnight at 4°C. Meat patties were cooked in non-stick electric skillets (Proctor Silex Plus 38520G, Hamilton Beach brands Inc., USA). Skillets were preheated to 176°C and meat patties were cooked to an internal temperature of 72°C. The meat patties were flipped frequently to prevent burning and when the internal core (centre) temperature reached 72°C, patties were removed from the skillets to prevent further cooking. To assess the effect of sugars on lipid oxidation during refrigerated storage, cooked patties were re-warmed to an internal temperature of 65°C for further analyses.

3.2.5. Colour measurements

Objective colour measurements were recorded on fresh meat patties before they were used to perform TBARS assay at three different locations on each patty for lightness (L*), (a*) red–green spectral axis, (b*) yellow–blue spectral axis (CIE, 1978) using a (Minolta CM2002 [Minolta Canada Inc., Mississauga, ON]). Hue and chroma were determined as hue [H_{ab} = arctan (b*/a*)*57.296] and chroma [$C_{ab} = (a^{*2} + b^{*2})^{0.5}$]. Deoxymyoglobin (DMB), metmyoglobin (MMB), and oxymyoglobin (OMB) relative contents were determined based on equation proposed by Krzywicki, (1979) which uses the reflectance values using the Lambert-Beer law and molar absorbance coefficients for the different myoglobin species by interpolation of the isobestic points at 473, 525, 572, and 700 nm.

3.2.6. Thio-barbaturic acid reactive substances (TBARS) assay

TBARS assay were performed on the fresh, frozen (8, 12 and 16 weeks), cooked and re-warmed meat patties as per the procedures outlined by McDonald & Hultin (1987) and Nielsen et al. (1997) which is followed as the standard operating procedure for TBARS assay at AAFC, Lacombe. Meat

patties of approximately 140g were formed using a single patty hamburger press (Cabelas Inc, Sidney, NE, USA). Fresh meat patties were processed in a food processor (Robot Coupe USA Inc., Ridgeland MS) and homogenised for 10 sec. From this homogenate, 10g were weighed into a 50mL centrifuge tube. Thirty mL of 7.5% TCA (trichloracetic acid) were transferred by pipette to a 50mL centrifuge tube. The mixture was then homogenized (Polytron Model 3100, Kinematica AG,Switzerland) for 30 sec and the homogenate filtered (Whatman # 4 filter paper, Fisher Scientific, Mississauga, ON) into a 50 mL centrifuge tube. A 2.5 mL aliquot of the filtrate was transferred by pipette in duplicate into 15 mL Pyrex screw top tubes. To this, a further 2.5mL of thiobarbituric acid solution was added. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario) and the samples incubated in water bath (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) at 94°C for 40 min. After incubation, the tubes were removed from the water bath and subjected to sonication for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into an Gilson 222 XL auto-sampler (Mandel Scientific Company, Inc., Ontario, Canada) and the concentration of the thiobarbituric acid reacting substances measured spectrophotometrically at 532nm (Ultraspec 3000, Pharmacia Biotech, Buckinghamshire, UK). TBARS results were expressed as mg malonaldehyde per kg of meat. For estimation of TBARS on the frozen meat patties, samples were thawed overnight and then were cooked and reheated as described in section 3.2.4. The TBARS assay was subsequently performed according to the procedure outlined above.

3.2.7. Sensory study

The sensory study was approved by the *PER/ALES research ethics board* at University of Alberta. Twenty four participants were screened for their sensory acuity in a single 60 min session in which the participants were asked to participate in four taste tests. Participants performed a taste identification test in which they were asked to taste food-grade aqueous solutions with specific concentrations of caffeine (0.2g/L), sucrose (20g/L), citric acid (0.8g/L), salt 2g/L) and distilled water and describe the taste of

each. They then performed a sweetness test in which they ranked the sweetness intensity from low to high concentration of water containing 2, 5, 7.5, 10 or 12% sucrose. Participants were then asked to taste an aqueous solution of 0.0032 M 6-thiopropyluracil (PROP test) to determine if the participants had the genetic ability to taste this compound. Finally, the participants completed a questionnaire (Table 3.7.1) in which the ability of the participants to describe the taste and characteristics of foods tasted in the past was evaluated.

Following the screening, 12 participants were selected as panellists based on their sensory acuity measured in the screening session for training. Training was conducted in six 1h sessions. During training, panellists were provided with a list of descriptors describing the aroma, texture and flavour of pork meat that were collected from the literature (Table 3.7.2) (Byrne et al., 1999; 2001a; 2001b; Delahunty et al., 1997). They were introduced to the use of line scales and were asked to develop their own list of descriptors by providing them with cooked pork patties. Panellists were instructed as to how to perform the test for aroma, texture and flavour of the meat patties and were then asked to record the aroma, texture and flavour of the meat patties without consulting other participants. After developing the list of descriptors individually, they then discussed as a group the initial descriptors developed. During all training sessions, the panellists were able to modify the list of descriptors developed. The last two training sessions were performed in individual booths and 8 experimental meat patties were served to the panellists to familiarise them with the actual tasting situation. Following this training, the list of descriptors developed by each panellist was finalised. No formal definition was developed for each attribute by each panellist; however, each was able to explain all their attributes. A lexicon was compiled for each panellist based on all the terms and definitions necessary for that panellist to describe the aroma, texture and flavour of meat patties.

3.2.8. Sample preparation, cooking and sensory presentation

Meat patties were thawed overnight at 4°C and cooked as described in Section 3.2.4. Immediately following cooking, each meat patty was cut into 4 identical portions. Each portion of the meat patty was kept warm at 65°C in a bain marie until served to the panellists. Samples were placed into labelled 250

mL glass jars (Bernardin Mason jars, Bernardin Ltd., Ontario, Canada), capped with metal lids and kept immersed in a water bath heated by an electric heating plate (IKA Hotplate stirrer C-MAG HP10 S1, IKA works Inc., NC, USA). For presentation to panellists, the samples were removed from the glass jars and placed into labelled 250 mL foam containers with plastic lids.

To assess the effect of sugars on lipid oxidation during refrigerated storage after cooking, patties were stored for 2 days at 4 °C in styrofoam hinged lid containers (SmartLock®, Scarborough, Ontario) and then reheated to an internal temperature of 65°C on the day of sensory evaluation. All subsequent sample preparation and presentation methods for the re-heated patties were similar to those described for the frozen patties.

3.2.9. Sensory evaluation

Following the training sessions, 8 panellists were selected from the 12 trained panellists for the actual evaluations of the pork patties. Assessment of aroma, texture and flavour of the cooked and re-warmed meat patties by the panellists was completed in 3 weeks with 4 sessions per week in an incomplete block design (Deppe et al., 2001). There were 16 treatment combinations (2 Vitamin E levels × 2 Flax levels × 4 sugar levels). Samples were serially numbered from 1 to 16. The replicates were identified with labels A, B or C. Thus each panellist evaluated 48 samples (16 treatments replicated thrice) in the study. All the samples were labelled using three digit codes. Each panellist tasted the same 8 samples in a session which were presented to the panellists based on an order generated by a William's Latin square design to avoid carry over and tasting position effects (MacFie et al., 1989). The samples were served in foam containers (Dixie 103.5 mL [Georgia Pacific Consumer Products, Inc., ON, Canada]) covered by plastic lids. Cooked and re-warmed samples were served in separate sessions and not compared. Samples were thus scored on 15 cm line scales using the assessors' own vocabularies, anchored at the ends by the terms "none" and "extreme". Data were recorded on paper ballots and entered into Microsoft Excel 2007 (Microsoft Corporation Inc., WA, USA) worksheets for analysis. Filtered water at room temperature and unsalted crackers were provided as palate cleansers before the start of evaluation and between samples. Panellists were rewarded at the end of the evaluation sessions each day with refreshments and received gift

certificates commensurate with hours of involvement for their participation at the end of the study.

3.3. Statistical analysis

Colour and oxidative stability (TBARS assay) data were analyzed as a split plot design PROC MIXED (SAS Version 9.2, SAS institute Inc., Cary, NC) with sources of variation including levels of Vitamin E, periods of flax treatments and their interactions in the main plot and sugar treatment and its interactions in the subplot. Pool was included as a random effect. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. For significant main or interaction effects ($P < 0.05$), differences between treatment or interaction means were computed using (LSMEANS) least square means and separated using *t*-tests with the PDIFF option.

Sensory analysis data were entered into a Microsoft Excel 2007 worksheet and analyzed by generalized Procrustes analysis (GPA), using XLStat 2010 (Addinsoft Inc., NY, USA). The attributes developed by the 8 panellists were pooled by whether they were an aroma, texture or flavour attribute prior to the GPA. These pooled attributes generated the GPA output separately for aroma, texture and flavour. Panellists that did not detect a particular attribute were assigned zero for that attribute so that the total number of attributes was made constant for all the panellists within the GPA.

3.4. Results

3.4.1. Colour and TBARS (Fresh)

Results of colour measurements and thiobarbituric acid reactive substances assay (TBARS) value for fresh uncooked meat patties are presented in table 3.7.4. There were no significant effects of vitamin E, flax supplementation or sugar type ($P>0.05$) on the lightness (L*), chroma (%), hue (°), and metmyoglobin and oxymyoglobin proportions, nor were the two way and three way interactions significant ($P>0.05$). TBARS values for the fresh uncooked meat patties showed significant interactions between vitamin E and flax ($P=0.0327$) (Figure 3.8.1), sugar and vitamin E ($P=0.0001$) (Figure 3.8.2) and sugar and flax treatments ($P=0.0004$) (Figure 3.8.3). Pigs fed 400 mg Vitamin E/kg of feed and supplemented with 10% flax for 6 weeks had greater mean concentrations of TBARS than all other dietary treatments (Figure 3.8.1). Addition of the sugars to the meat grinds also interacted with both

vitamin E and 10% flax supplementation, with vitamin E supplementation decreasing mean TBARS levels in pork grinds without reducing them to control levels (Figure 3.8.2). Regardless of dietary treatment, the addition of xylose to pork patty grinds produced mean TBARS concentrations far in excess of those from the addition of other sugars (Figures 3.8.2 and 3.8.3). The addition of sucrose also increased mean TBARS levels compared to those of control, and the TBARS concentration resulting from the addition of glucose did not differ from those of control regardless of dietary treatment (Figures 3.8.2 and 3.8.3). Vitamin E had no effect of the mean TBARS concentrations produced by sucrose (Figure 3.8.2) but supplementation of the pigs with 10% flax for 6 weeks increased the mean TBARS levels in pork grinds to which sucrose had been added (Figure 3.8.3). Higher levels of vitamin E acted as a prooxidant and increased the mean TBARS produced in control and glucose-added pork grinds (Figure 3.8.2). Similarly, supplementation of pigs with 10% flax increased the mean TBARS levels measured in control and glucose-added pork grinds (Figure 3.8.3).

3.4.2. TBARS (frozen, cooked and re-warmed)

Results of TBARS measurements of uncooked patties stored frozen for 8, 12 and 16 weeks as well as those that were cooked and re-warmed are shown in table 3.7.5. Dietary vitamin E had no effect on patty mean TBARS values after 8, 12 or 16 weeks frozen storage or that of cooked patties. Dietary supplementation of the pigs with vitamin E reduced TBARS values of re-warmed patties (Table 3.7.5). Dietary flax treatment had no effect on patty mean TBARS values of 8 weeks of frozen storage or on that of cooked patties; but produced increased TBARS values in samples after 12 or 16 weeks of frozen storage and in re-warmed meat patties (Table 3.7.5). Addition of sugars (glucose and sucrose) lowered patty mean TBARS values after 12 or 16 weeks of frozen storage as well as of cooked patties. The addition of sugars had no effect on TBARS levels produced in re-warmed patties (Table 3.7.5).

3.4.2.1. TBARS (frozen 8 weeks)

For TBARS values of uncooked patties that were stored frozen for 8 weeks, the interactions between sugar and vitamin E (P=0.0001) as well as between sugar and flax (P=0.0353) were found to be significant (Figures 3.8.4 and 3.8.5 respectively). Irrespective of the dietary treatments, addition of

xylose to pork patty grinds stored frozen for 8 weeks produced the highest mean TBARS levels compared with those of other sugars (Figure 3.8.4 and 3.8.5). Mean TBARS levels in meat patties that contained glucose and sucrose did not differ from controls regardless of dietary vitamin E and flax treatments (Figure 3.8.4 and 3.8.5). Dietary supplementation of 400mg/kg vitamin E reduced the mean TBARS levels only in meat patties that contained xylose (Figure 3.8.4). Supplementation of pigs with 10% flax for 6 weeks had no effect on mean TBARS levels in any of the meat patties after 8 weeks of frozen storage (Figure 3.8.5).

3.4.2.2. TBARS (frozen 12 and 16 weeks)

None of the interactions was found to be significant with respect to TBARS values of uncooked patties stored frozen for 12 or 16 weeks ($P>0.05$). Pork patties that had xylose added and were frozen for 12 and 16 weeks had the highest mean TBARS concentrations (Table 3.7.5). Patties with sucrose added that were stored for 12 and 16 weeks had mean TBARS levels that did not differ from those of control patties (12 weeks) or were less than those of control (16 weeks) (Table 3.7.5). The addition of glucose reduced mean TBARS levels to less than those of controls after 12 and 16 weeks storage (Table 3.7.5).

3.4.2.3 TBARS (cooked)

TBARS values of cooked meat patties showed a three way interaction between dietary vitamin E, flax supplementation and sugar treatments ($P>0.05$). Irrespective of the dietary treatments, mean TBARS levels in pork patties that contained xylose were highest compared with other sugars and samples that contained glucose and sucrose did not differ among each other in their mean TBARS values (Figure 3.8.6). Mean TBARS levels in pork patties that contained glucose and sucrose did not differ from control samples except in those in which the dietary treatments were 400mg/kg vitamin E and 10% flax for 0 weeks (Figure 3.8.6) where mean TBARS levels of control samples were higher than those of patties that had glucose and sucrose. In these patties mean TBARS values of the control samples did not differ from the patties that contained xylose (Figure 3.8.6).

3.4.2.4 TBARS (re-warmed)

None of the interactions was found to be significant in case of TBARS values of re-warmed meat patties (P>0.05). Dietary supplementation of vitamin E (400mg/kg) reduced mean TBARS levels of the re-warmed meat patties (Table 3.7.5) while dietary supplementation of 10% flax for 6 weeks caused an increase in mean TBARS levels (Table 3.7.5).

3.4.3. Sensory data results

There were 21 attributes for aroma, 17 attributes for texture and 19 attributes for flavour developed by the panellists (Table 3.7.3).

3.4.3.1. Fresh cooked pork patty aroma

For the aroma of the fresh cooked meat patties, the first four dimensions explained 38.36%, 24.77%, 10.23%, and 8.19% of the variation, respectively. The correlation plot showing the distribution of the aroma attributes is shown in Figure 3.8.7 and the placement of the samples relative to the attributes is shown in Figure 3.8.8. Comparison of the two plots indicated that the presence of sugars produced distinct characteristics irrespective of vitamin E and flax levels. Panellists described the aroma of cooked pork patties that contained xylose as warmed over, fishy, chemical, cardboardy and burnt. Cooked pork patties that contained either glucose or sucrose shared along with control cooked pork patty aromas and was described as pork meaty, roasted pork meaty, fried and browned. The aroma of the cooked pork patties that did not have sugars were described as being warmed over and reheated porky. Addition of sucrose generated a sweet aroma specifically indicating that sugars produced distinct aroma characteristics. For cooked pork patties that did not have sugars, patties made from pork of pigs fed high vitamin E and 10% flax as well as those from pigs fed no flax and no vitamin E were described as having pork meaty aroma. High vitamin E and no flax samples were described as having roasted pork aroma whereas samples that contained low vitamin and high flax were described as having fishy and burnt aromas (Figure 3.8.7 and 3.8.8).

3.4.3.2. Re-warmed cooked pork patty aroma

The first four dimensions explained 81.60% of the total variance associated with the aroma of re-warmed pork patties with the first, second, third and the fourth dimensions explaining 42.76%, 16.83%, 13.73%, and

8.28% respectively. Figure 3.8.9 shows the correlation plot which indicates the distribution of the attributes. Figure 3.8.10 shows the plot of the samples relative to the attributes in Figure 3.8.9. The presence of sugars produced distinct characteristics irrespective of vitamin E and flax levels. Panellists described the aroma of samples which contained xylose as warmed over, fishy, chemical, cardboardy and burnt with more agreement than in the case of fresh cooked samples. Meat patties that contained glucose and sucrose were described as having pork meaty, roasted pork meaty, fried, oily and browned aromas. In the case of control (no sugar) samples dietary vitamin E and flax treatments seemed to have an influence on the attributes described by the panellists. Re-warmed patties that did not have sugars but contained high vitamin E (400mg/kg) and flax (10% flax for 6 weeks) were described as having browned aromas whereas no flax and vitamin E samples (40mg/kg) were described as cardboard and stale. High vitamin E and no flax samples were described as having pork meaty aroma whereas samples that contained low vitamin and high flax were described as having fishy and burnt aromas (Figure 3.8.9 and 3.8.10).

3.4.3.3. Texture of fresh cooked pork meat patties

Approximately 71% of the total variance associated with the texture of the fresh cooked pork patties was explained by the first four dimensions, which explained 34.89%, 17%, 10.66%, and 9.09% of the variation, respectively. The correlation plot showing the distribution of the texture attributes is shown in Figure 3.8.11 and the placement of the samples relative to the attributes is shown in Figure 3.8.12. The samples varied significantly in their texture attributes. Levels of flax and vitamin E were not found to be associated with a particular texture; however, panellists described the texture of the cooked pork patties that contained sucrose and glucose as soft, juicy and moist. Patties that contained xylose were rated as fibrous and spongy. Control samples without sugars were described as crunchy, stringy and sticky (Figures 3.8.11 and 3.8.12).

3.4.3.4. Texture of re-warmed pork meat patties

The first four dimensions explained 80.20% of the total variance associated with the texture of re-warmed meat patties with the first, second, third and the fourth dimensions explaining 52.26%, 11.86%, 8.88%, and

7.20%, respectively. Figure 3.8.13 shows the correlation plot which illustrates the distribution of the attributes. Figure 3.8.14 shows the plot of the samples relative to the attributes shown in Figure 3.8.13.

The samples varied significantly in their texture attributes. Levels of flax and vitamin E were not found to be associated to a particular texture. But still overall panellists described the texture of the meat patties that contained sucrose, glucose and xylose as soft, juicy and moist. Patties that contained no sugars were rated as fibrous, dry, crunchy and hard (Figure 3.8.13 and 3.8.14).

3.4.3.5. Flavour of fresh cooked pork meat patties

Approximately 77% of the total variance associated with the flavour of fresh cooked pork meat patties was explained by the first three dimensions with the first, second and third dimensions explaining 48.25%, 21.07% and 7.72%, respectively. Figure 3.8.15 shows the correlation plot illustrating the distribution of the attributes. Figure 3.8.16 shows the plot of the samples relative to the attributes shown in Figure 3.8.15.

Addition of sugars produced distinct flavours irrespective of the vitamin E and flax levels. Panellists described the flavour of the meat patties that contained sucrose predominantly as having smoked, caramelized and sweet flavour whereas those having glucose were described as having roasted pork, fishy, umami and tangy flavours. Meat patties that contained xylose were described as having a chemical, burnt, sour and oily flavour. Meat patties that did not have sugar added were described as having boiled and warmed over flavour (Figure 3.8.15 and 3.8.16).

3.4.3.6. Flavour of re-warmed pork meat patties

The first four dimensions explained 79.95 % of the total variance associated with the flavour of re-warmed pork patties with the first, second, third and fourth dimensions explaining 37.95%, 19.06%, 13.55% and 9.39% respectively. Figure 3.8.17 shows the correlation plot which shows the distribution of the attributes. Figure 3.8.18 shows the plot of the samples relative to the attributes.

The addition of sugars produced distinct flavours irrespective of the vitamin E and flax levels. Panellists described the flavour of the meat patties that contained sucrose predominantly as having roasted pork and sweet flavour whereas those having glucose were described as having boiled, fishy, smoked

and oily flavours. Meat patties that contained xylose were described as having chemical, burnt, sour, bitter and chemical flavours. Meat patties that did not have sugar added were described as having rancid, stale and warmed over flavours irrespective of the vitamin E and flax levels (Figure 3.8.17 and 3.8.18).

3.5. Discussion

Ever since the beneficial effects of *n*-3 fatty acids became apparent, there has been an increased trend towards *n*-3 enrichment of food products (Ian et al., 2008). Products that are *n*-3 enriched such as eggs, juices and meat are available in the market (Simopoulos, 2000) and considerable awareness has also generated regarding the potential advantages of consumption of *n*-3 fatty acids and need for inclusion of *n*-3 enriched products in the human diet (Gogus & Smith, 2010). Omega -3 fatty acids in food tend to oxidize quickly during cooking and this poses a serious problem in *n*-3 enriched foods as most often lipid oxidation reactions will eventually lead to the generation of off odours and off-flavours (Morrissey et al., 1998). Lipid oxidation and the resultant off flavour generation leads to serious consumer dissatisfaction. Addition of antioxidants is a common approach to prevent lipid oxidation reaction thus preventing the generation of off-flavours to a particular extent (Nam & Ahn, 2003). In the case of meat and meat products, cooking causes considerable lipid oxidation and the situation becomes worse when cooked foods are reheated. This will lead to significant generation of off- flavours often referred to as warmed-over flavour (Tims & Watts, 1958).

All the steps towards *n*-3 enrichment will be in vain if the generation of off odours and off-flavours is not addressed. Sugars play an important role in the generation of pork meat flavour through involvement in the Maillard reaction (Mottram, 1998) and the role of sugars in the generation of meat flavour has also been extensively studied (Campo et al., 2003; Elmore et al., 2002; Hudson & Loxley, 1983; Meinert et al., 2009). Addition of Maillard reaction products obtained by heating sugars and amino acids to pork meat patties has caused a reduction in lipid oxidation reaction over 10 days of refrigerated storage (Bedinghaus & Ockerman, 1995).

Off-flavour generation in meat is highly correlated to the production of compounds like aldehydes and 2-thiobarbituric acid reacting substances

(TBARS) (Ahn et al., 2000). The present study evaluated the effects of addition of food grade sugars (xylose, glucose and sucrose) at 2% levels to pork meat grinds obtained from pigs fed with high levels of flax (10%) and vitamin E (400mg/kg) for 6 weeks on oxidative stability and sensory characteristics.

Thiobarbituric acid reactive substances (TBARS) assay of patties that were fresh or frozen for 8, 12 or 16 weeks showed that the addition of sugars (glucose and sucrose) improved oxidative stability after 12 and 16 weeks of frozen storage. Sugars interacted with dietary vitamin E and flax treatments and improved the oxidative stability as indicated by lower TBARS of cooked samples that had high vitamin E (400mg/kg) and no flax. The ability of the sugars to improve the oxidative stability of the meat patties suggested that Maillard reaction products (MRP) may have been formed that possessed antioxidant properties (Kim & Lee, 2010). However different factors like reactant concentration, temperature, time, initial pH and water activity could affect the reaction and can influence the subsequent generation of MRP's (Naranjo et al., 1998) and MRP's were not measured in the present study.

Free choice profiling (FCP) sensory approach was used to characterise aroma, texture and flavour attributes of freshly cooked as well as re-warmed pork meat patties. FCP is considered to be a simple but powerful sensory tool that enables easy characterization of the products (Murray et al., 2001). FCP differs from other descriptive sensory methods in that panellists use their own words to describe product sensory attributes (Williams & Langron, 1984). The principle behind FCP is that panellists do not differ in their sensory perceptions, but merely in the way in which they describe them. The assessors must be able to detect differences between the samples, describe the perceived attributes in their own words and quantify them (Oreskovich et al., 1991). It is often considered as a useful tool to obtain important insights into consumer differentiation of products and establish relationships between consumer preferences and sensory characteristics (Jack & Piggott, 1992).

FCP data are analyzed by Generalized Procrustes Analysis (Gower, 1975). In this statistical technique, data obtained from individual panellists are transformed to obtain a consensus configuration that shows the interrelationships between the samples for the panel as a whole (Williams &

Langron, 1984). FCP provides a generalized trend towards consumers perception of products without detailed technical descriptions of the products, which are typically provided by trained sensory panels (Murray et al., 2001).

Sensory characterization of the samples using FCP showed that the addition of sugars produced distinct sensory characteristics irrespective of the source grind fatty acid or tocopherol composition. Differences in the chemical structure of the sugars might be a factor that could influence the way these sugars react in Maillard reaction and their subsequent roles in the generation of compounds that could influence lipid oxidation and/or flavour formation. Spark (1969) compared the reactivity of reducing sugars in Maillard reaction and found that they followed the order: aldopentoses > aldohexoses > ketohexoses > disaccharides. The reactivity of reducing sugars in Maillard reaction is often assessed by the extent of browning they cause (Jing & Kitts, 2002; Jing & Kitts, 2004). Other possible methods that have used been to assess the reactivity of sugars in Maillard reaction systems include monitoring loss of available primary amino groups in the reaction medium (Lertittikul et al., 2007), loss of sugar from the reaction medium (Brands & van Boekel, 2002) and formation of reaction products (Benjakul et al., 2005). Recently Laroque et al. (2008) compared the reactivity of fructose, glucose, xylose, ribose and arabinose in Maillard reaction and found that the order of reactivity of sugars was fructose ~ glucose < arabinose < xylose < ribose when parameters like measurement of browning intensity, monitoring of the disappearance of reactants and chromatographic data were assessed. Further Laroque et al. (2008) also stated that the degree of reactivity of sugars in Maillard reaction follows the order: pentoses > hexoses > disaccharides and was in agreement with that observed by Spark (1969). In the present study there was some comparable results exhibited between sucrose and glucose in TBARS assay. This could be attributed to the similar reactivity of glucose and fructose, which are monosaccharides, and sucrose, which is a disaccharide consisting of one molecule each of glucose and fructose. Further it is also interesting to note that the sensory panellists also identified similar sensory attributes in the samples that contained glucose and sucrose which supports this hypothesis. The sugar specific perception of attributes was more seen for aroma and flavour and not for texture. This shows

the the role of the sugars present in the samples in generation of the volatiles responsible for aroma and flavour generation. However further studies focussing on the identification of the compounds produced would provide substantial scientific evidence.

Xylose is a pentose sugar and has an open ring structure that is more reactive than that of hexoses and thus an amino acid – sugar complex will be formed more easily than with other sugars (Young et al., 2009). Bedinghaus & Ockerman (1995) observed that Maillard reaction products obtained from xylose had potent antioxidant activity when added to pork meat patties and were present in higher concentrations than the MRP's derived from glucose. Young & Cummings (2008) also proposed that xylose derived MRP's have potent antioxidant activity. In the present study which compared the antioxidative ability of xylose, glucose and sucrose, xylose appeared to acted as a prooxidant. However previous studies did not compare the antioxidative ability as did in this research. They were more focussed on the reactivity of sugars and hence even though xylose might react fast due to its open ring structure, the exact mechanism that caused the difference in the anti-oxidative stability is unknown. Furthermore, the reaction varies considerably depending on the reaction conditions and hence this warrants further study. But in general, addition of sugars (sucrose and glucose) improved the oxidative stability of 15% fat pork patties and this might be attributed to the ability of MRP's generated to sequester the transition metals that accelerate lipid oxidation reactions and also by their ability to scavenge free radicals that are involved in the autooxidation propagation reactions (Wijewickreme & Kitts, 1997).

Lauridsen et al (2006) found that addition of sugars altered the odour produced from pork meat. These researchers did a descriptive odour profiling study in pork samples by adding sugars like glucose, glucose -6- phosphate, ribose, ribose -5- phosphate, fructose and lactose and found that addition of lactose specifically produced sour odour whereas other sugars produced caramel and grilled odours. Further analysis of Maillard reaction volatiles using GC-MS showed an increase in the production of flavour volatiles when sugars were added. The specific aroma generating potential of sugars added to pork has also been studied by Farmer et al. (1999) in which addition of ribose

and glucose increased ‘meaty’ aroma whereas glucose -6- phosphate produced ‘roasted’ aroma. Aliani & Farmer (2005) did studies with chicken meat with added ribose and found that addition of ribose produced ‘roasted’ odour. Young et al. (2009) did studies with sheep meat and found that addition of xylose to sheep meat modified the flavour of sausages and increased the consumer liking. These researchers demonstrated the ability of sugars to influence the generation of odour and flavour in meat and meat products. In the present sensory study, even though it could not be characterized that addition of sugars produced good or bad flavour/aromas, it could be said that the addition of sugars produced distinct aroma and flavour characteristics, suggesting that each sugar may participate in the Maillard reaction differently. For example, sucrose was related to ‘caramelized’ flavour notes, which could be due to the caramelization of sucrose. Further, xylose produced ‘burnt’ notes, which may arise from reactions between xylose and amino acids in the Maillard browning reaction leading to the formation of strong amino acid – sugar complexes (Hodge et al., 1972).

3.6. Conclusion

The Maillard reaction is a very important reaction that has tremendous applications in food industry. Ever since this reaction was first explained by Louis Camille Maillard in the 1910’s, the scientific community has conducted constant research to unfold its complexity. The antioxidative potential of Maillard reaction products has also been used in the food industry in various applications. The present study indicated that the addition of glucose to *n*-3 enriched pork meat could be used to enhance the oxidative stability of *n*-3 enriched pork meat and generate specific aroma and flavour characteristics. Further research into the effect of sugars in meat may lead to the ability to generate MRP’s with specific antioxidant activity and could therefore be valuable for use in meat processing strategies.

3.7. Tables

Table 3.7.1 Panellist Screening Questionnaire

Panelist screening questionnaire

Contact information:

Name: _____

Phone number (lab/office): _____

Email: _____

Availability:

1. Are there any weekdays (Monday – Friday) that you will not be available between July 1st to August 30?

_____
_____
_____

Health:

1. Do you have any of the following?
   Dentures _____
   Diabetes _____
   Oral or gum disease _____
   Hypoglycemia _____
   Food allergies _____
   Hypertension _____
   Thyroid condition _____
   Pregnant _____

2. Do you take any medications which affect your senses, especially taste and smell?
   _____

Food Habits:

1. Are you currently on a restricted diet? If yes, please explain.

_____

2. What is (are) your favorite foods? _____

3. What is (are) your least favorite foods? _____

4. What foods do you <u>not</u> eat because of insensitivities, intolerances, allergies or dislikes?

Insensitivities →

_____

Intolerances →

_____

Allergies →

_____

1. How would you rate your ability to distinguish smells and tastes?

	Smell	Taste
Better than average	_____	_____
Average	_____	_____
Worse than average	_____	_____

2. Does anyone in your immediate family work for a food company?

_____

_____

_

7. Does anyone in your immediate family work for an advertising company or a marketing research agency?

_____

_____

__

Flavour Quiz:

1. What are some other foods that taste like yogurt?

_____

2. What would you say is the difference between flavour and aroma?

_____

_____

__

_____

__

_____

__

3. What would you say is the difference between flavour and texture?

_____

_____

_

_____

_

_____

_

Table 3.7.2. List of descriptors presented to the panellists obtained from literatures

List of descriptors

Aroma	Flavour	Texture
Pork meaty	Rancid	Stringy
Browny	Smoked	Springy
Boar taint	Sweet	Chewy
Rancid	Salt	Juicy
Oily	Sour	Crumbly
Cardboardy	Bitter	Crunchy
Serum	Cooked porky	Mealy
Sweet	Processed meaty	Pasty
Fishy	Cured pork meaty	Grainy
	Plastic	Fibrous
	Boiled	Rubbery
	Bread like	Tough
	Broth like	Moist
	Paint like	Coarse
	Cereal like	Plastic
	Chemical	Firm
	Rubbery	Brittle
	Woody	Puffy
	Oxidised	Sandy
	Greasy	Lumpy
	Livery	
	Cooked pork fat	
	Cooked pork meat	
	Roasted pork	
	Fishy	
	Cardboardy	
	Oily	
	Metallic	
	Nutty	
	Artificial	

Table 3.7.3. List of descriptors developed by the panellists

Aroma	**Flavour**	**Texture**
Brothy	Boiled	Chewy
Browny	Burnt	Crumbly
Burnt	Caramelized	Crunchy
Cardboard	Cardboard	Dry
Chemical	Chemical	Fibrous
Fishy	Fishy	Grainy
Fried	Oily	Hard
Grilled porky	Overcooked	Juicy
Metallic	Pork meaty	Moist
Oily	Rancid	Oily
Pork meaty	Roasted pork	Soft
Rancid	Salty	Spongy
Reheated pork	Smoked	Sticky
Roasted pork	Sour	Stringy
Salty	Stale	Firm
Smoked	Sweet	Springy
Sour	Tangy	
Stale	Umami	
Sweet	Warmed over	
Umami	Bitter	
Warmed over		

Table 3.7.4. Effects of dietary vitamin E, duration of 10% flax and sugar treatments on patties on colour characteristics and TBARS of fresh meat patties

Effect	n	Lightness (L*)	Chroma (%)	Hue (°)	MMB[2]	OMB[3]	TBARS[4]
Vitamin E (mg/kg)							
40	24	62.83	19.24	63.92	0.35	0.68	0.21
400	24	60.19	18.85	62.40	0.35	0.67	0.23
10% flax (weeks)							
0	24	63.02	19.35	62.98	0.34	0.68	**0.20[b]**
6	24	59.99	18.74	63.34	0.36	0.67	**0.25[a]**
SEM[1]		0.98	0.24	0.59	0.005	0.003	0.008
Sugars							
Xylose	12	60.65	19.00	63.00	0.35	0.68	**0.57[a]**
Sucrose	12	51.01	19.03	63.53	0.35	0.68	**0.19[b]**
Glucose	12	62.13	19.53	62.80	0.35	0.67	**0.06[c]**
Control	12	62.25	18.63	63.31	0.35	0.67	**0.05[c]**
SEM		1.05	0.33	0.53	0.004	0.004	0.009

[1]Standard error of mean
[2]Metmyoglobin
[3]Oxymyoglobin
[4]Thiobarbituric acid reacting substances
[abc] LSMEANS within column which are bold and with different superscript letters are significantly different at P <0.05

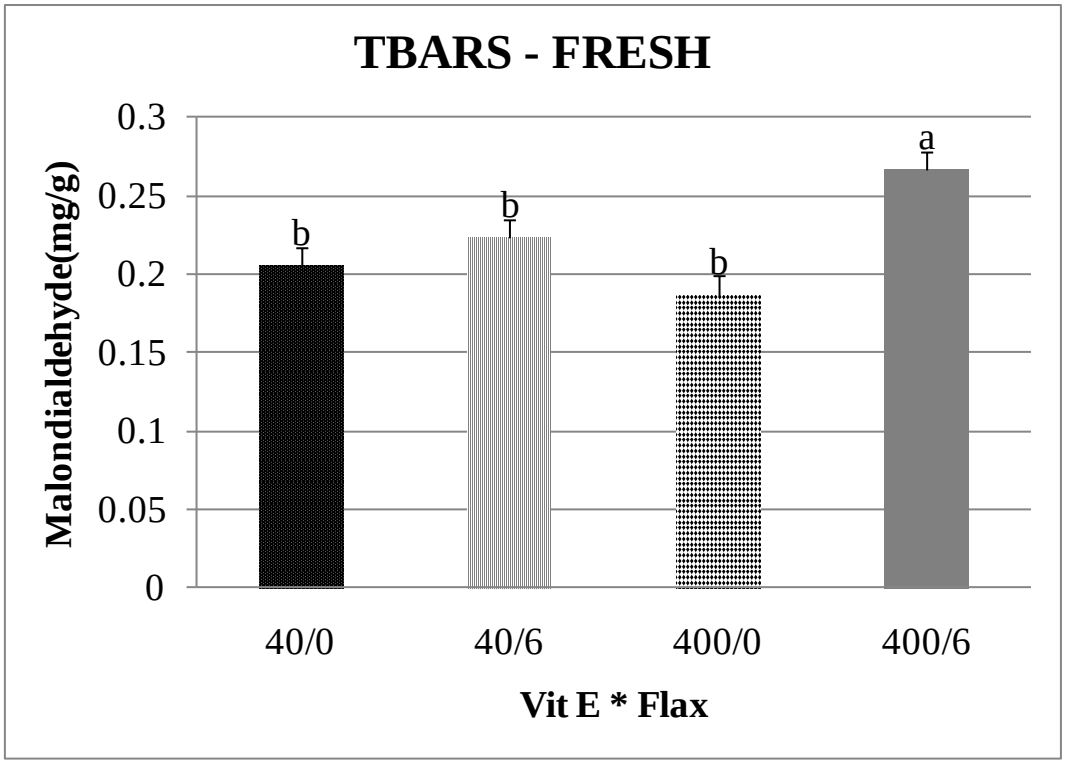
Table 3.7.5. Effects of dietary vitamin E, duration of 10% flax and sugar treatments on TBARS of frozen (8, 12 and 16 weeks), cooked and re-warmed meat patties

Effect	n	TBARS[2] 8 weeks	TBARS 12 weeks	TBARS 16 weeks	TBARS Cooked	TBARS Re-warmed
Vitamin E (mg/kg)						
40	24	0.50	0.64	0.98	0.94	**3.24[a]**
400	24	0.40	0.56	0.87	0.92	**2.76[b]**
10% flax (weeks)						
0	24	0.41	**0.51[b]**	**0.74[b]**	0.87	**2.33[b]**
6	24	0.50	**0.69[a]**	**1.11[a]**	0.99	**3.67[a]**
SEM[1]		0.05	0.04	0.09	0.06	0.11
Sugars						
Xylose	12	**0.89[a]**	**1.00[a]**	**1.24[a]**	**1.72[a]**	3.02
Sucrose	12	**0.37[b]**	**0.50[b]**	**0.76[c]**	**0.61[c]**	3.09
Glucose	12	**0.25[c]**	**0.41[c]**	**0.77[c]**	**0.55[c]**	2.69
Control	12	**0.29[c]**	**0.49[b]**	**0.92[b]**	**0.85[b]**	3.20
SEM		0.04	0.03	0.08	0.07	0.15

[1]Standard error of mean
[2]Thiobarbituric acid reacting substances
[abc]LSMEANS within column which are bold and with different superscript letters are significantly different at P <0.05

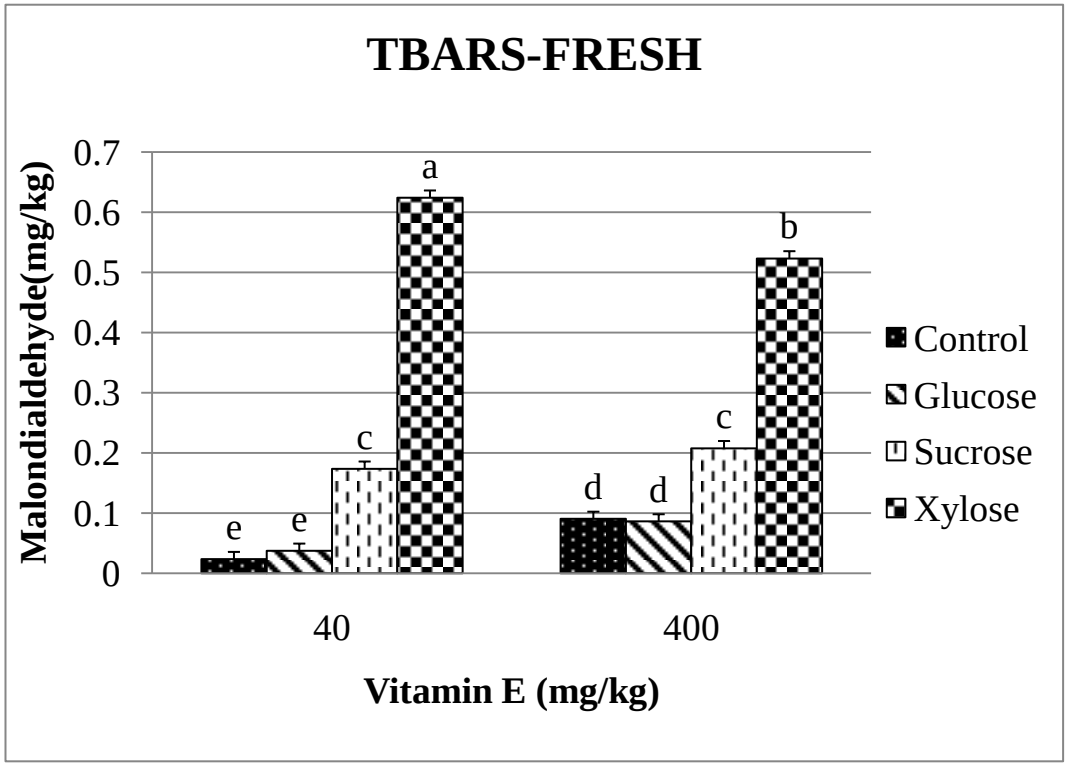
3.8. Figures

Figure 3.8.1. Interaction between vitamin E and duration of flax supplementation on TBARS of fresh (uncooked) meat patties.



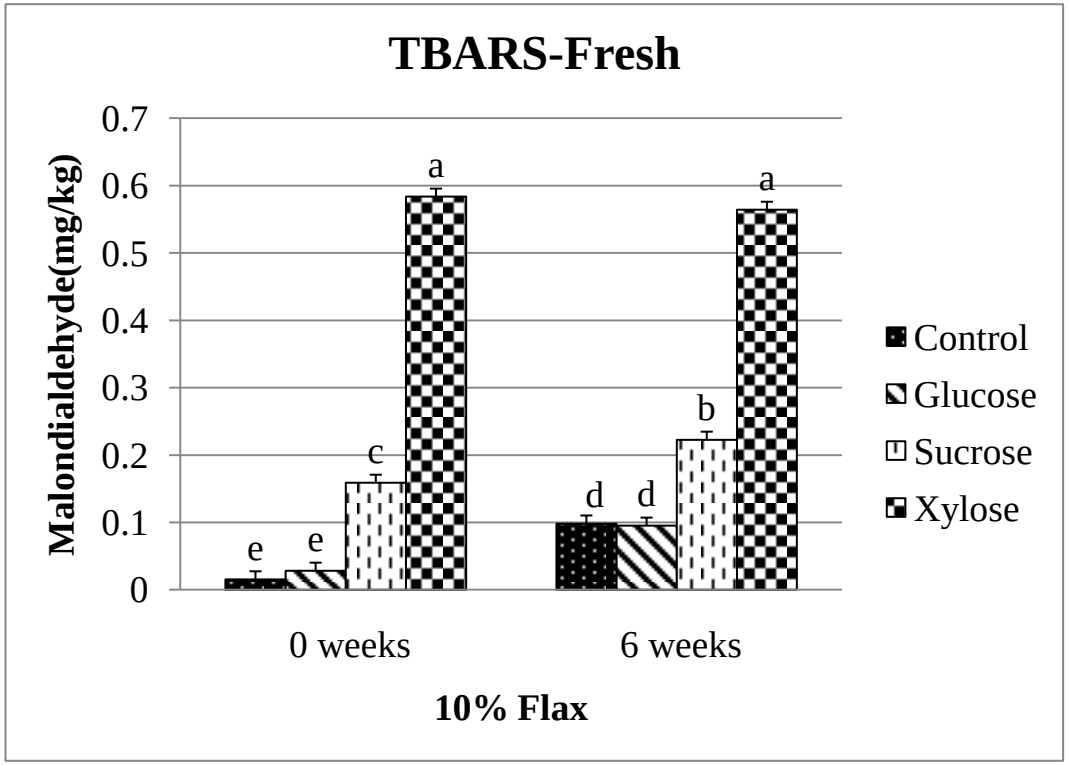
Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
40/0 – Interaction between 40mg/kg vitamin E and no flax
40/6 – Interaction between 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – Interaction between 400mg/kg vitamin E and no flax
400/0 – Interaction between 400mg/kg vitamin E and 10% flax for 6 weeks

Figure 3.8.2. Interaction between vitamin E and sugar on TBARS of fresh (uncooked) meat patties.



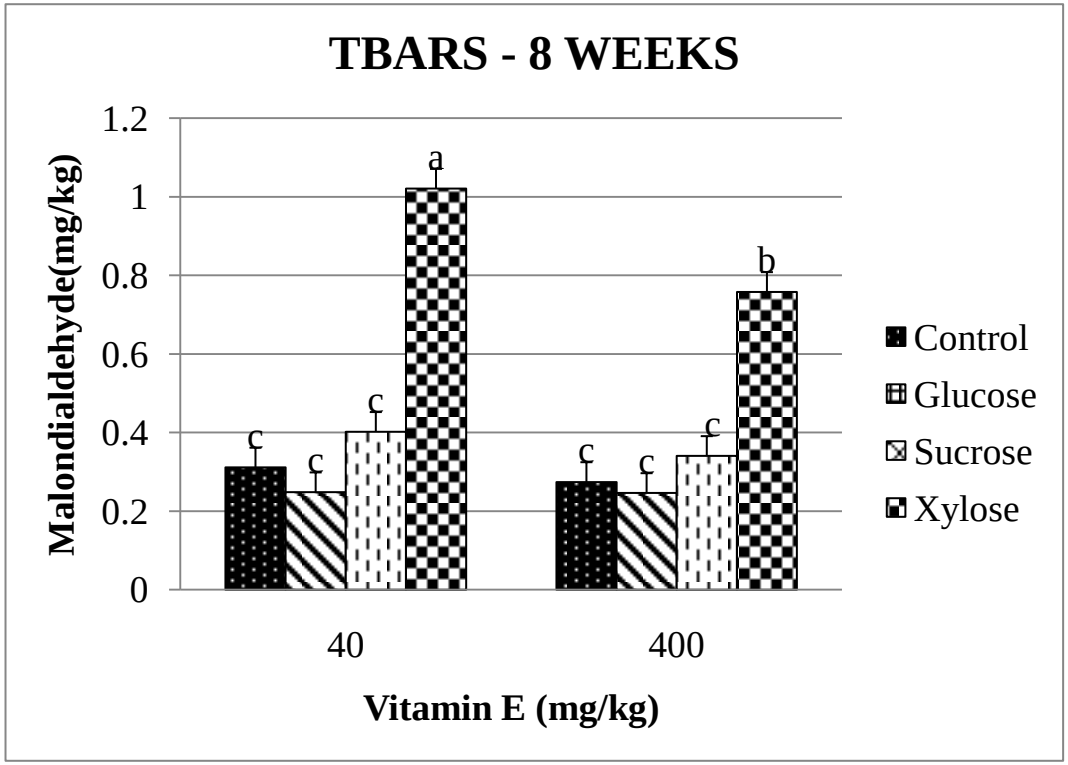
Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)

Figure 3.8.3. Interaction between duration of flax and sugar on TBARS of fresh (uncooked) meat patties.



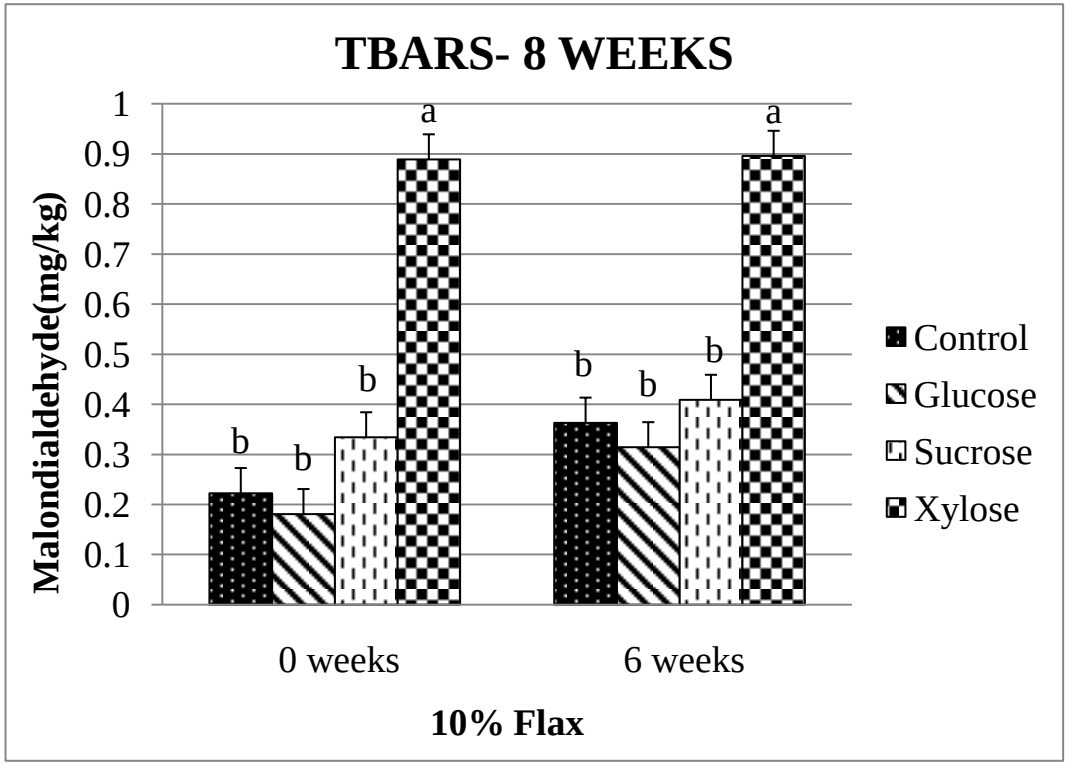
Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)

Figure 3.8.4. Interaction between vitamin E and sugar on TBARS of meat patties kept frozen for 8 weeks



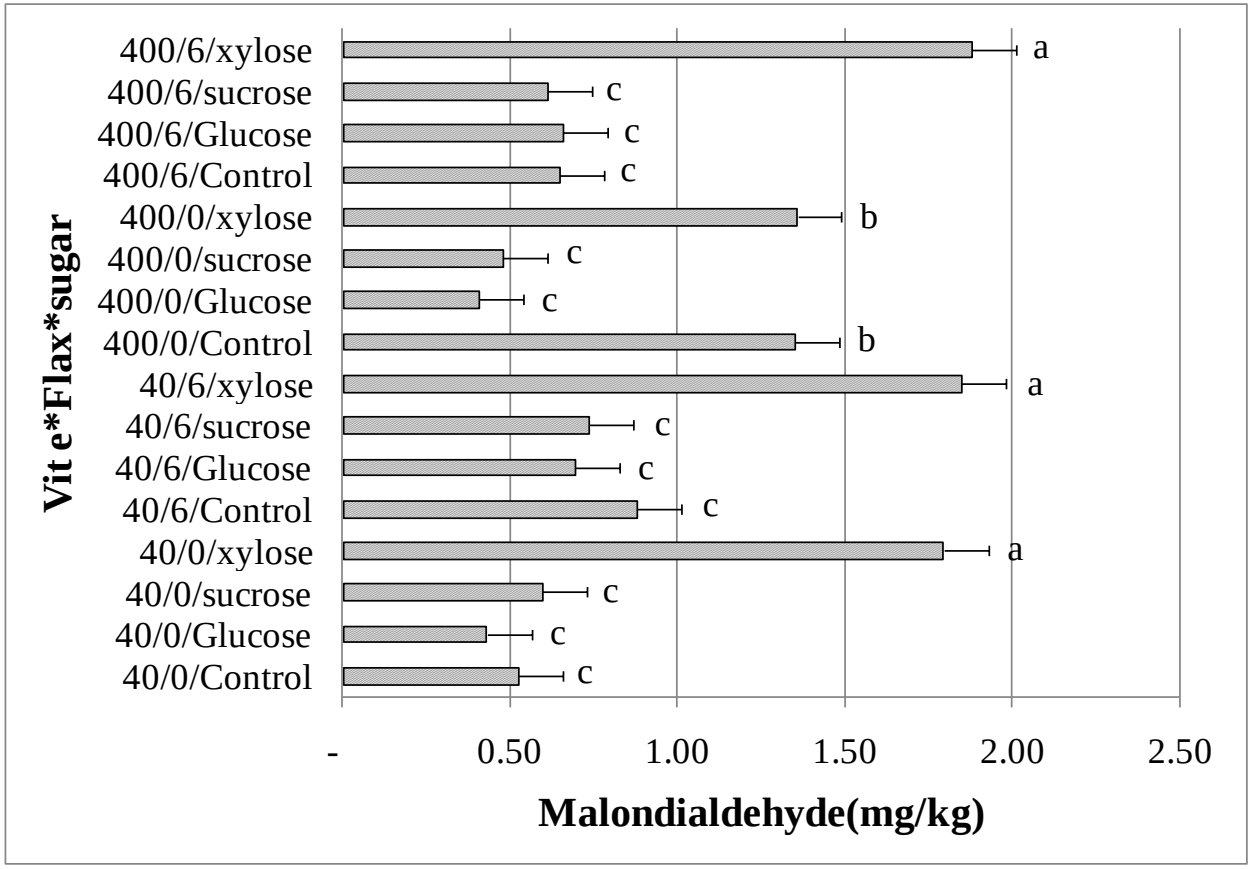
Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)

Figure 3.8.5. Interaction between duration of flax and sugar on TBARS of meat patties kept frozen for 8 weeks



Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)

Figure 3.8.6. Interaction between duration of flax, vitamin E and sugar on TBARS of cooked meat patties



Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
40/0 – Interaction between 40mg/kg vitamin E and no flax
40/6 – Interaction between 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – Interaction between 400mg/kg vitamin E and no flax
400/0 – Interaction between 400mg/kg vitamin E and 10% flax for 6 weeks.

Figure 3.8.7. GPA plot of the aroma attributes of cooked pork meat patties

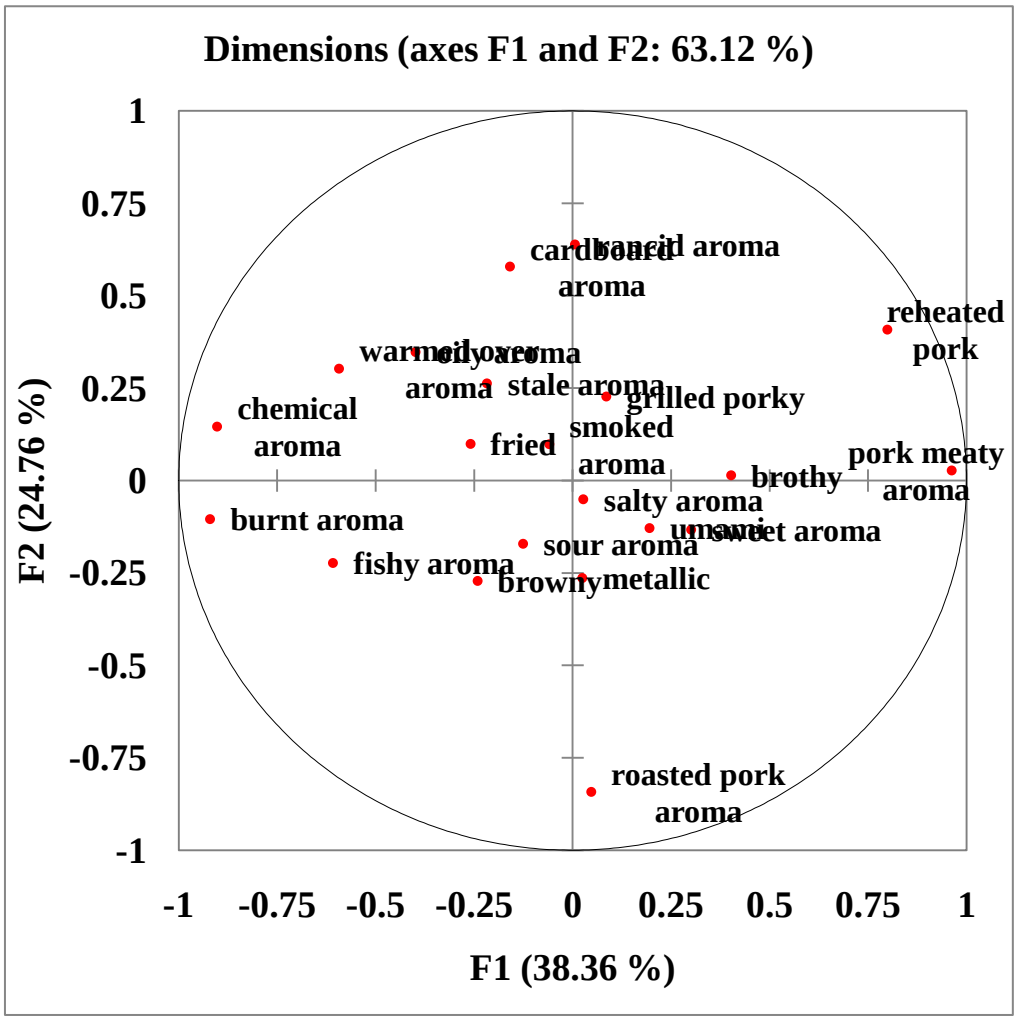
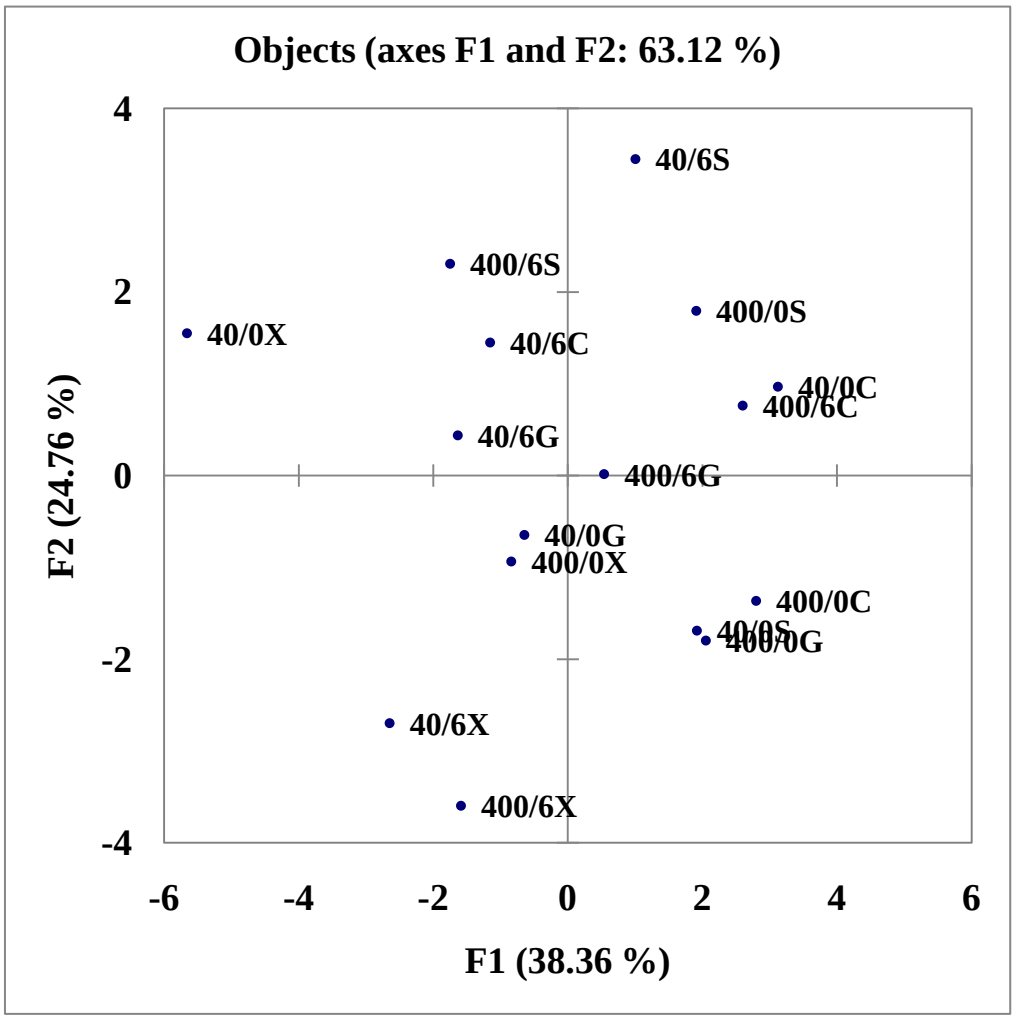


Figure 3.8.8. GPA plot of the samples for the aroma of cooked pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

Figure 3.8.9. GPA plot of the aroma attributes of warmed over (reheated) pork meat patties,

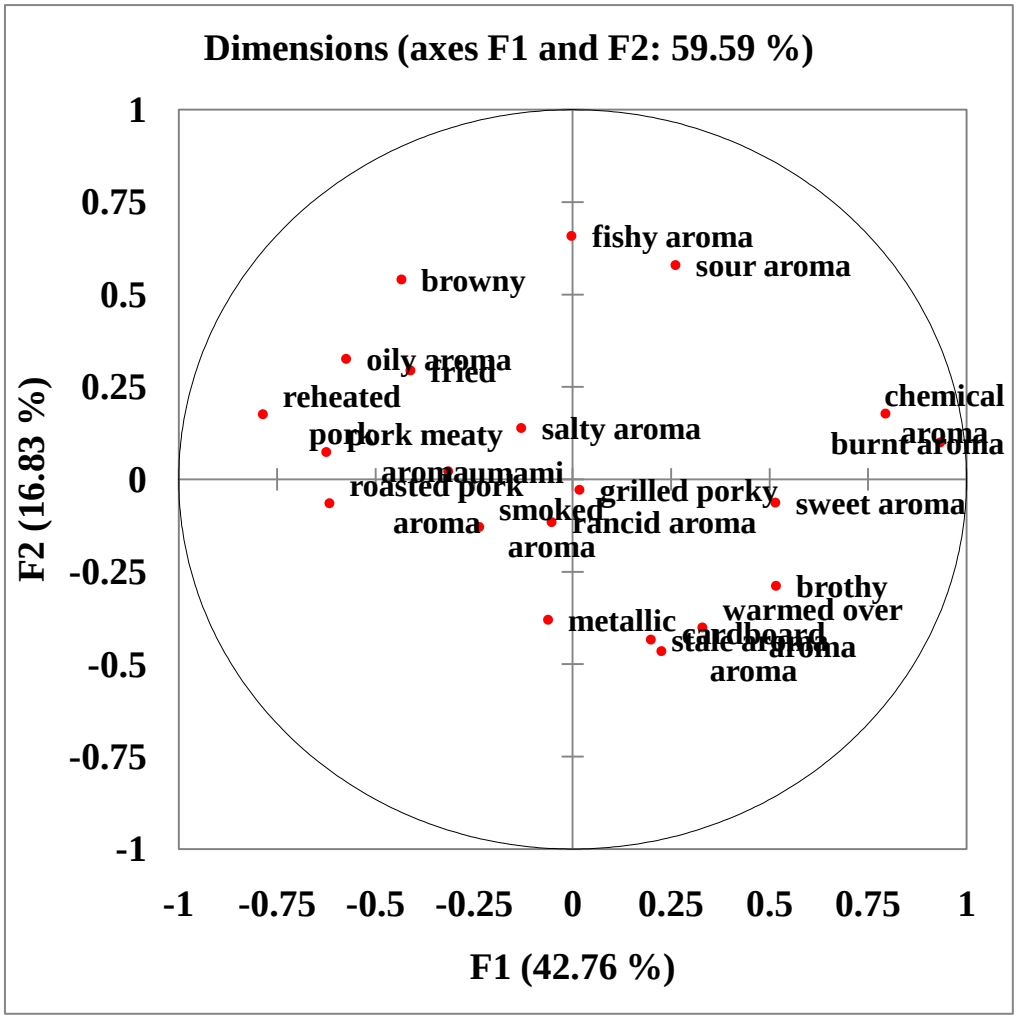
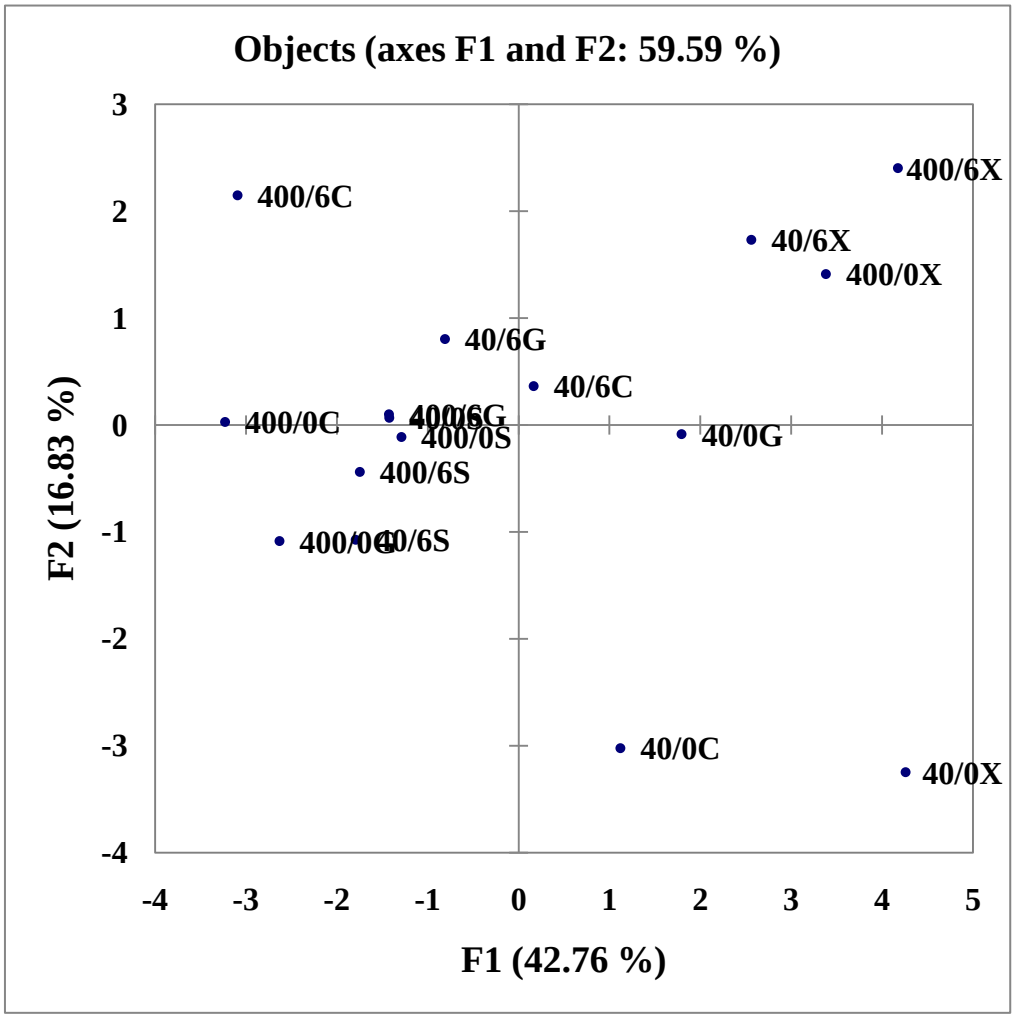


Figure 3.8.10. GPA plot of the samples for the aroma of warmed over (reheated) pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

Figure 3.8.11. GPA plot of the texture attributes of cooked pork meat patties

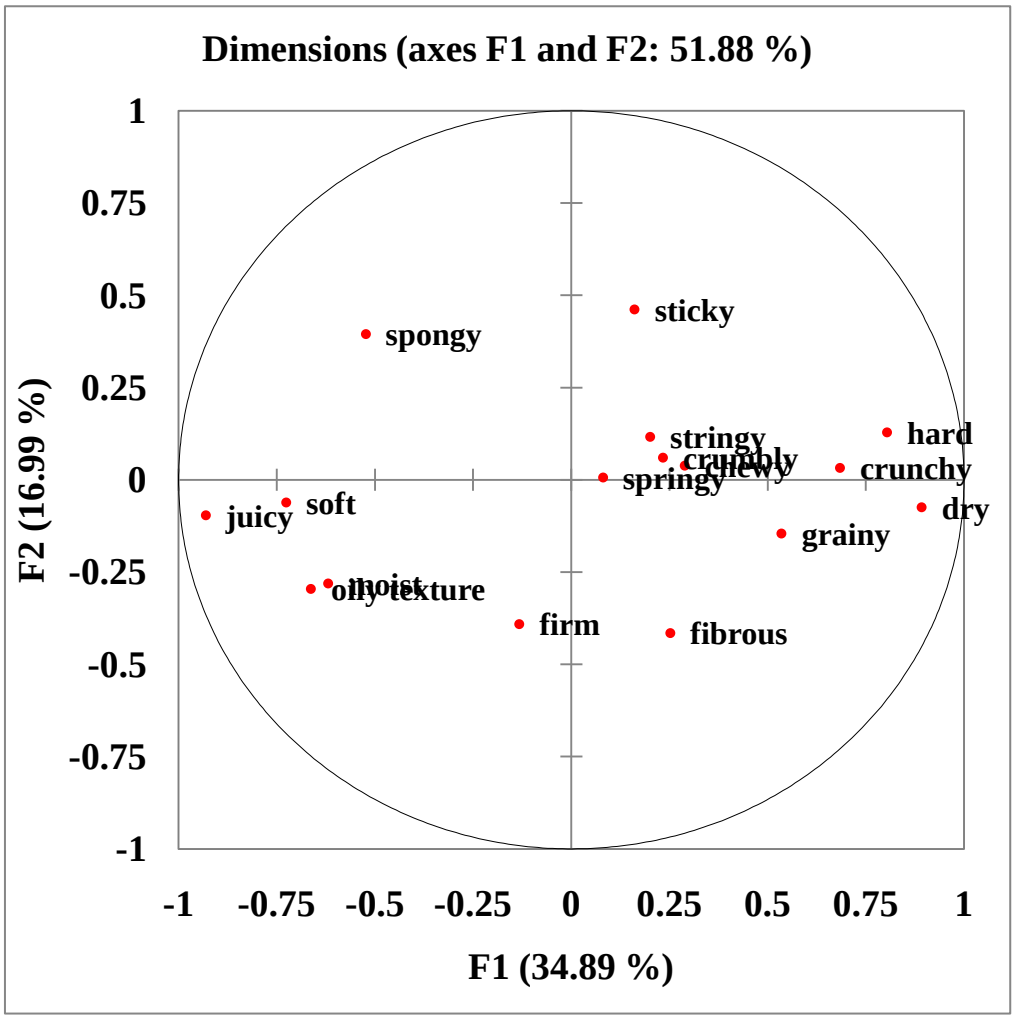
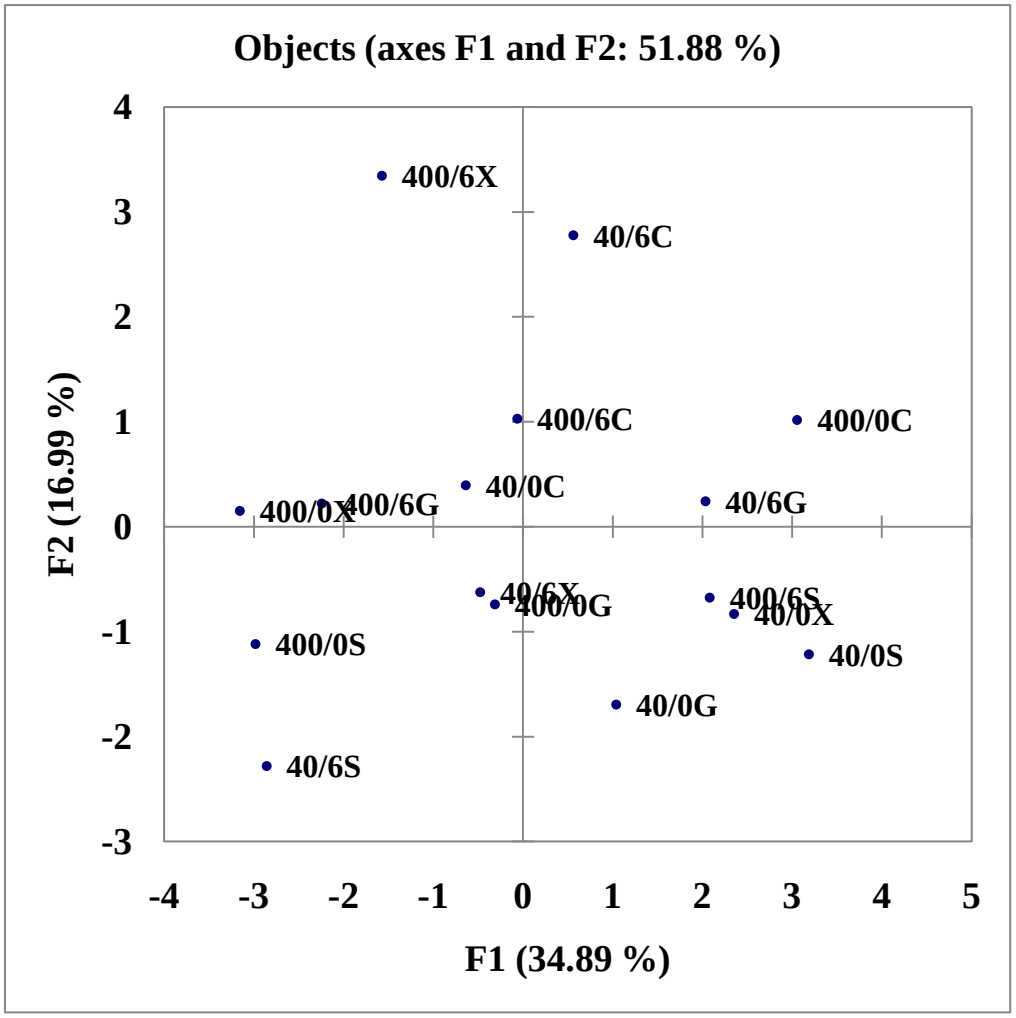


Figure 3.8.12. GPA plot of the samples for the texture of cooked pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

Figure 3.8.13. GPA plot of the texture attributes of warmed over (reheated) pork meat patties

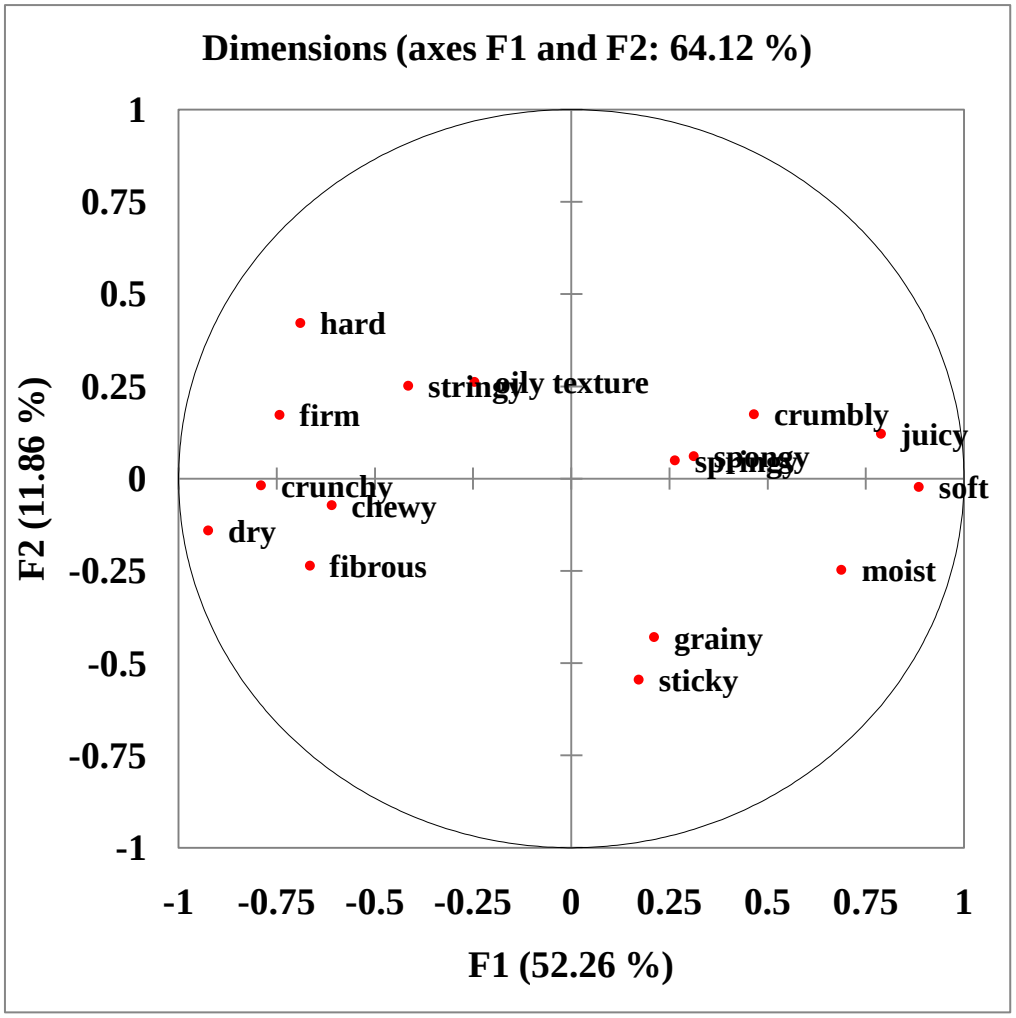
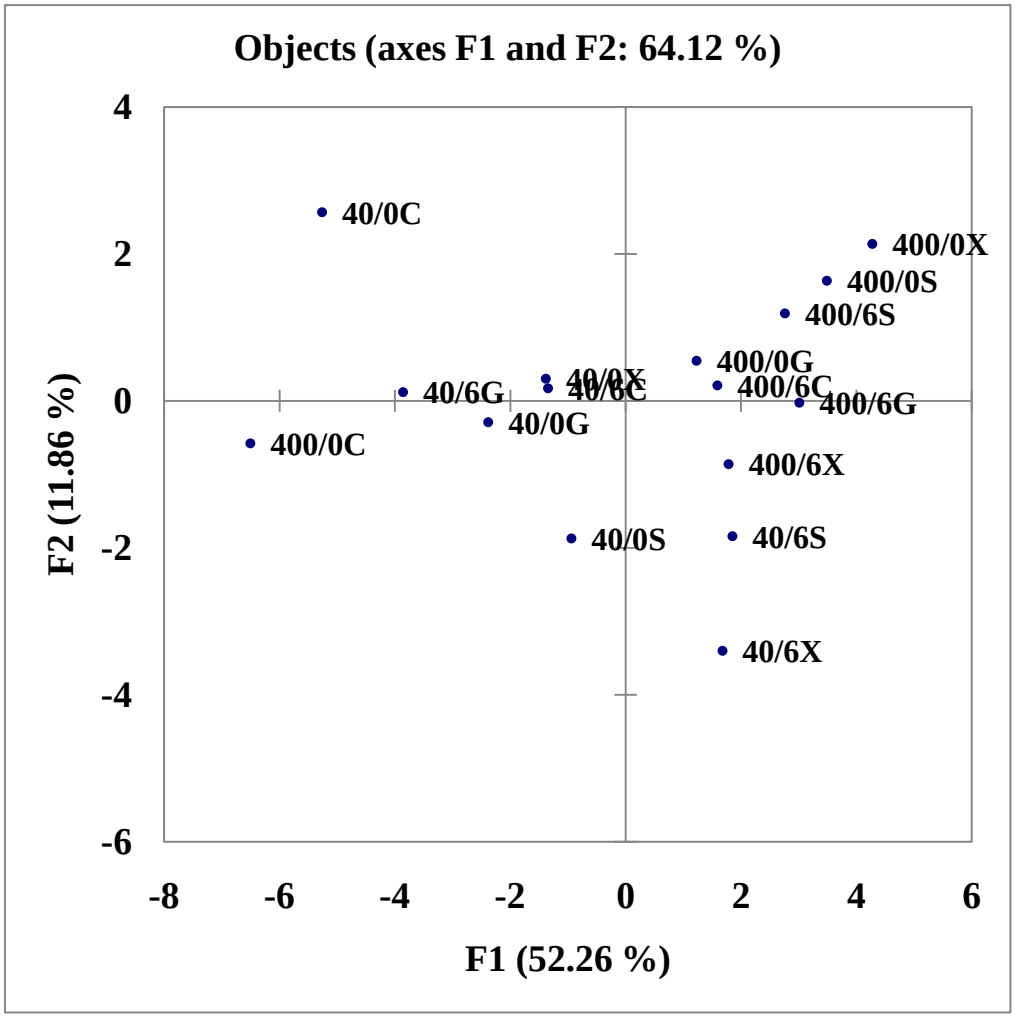


Figure 3.8.14. GPA plot of the samples for the texture of warmed over (reheated) pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

Figure 3.8.15. GPA plot of the flavour attributes of cooked pork meat patties

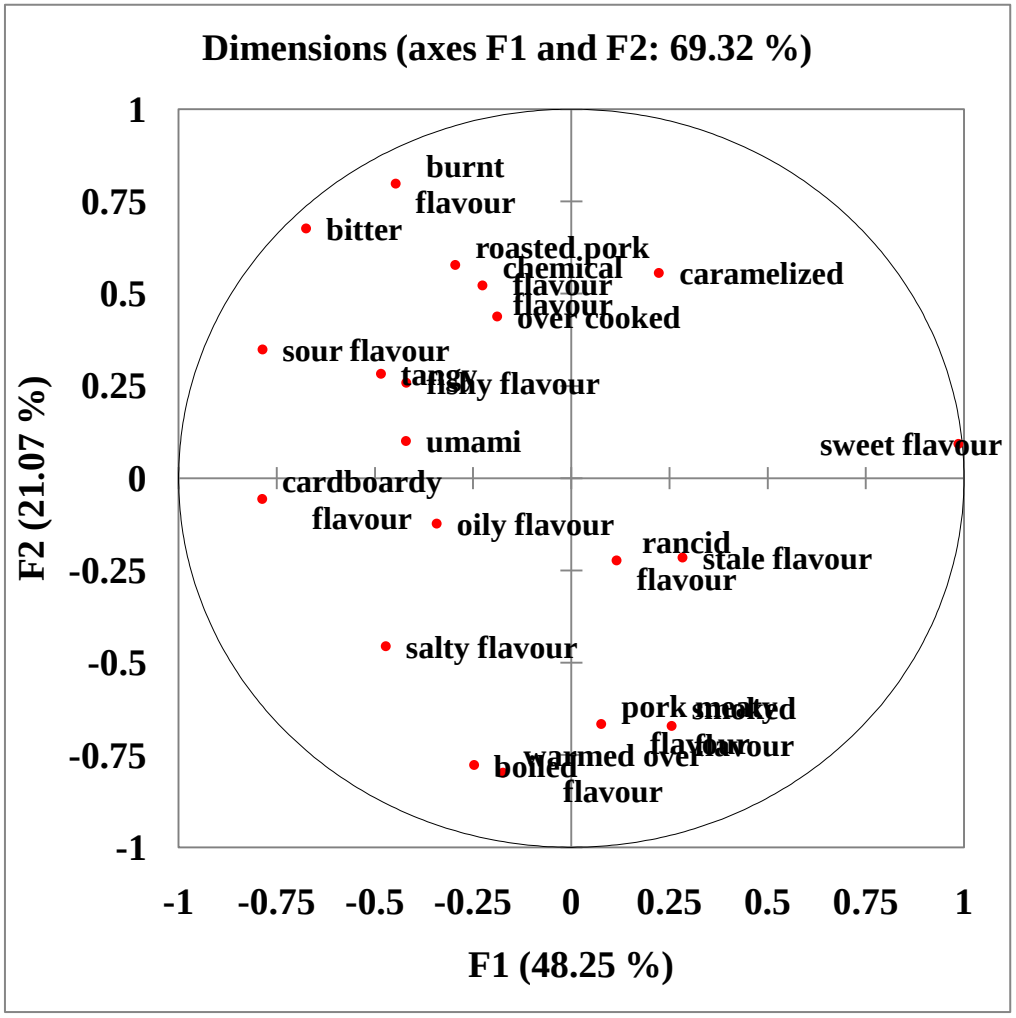
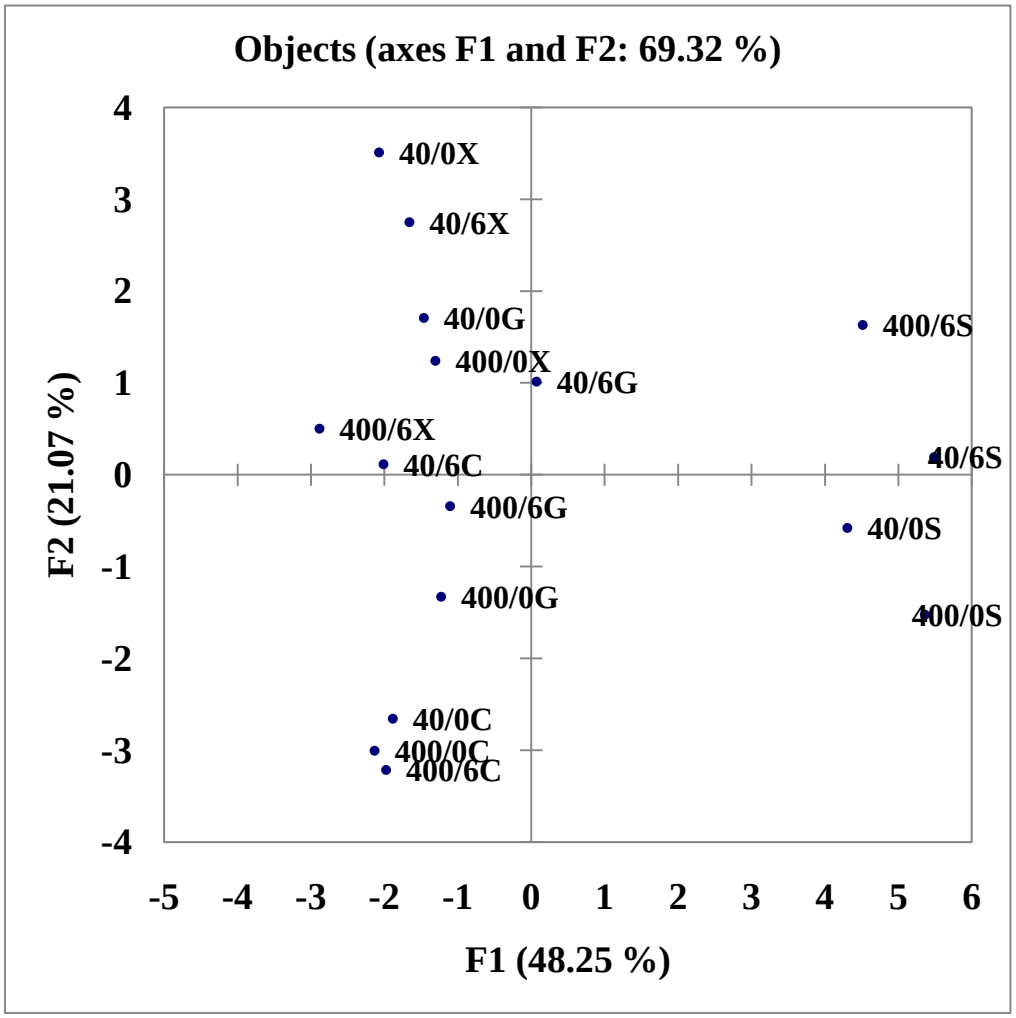


Figure 3.8.16. GPA plot of the samples for the flavour of cooked pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

Figure 3.8.17. GPA plot of the flavour attributes of warmed over (reheated) pork meat patties

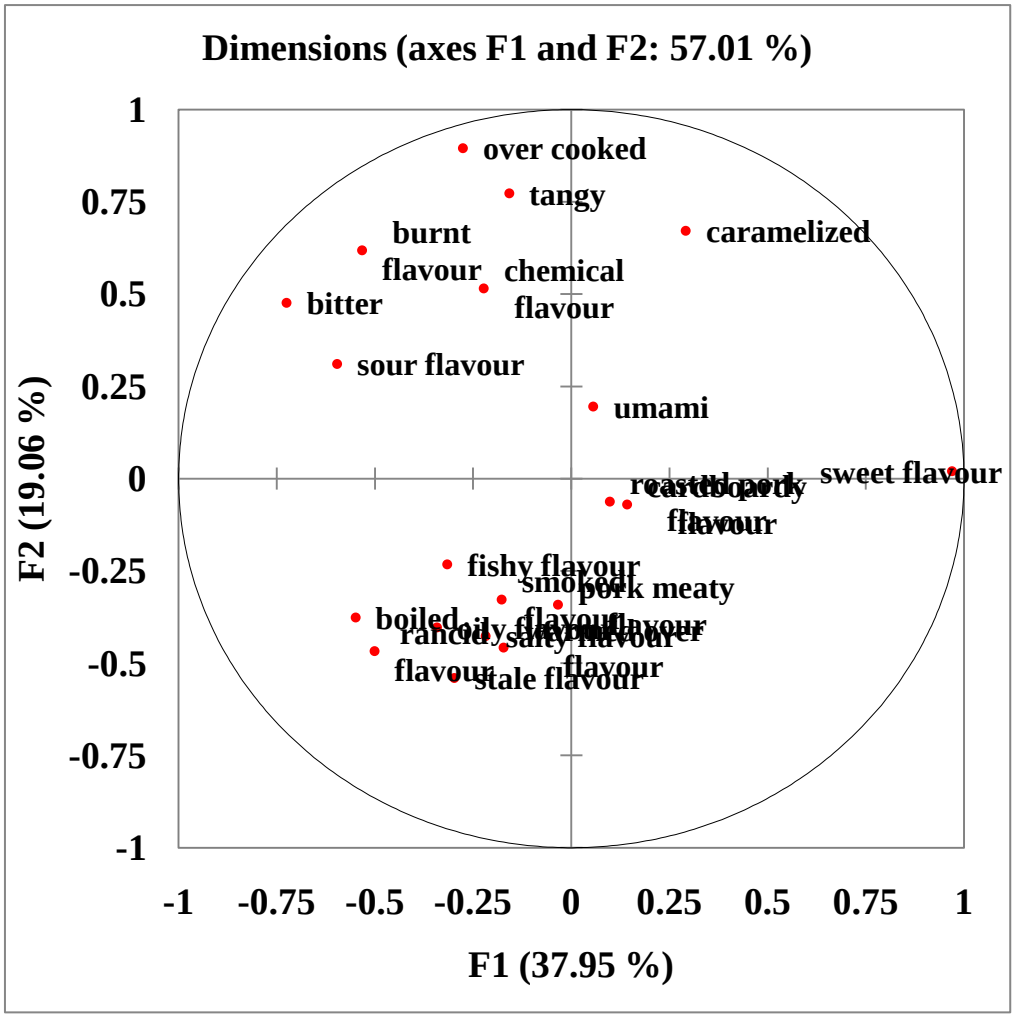
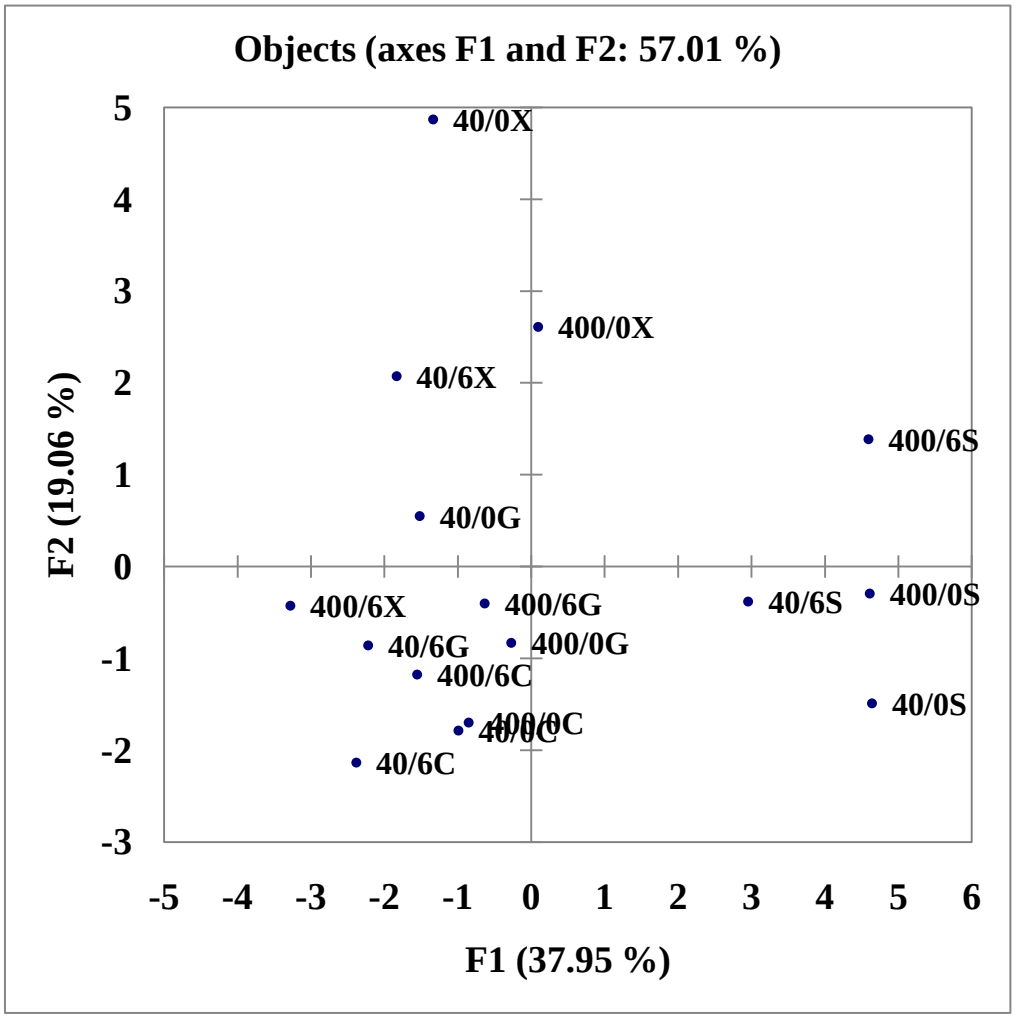


Figure 3.8.18. GPA plot of the samples for the flavour of warmed over (reheated) pork meat patties



40/0 – 40mg/kg vitamin E and no flax
40/6 – 40mg/kg vitamin E and 10% flax for 6 weeks
400/0 – 400mg/kg vitamin E and no flax
400/0 – 400mg/kg vitamin E and 10% flax for 6 weeks
X – Xylose; G – Glucose; S – Sucrose; C – Control

3.9. Bibliography

Ahn, D. U., Jo, C., Du, M., Olson, D. G., & Nam, K. C. (2000). Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Science, 56*(2), 203-209.

Aliani, M., & Farmer, L. J. (2005). Precursors of chicken flavour. II. identification of key flavour precursors using sensory methods. *Journal of Agricultural Food Chemistry, 53*(16), 6455-6462.

Bailey, M. E. (1988). Inhibition of warmed-over flavour, with emphasis on maillard reaction products. *Food Technology, 42*(6), 123-126.

Bedinghaus, A. J., & Ockerman, H. W. (1995). Antioxidative maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *Journal of Food Science, 60*(5), 992-995.

Benjakul, S., Lertittikul, W., & Bauer, F. (2005). Antioxidant activity of maillard reaction products from a porcine plasma protein-sugar model system. *Food Chemistry, 93*(2), 189-196.

Bousquet, M., Saint-Pierre, M., Julien, C., Salem Jr, N., Cicchetti, F., & Calon, F. (2008). Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of parkinson's disease. *The FASEB Journal, 22*(4), 1213-1225.

Brands, C. M. J., & van Boekel, M. A. J. S. (2002). Kinetic modeling of reactions in heated monosaccharide− casein systems. *Journal of Agricultural Food Chemistry, 50*(23), 6725-6739.

Bryhni, E. A., Kjos, N. P., Ofstad, R., & Hunt, M. (2002). Polyunsaturated fat and fish oil in diets for growing-finishing pigs: Effects on fatty acid composition and meat, fat, and sausage quality. *Meat Science, 62*(1), 1-8.

Byrne, D. V., Bak, L. S., Bredie, W. L. P., Bertelsen, C., & Martens, M. (1999). Development of a sensory vocabulary for warmed-over flavour: Part I. in porcine meat. *Journal of Sensory Studies, 14*(1), 47-65.

Byrne, D. V., Bredie, W. L. P., Bak, L. S., Bertelsen, G., Martens, H., & Martens, M. (2001a). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science, 59*(3), 229-249.

Byrne, D. V., O'Sullivan, M. G., Dijksterhuis, G. B., Bredie, W. L. P., & Martens, M. (2001b). Sensory panel consistency during development of a vocabulary for warmed-over flavour. *Food Quality and Preference, 12*(3), 171-187.

Campo, M. M., Nute, G. R., Wood, J. D., Elmore, S. J., Mottram, D. S., & Enser, M. (2003). Modelling the effect of fatty acids in odour development of cooked meat in vitro: Part I--sensory perception. *Meat Science, 63*(3), 367-375.

Delahunty, C. M., McCord, A., O'Neill, E. E., & Morrissey, P. A. (1997). Sensory characterisation of cooked hams by untrained consumers using free-choice profiling. *Food Quality and Preference, 8*(5-6), 381-388.

Deppe, C., Carpenter, R., & Jones, B. (2001). Nested incomplete block designs in sensory testing:: Construction strategies. *Food Quality and Preference, 12*(5-7), 281-290.

D'Souza, D. N., Mullan, B. P., & Dunshea, F. R. (2005). Pork as a functional food. In Manipulating pig production X. Proceedings of the Tenth Biennial Conference of the Australasian Pig Science Association (APSA), held in Christchurc, New Zealand, 27th to 30th November, 2005 pp. 256-267.

Elmore, J. S., Campo, M. M., Enser, M., & Mottram, D. S. (2002). Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. *J.Agric.Food Chem, 50*(5), 1126-1132.

Farmer, L. J., Hagan, T. D. J., & Paraskevas, O. (1999). Role of selected precursors in meat flavour formation. In XiongYL, HoC-T, Shahidi F, Editors. Quality Attributes of Muscle Foods. Kluwer Academic /Plenum Publishers New York.159-172.

Gogus, U., & Smith, C. (2010). n-3 omega fatty acids: A review of current knowledge. *International Journal of Food Science and Technology, 45*(3), 417-436.

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika, 40*(1), 33-51.

Guo, Q., Richert, B. T., Burgess, J. R., Webel, D. M., Orr, D. E., Blair, M., et al. (2006). Effect of dietary vitamin E supplementation and feeding period on pork quality. *Journal of Animal Science, 84*(11), 3071-3078.

Health Canada. (1998).Policy paper-Nutraceuticals /functional foods and health claims on foods. Retrieved from http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/nutra-funct_foods-nutra-fonct_aliment-eng.php on February 7, 2011

He, M. L., Hollwich, W., & Rambeck, W. A. (2002). Supplementation of algae to the diet of pigs: A new possibility to improve the iodine content in the meat. *Journal of Animal Physiology and Animal Nutrition, 86*(3-4), 97-104.

Hodge, J. E., Mills, F. D., & Fisher, B. E. (1972). Compounds of browned flavour derived from sugar-amine reactions. *Cereal Science Today, 17*(2), 34-40.

Hudson, J. E., & Loxley, R. A. (1983). The effect of pentose sugars on the aroma and flavour of mutton. *Food Technology in Australia, 83*, 174-175.

Ian Givens, D., & Gibbs, R. A. (2008). Current intakes of EPA and DHA in european populations and the potential of animal-derived foods to increase them. *Proceedings of the Nutrition Society, 67*(03), 273-280.

Jack, F. R., & Piggott, J. R. (1992). Free choice profiling in consumer research. *Food Quality and Preference, 3*(3), 129-134.

Jing, H., & Kitts, D. D. (2002). Chemical and biochemical properties of casein-sugar maillard reaction products. *Food and Chemical Toxicology, 40*(7), 1007-1015.

Jing, H., & Kitts, D. D. (2004). Antioxidant activity of sugar-lysine maillard reaction products in cell free and cell culture systems. *Archives of Biochemistry and Biophysics, 429*(2), 154-163.

Kim, J. S., & Lee, Y. S. (2010). Characteristics and antioxidant activity of maillard reaction products from fructose-glycine oligomer. *Food Science and Biotechnology, 19*(4), 929-940.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., & Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science, 81*(8), 1967-1979.

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science, 3*(1), 1-10.

Lanari, M. C., Schaefer, D. M., & Scheller, K. K. (1995). Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science, 41*(3), 237-250.

Laroque, D., Inisan, C., Berger, C., Vouland, É., Dufossé, L., & Guérard, F. (2008). Kinetic study on the maillard reaction. consideration of sugar reactivity. *Food Chemistry, 111*(4), 1032-1042.

Lauridsen, L., Miklos, R., Schäfer, A., Aaslyng, M. D., & Bredie, W. L. P. (2006). Influence of added carbohydrates on the aroma profile of cooked pork. *Developments in Food Science, 43*, 355-358.

Lawless, H. T., & Heymann, H. (1999). *Sensory evaluation of food: Principles and practices* Aspen Publishers.

Lertittikul, W., Benjakul, S., & Tanaka, M. (2007). Characteristics and antioxidative activity of maillard reaction products from a porcine plasma protein-glucose model system as influenced by pH. *Food Chemistry, 100*(2), 669-677.

MacFie, H. J., Bratchell, N., Greenhoff, K., & Vallis, L. V. (1989). Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies, 4*(2), 129-148.

Mcdonald, R. E., & Hultin, H. O. (1987). Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. *Journal of Food Science, 52*(1), 15-21.

Meadus, W. J., Duff, P., Uttaro, B., Aalhus, J. L., Rolland, D. C., Gibson, L. L., et al. (2010). Production of docosahexaenoic acid (DHA) enriched bacon. *Journal of Agricultural and Food Chemistry, 58*(1), 465-472.

Meinert, L., Schäfer, A., Bjergegaard, C., Aaslyng, M. D., & Bredie, W. L. P. (2009). Comparison of glucose, glucose 6-phosphate, ribose, and mannose as flavour precursors in pork; the effect of monosaccharide addition on flavour generation. *Meat Science, 81*(3), 419-425.

Morrissey, P. A., Buckley, D. J., Sheehy, P. J. A., & Monahan, F. J. (1994). Vitamin E and meat quality. *Proceedings of the Nutrition Society, 53*(02), 289-295.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science, 49*, S73-S86.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S., & Whitfield, F. B. (1995). Maillard-lipid interactions in nonaqueous systems: Volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *Journal of Agricultural and Food Chemistry, 43*(5), 1302-1306.

Murray, J. M., Delahunty, C. M., & Baxter, I. A. (2001). Descriptive sensory analysis: Past, present and future. *Food Research International, 34*(6), 461-471.

Musella, M., Cannata, S., Rossi, R., Mourot, J., Baldini, P., & Corino, C. (2009). Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. *Journal of Animal Science, 87*(11), 3578-3588.

Nam, K. C., & Ahn, D. U. (2003). Use of antioxidants to reduce lipid oxidation and off-odor volatiles of irradiated pork homogenates and patties* 1. *Meat Science, 63*(1), 1-8.

Naranjo, G. B., Malec, L. S., & Vigo, M. (1998). Reducing sugars effect on available lysine loss of casein by moderate heat treatment. *Food Chemistry, 62*(3), 309-313.

Nielsen, J. H., Sørensen, B., Skibsted, L. H., & Bertelsen, G. (1997). Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Science, 46*(2), 191-197.

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G., et al. (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science, 70*(1), 63-74.

Oreskovich, D. C., Klein, B. P., & Sutherland, J. W. (1991). Procrustes analysis and its applications to free-choice and other sensory profiling. *LAWLESS, HT; KLEIN, BP Sensory Science Theory and Applications in Foods.New York, Marcel Dekker*, 353-394.

Santos, C., Hoz, L., Cambero, M. I., Cabeza, M. C., & Ordóñez, J. A. (2008). Enrichment of dry-cured ham with [alpha]-linolenic acid and [alpha]-tocopherol by the use of linseed oil and [alpha]-tocopheryl acetate in pig diets. *Meat Science, 80*(3), 668-674.

SAS Institute Inc.2009.SAS User's guide:Statistics.SAS for Windows,Version 9.2 SAS Institute,Inc.,Cary,NC.

Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poultry Science, 79*(7), 961-970.

Spark, A. A. (1969). Role of amino acids in non‐enzymic browning. *Journal of the Science of Food and Agriculture, 20*(5), 308-316.

Tims, M. J., & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technology, 12*(5), 240-243.

Wijewickreme, A. N., & Kitts, D. D. (1997). Influence of reaction conditions on the oxidative behavior of model maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*(12), 4571-4576.

Williams, A. A., & Langron, S. P. (1984). The use of free‐choice profiling for the evaluation of commercial ports. *Journal of the Science of Food and Agriculture, 35*(5), 558-568.

Yashodhara, B. M., Umakanth, S., Pappachan, J. M., Bhat, S. K., Kamath, R., & Choo, B. H. (2009). Omega-3 fatty acids: A comprehensive review of their role in health and disease. *Postgraduate Medical Journal, 85*(1000), 84-90.

Young, O. A., & Cummings, T. L. (2008). Effect of xylose on sheepmeat flavours in Casserole-Style cooking. *Journal of Food Science, 73*(6), S308-S313.

Young, O. A., Cummings, T. L., & Binnie, N. S. (2009). Effect of several sugars on consumer perception of cured sheepmeat. *Journal of Food Science, 74*(5), S198-S204.

Chapter 4

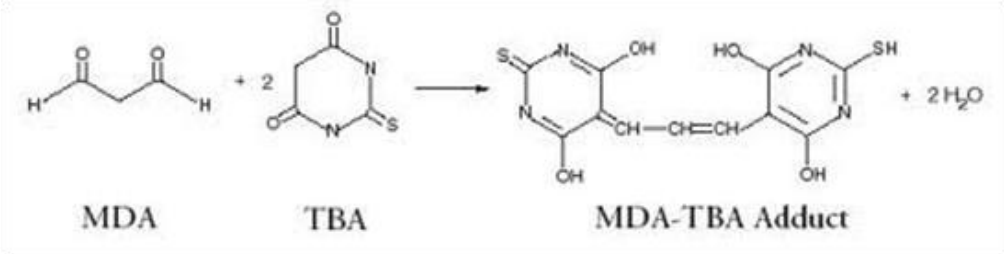
Interference of exogenous sugars during the spectrophotometric estimation of thiobarbaturic acid reactive substances (TBARS) in cooked and reheated pork meat patties

4.1. Introduction

Addition of food grade sugars may improve the oxidative stability and sensory quality of *n*-3 enriched meat due to the involvement of sugars in the Maillard reaction, which may generate reaction products that have antioxidant properties (Mottram, 1998). The thiobarbituric acid reactive substances (TBARS) assay is widely used to estimate the extent of lipid oxidation in raw or cooked meats (Guillen-Sans & Guzman-Chozas, 1998; Raharjo et al., 1993) and is considered to be a fast, sensitive and low cost method (Shlafer & Shepard, 1984). The TBARS assay involves thiobarbituric acid (TBA) reacting with malonaldehyde (MDA), which is a secondary reaction product of lipid peroxidation, thereby generating a TBA-MDA adduct that produces a red colour that can be detected spectrophotometrically (Tarladgis et al., 1960). In the early 1950's, Keeney & Doan (1951) observed that a three carbon compound arising from the oxidation of mono- and poly-enoic fatty acids was producing this red colour and Patton & Kurtz (1951) identified the compound as malonaldehyde based on spectral curves.

The TBARS assay method varies significantly between laboratories and the assay as it is performed on foods may be divided into four types according to Hoyland & Taylor (1991): "1.whole sample; 2. acid extract of the sample; 3. steam distillate of the sample; 4. lipid extract of the sample". The TBARS assay involves heating the sample and adding strong acid for extraction of the malonaldehyde and its reaction with TBA so that maximum colour development is achieved (Patton & Kurtz, 1951; Sinnhuber & Yu, 1958; Tarladgis et al., 1960). Research conducted to study the conditions affecting colour development within the TBARS assay has however yielded conflicting results. Tarladgis et al. (1964) stated that maximum colour development could be obtained after 15 h of incubation using an un-acidified aqueous extract and that the acid-heat treatment only served to accelerate the

reaction. Salih et al. (1987) used an aqueous extraction method and incubated samples at room temperature for 15-17 h and compared this to incubation at boiling temperature for 30 min and stated that there was no significant difference in the colour formed or on the estimates of lipid oxidation. However, using the same reaction conditions, Pikul et al. (1989) obtained TBARS values 1.4 times higher than that obtained by Salih et al. (1987) when incubated at a boiling temperature. According to Guillen-Sans & Guzman-Chozas (1998), during the course of TBARS a yellow pigment will be formed that absorbs at 450 nm followed by orange pigment at 495 nm and then a red pigment that has a maximum absorption at 532 nm. Even though different theories exist regarding the TBARS reaction and the absorption spectra, colour development during the TBA test is usually assessed by measuring the absorbance of the pink chromagen between 530 and 537 nm (Melton, 1983) and maximum absorption of MDA-TBA adduct corresponds to the red pigment formation at 532 nm (Janero et al., 1990). The extent of oxidation is reported as the TBA value and is expressed as milligrams of MA equivalents per kilogram sample or as micromoles of MA equivalents per gram of sample. It must, however, be noted that other lipid oxidation products such as alkenals and alkadienals also react with the TBA reagent and produce a pink color. Thus, the term thiobarbituric acid reactive substances (TBARS) is now used instead of malonaldehyde.



Even though the reaction between malonaldehyde and TBA indicates the oxidation of fats in foods, there are some caveats when using the TBARS assay for this purpose. TBA can also react with other compounds present in foods such as sugars, which can interfere with the spectrophotometric measurements of the MDA-TBA complex (Baumgartner et al., 1975; Wang et

al., 2002). Apart from sugars, other compounds that may react with TBA and cause interference in the TBARS assay readings are acids, esters, amides and pyrimidine compounds (Guillen-Sans & Guzman-Chozas, 1998). These interferences could produce false readings of lipid oxidation as reaction products of sugars with TBA cannot be differentiated from malonaldehyde adducts and so may erroneously be measured as derivatives of lipid peroxidation rather than artefactual compounds (Shlafer & Shepard, 1984). The reaction of sugars with TBA to yield different coloured substances in different reaction systems has been studied (Du & Bramlage, 1992; Guillen-Sans & Guzman-Chozas, 1998; Shlafer & Shepard, 1984; Wang et al., 2002), but the level of sugar that is needed to cause interference is not clear. Baumgartner et al. (1975); Biggs & Bryant (1953); Wertheim & Proctor (1956) in their studies found that sugars reacted with TBA and produced a yellow colour. These researchers demonstrated the formation of yellow colours with galactose, maltose, dextrose, fructose, lactose and sucrose when reacted with TBA in their studies using milk and milk products. Further, Salih et al. (1987) found production of yellow colour by sugars and observed interference from yellow colouring pigment during TBARS assay when sugar was added to poultry meat.

Different approaches have been proposed to avoid interference of sugars in the colorimetric measurement of TBARS. Sugars that react with TBA produce compounds that have an absorption band located between 450 and 460 nm (Yu & Sinnhuber, 1962), which may allow chromatographic separation of the interfering yellow colour from the pink chromagen before estimation of the TBARS. Asakawa et al. (1975) stated that addition of sodium sulphite prevented the production of the yellow chromagen and enhanced the development of the pink chromagen. According to Angelo et al. (1987), the yellow colour disappeared with the addition of sodium hydroxide. Addition of sodium nitrite to TBA was also shown to reduce the yellow pigment formation and increase formation of the red adduct (Asakawa et al., 1975). Raharjo et al. (1993) tried to remove the interfering substances by filtering the extract through a C18 cartridge before assessing TBARS, while Shlafer & Shepard (1984) successfully corrected for the interference of sucrose in the reactions by using a reagent blank containing sucrose.

Another approach to separate sugar derivatives from the TBA-malonaldehyde adduct is by changing the reaction parameters, thereby avoiding the interference produced by sugars. Wang et al. (2002) proposed TBARS estimation using 80mM of TBA at an incubation temperature of 40°C for 90 min and found that even 10% sucrose added to beef did not produc interference in the TBARS assay readings. In the present study, 2% food grade sugars (glucose, sucrose and xylose) were added to pork meat grinds to study the effects of the addition of food grade sugars on lipid oxidative stability of *n*-3 enriched pork meat patties. However there is much confusion regarding the amount of interference produced by sugars in the TBARS assay and modification of the reaction conditions could possibly overcome the interference, if any, produced.

The objective of the study was to see whether sugars added at the level of 2% to the meat grinds in this study caused interference in the colorimetric readings of TBARS assay performed as per the procedure outlined by McDonald & Hultin (1987) and Nielsen et al. (1997) and whether modification of the reactions conditions was needed. The hypothesis tested was that addition of sugars at the level of 2% is not causing interference in the TBARS assay when performed as per the procedure outlined by McDonald & Hultin (1987) and Nielsen et al (1997) and that no modification is needed.

4.2. Materials and methods

A series of experiments were performed to test the efficacy of the TBARS assay under different assay reaction conditions (Tables 4.2.1 to 4.2.4). Incubation time, temperature and concentration of TBA were the parameters chosen as the variables and the reaction conditions were optimized by animal diet and the type of sugar added.

Table 4.2.1. Design for testing of effect of sugar type on TBARS assay conditions for Experiment 1 for fresh cooked patties

TBA concentration (mM)	Incubation time (minutes)	TBARS incubation temperature (°C)
60	60, 90, 120, 150	40
80	60, 90, 120, 150	40

Table 4.2.2. Design for testing of effect of sugar type on TBARS assay conditions for Experiment 2 for re-heated patties

TBA concentration (mM)	Incubation time (minutes)	Incubation temperature (°C)
20	60, 90, 120, 150, 240	40
40	60, 90, 120, 150, 240	40
60	60, 90, 120, 150, 240	40

Table 4.2.3. Design for testing of effect of sugar type on TBARS assay conditions for Experiment 3

TBA concentration (mM)	Incubation time (minutes)	Incubation temperature (°C)
20	20, 40, 60	94
40	20, 40, 60	94
60	20, 40, 60	94

Table 4.2.4. TBARS reaction conditions for measurement of absorption spectra obtained using sugars only

TBA concentration (mM)	Incubation time (minutes)	Incubation temperature (°C)
20	40	94
60	90	40

In the first experiment (Table 4.2.1), a single meat patty from each dietary vitamin E*flax*sugar treatment was prepared and cooked as per the procedure outlined in Sections 3.2.3 and 3.2.4 respectively. Immediately after cooking, meat patties were processed in a food processor (Robot Coupe USA Inc., Ridgeland MS) and homogenised for 10 sec. From this homogenate, 10 g were weighed into a 50 mL centrifuge tube. Thirty mL of 7.5% TCA (trichloracetic acid) were transferred by pipette into the 50 mL centrifuge tube. The mixture was then homogenized (Polytron Model 3100, Kinematica AG,Switzerland) for 30 sec and the homogenate filtered (Whatman # 4 filter paper, Fisher Scientific, Mississauga, ON) into a 50 mL centrifuge tube. Filtrate obtained from the patties was pooled by the type of the sugar present. 2.5 mL aliquots of the filtrate were transferred by pipette into eight 15 mL Pyrex screw top tubes for each sugar treatment. The eight 15 mL Pyrex screw

top tubes for each sugar treatment were then divided into two sets of four tubes and to one set of four tubes 2.5 mL of 60 mM thiobarbituric acid solution were added. To the remaining four tubes, 2.5 mL of 80 mM thiobarbituric acid solution were added. This was repeated for all the sugars, producing a total of 32 tubes. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario) and the samples incubated in a water bath (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) at 40°C. One tube from each set was removed from the water bath after 60, 90, 120 or 150 min and was subjected to sonication for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into a Gilson 222 XL auto-sampler (Mandel Scientific Company, Inc., Ontario, Canada) and the absorbance was measured spectrophotometrically at 532 nm (Ultrospec 3000, Pharmacia Biotech, Buckinghamshire, UK) (Figure 4.7.1).

A second experiment was conducted using reheated meat patties as per the reaction conditions outlined in table 4.2.2. A single re-heated meat patty from each dietary vitamin E*flax*sugar treatment was prepared, cooked and reheated as per the procedure outlined in 3.2.3 and 3.2.4 respectively. Sample filtrate was obtained from each patty as per the procedure outlined above. Filtrate obtained from the samples was pooled by the type of the sugar present in it. A 2.5 mL aliquot of the pooled filtrate was transferred by pipette into fifteen 15 mL Pyrex screw top tubes and the tubes were divided into three sets of five tubes for each sugar, for a total of 60 tubes. To each of the three sets of five tubes, 2.5mL of either 20, 40 or 60 mM thiobarbituric acid solution were added. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario) and the samples incubated in water bath (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) at 40°C. One tube each from each concentration of TBA was removed from the water bath at 60, 90, 120, 150 or 240 min and was subjected to sonication for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into a Gilson 222 XL autosampler (Mandel Scientific Company, Inc., Ontario, Canada) and the absorbance was

measured spectrophotometrically at 532nm (Ultrospec 3000, Pharmacia Biotech, Buckinghamshire, UK) (Figure 4.7.2).

A third experiment was conducted also using reheated meat patties as per the reaction conditions outlined in table 4.2.3. A single meat patty from each dietary vitamin E*flax*sugar treatment was prepared, cooked and reheated as per the procedure outlined in 3.2.3 and 3.2.4 respectively. Sample filtrate was obtained from each patty as per the procedure outlined above. Filtrate obtained from the samples was pooled by the type of the sugar present in it. Three sets of 15 mL Pyrex screw top tubes were prepared from filtrate for each sugar treatment, with each set containing three tubes each and all of them contained 2.5mL of the filtrate. For each sugar treatment, the first set of three tubes received 2.5mL of 20mM thiobarbituric acid solution, the second set 2.5 mL of 40 mM thiobarbituric acid solution, and the third set 2.5 mL of 60 mM thiobarbituric acid solution. This was repeated for patties from all the sugars, producing a total of 36 tubes. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario) and the samples incubated in water bath (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) at 94°C. One tube each from the three sets was removed from the water bath at 20, 40, or 60 min and was subjected to sonication for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into an Gilson 222 XL autosampler (Mandel Scientific Company, Inc., Ontario, Canada) and the absorbance was measured spectrophotometrically at 532nm (Ultrospec 3000, Pharmacia Biotech, Buckinghamshire, UK)(Figure 4.7.3). Simultaneously absorbance values at different wavelengths starting from 400 nm to 600 nm were measured spectrophotometrically (Ultraspec3000, Pharmacia Biotech, Buckinghamshire, UK) (Figure 4.7.4).

A fourth experiment was performed using sugar solutions. Absorption scans of the sugars were done without adding TBA. For this, a 2% aqueous solution of each respective sugar was made. Two sets of three 15 mL Pyrex screw top tubes were assembled into which 0.18 mL of each sugar solution was added in duplicate to the tubes so that each tube in each set contained a different sugar. The quantity of sugar needed was calculated based on a

relative approximation of the amount of sugar that might be present in the meat filtrate. To all the tubes, 2.32 mL of 7.5% TCA were added to make a final volume of 2.5 mL in all tubes. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario). One set of the tubes was incubated at 94°C for 40 min (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) and the second set of tubes was incubated at 40°C for 90 min. Following incubation, samples were subjected to sonication for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into an Gilson 222 XL autosampler (Mandel Scientific Company, Inc., Ontario, Canada) and the absorbance at different wavelengths starting from 450 nm to 600 nm was measured spectrophotometrically and was referenced against standard pink buffer solution incubated at 94°C for 40 min (Ultraspec 3000, Pharmacia Biotech, Buckinghamshire, UK) (Figure 4.7.5).

A fifth experiment was performed using sugar solutions after addition of thiobarbituric acid (Table 4.2.4). For this experiment, 2% aqueous solutions of the respective sugars were made. Two sets of four 15 mL Pyrex screw top tubes were assembled and 0.18 mL each of the sugar solution added in duplicate to the tubes so that each tube in each set contained a different sugar. To all the tubes, 2.32 mL of 7.5% TCA were added and control samples were included that contained only 2.5 mL of 7.5% TCA. To the first set of tubes, 2.5 mL each of 20 mM TBA were added. To the second set of tubes 2.5 mL each of 60 mM TBA were added. The tubes were capped and mixed by vortex (Fisher Scientific Digital Vortex mixer 945415, Fisher Scientific Company, Ottawa, Ontario). The set of the tubes into which 20 mM TBA was added were incubated at 94°C for 40 min (Precision reciprocal shaking water bath 66800, Thermo Scientific Company, Mississauga, ON) and the second set of tubes that contained 60 mM TBA was incubated at 40°C for 90 min. Following incubation, samples were sonicated for 10 sec (VWR Scientific Aquasonic 50T, Mississauga, ON). The samples were then poured into borosilicate glass test tubes and loaded into an Gilson 222 XL autosampler (Mandel Scientific Company, Inc., Ontario, Canada) and the absorbance at different wavelengths starting from 400 nm to 600 nm was measured

spectrophotometrically (Ultrospec3000, Pharmacia Biotech, Buckinghamshire, UK) (Figure 4.7.6).

4.3. Statistical analysis

There were not enough replicates for a sound statistical analysis. Further due to limitations in the availability of grind samples, dietary vitamin E and flax treatment effects was not assessed. A speculative approach comparing absorbance values from pooled experimental samples based on the type of sugar present were done. Absorbance values noted using the different reaction parameters (concentration of TBA, incubation time and temperature) were plotted and compared among sugar treatments in the first, second and third set of experiments using meat patties. The absorbance measurements using sugar solutions were compared with or without addition of TBA in the fourth and fifth set of experiments respectively.

4.4. Results

Absorbance values obtained for the cooked meat patties when subjected to the experiment conditions as per the reaction parameters given in table 4.2.1 are shown in Figure 4.7.1. Reaction parameters of 80 mM thiobarbituric acid, incubation temperature of 40°C and incubation time of 90 min as proposed by Wang et al. (2002) were tested. Absorbance at 532 nm after an incubation time of 60 min, 90, 120 or 150 min at 40°C at thiobarbituric acid concentration of either 60 mM or 80 mM showed that absorbance maximums were reached after 90 min of incubation regardless of TBA concentrations when incubated at 40°C and were unaffected by the presence of sugar or its type. Increasing the concentration of TBA to 80mM tended to decrease the absorbance values, regardless of sugar type added (Figures 4.7.1).

Results of the second experiment in which re-heated pork patties were used (Table 4.2.2) are plotted in Figure 4.7.2. Absorbance at 532 nm after an incubation time of 60 min, 90, 120, 150 and 240 min at 40°C at thiobarbituric acid concentration of 20 mM, 40 mM and 60 mM showed that 60 mM TBA incubated at 40°C for 90 min gave the maximum absorbance regardless of sugar type added and absorbance values were very similar to those of the 40mM TBA samples incubated at 40°C for 90 min (Figure 4.7.2).

Absorbance values from the third experiment with reheated meat patties subjected to the reaction parameters given in table 4.2.3 are shown in Figure 4.7.3. Reaction parameters proposed by McDonald & Hultin (1987) and Nielsen et al. (1997) using 20 mM TBA incubated at 94°C for 40 min were tested. Absorbance values compared at TBA concentrations of 20, 40 and 60 mM after incubation for 20, 40 or 60 min showed little difference in absorbance due to TBA concentration and incubation time for meat patties containing glucose, sucrose or no sugar (control); (Figure 4.7.3). However samples containing xylose showed increased absorbance values at 40 and 60 mM TBA after 40 min incubation.

Spectral absorbance values of filtrate from the reheated meat patties from 400 nm to 600 nm are shown in Figure 4.7.4. Absorbance values for xylose appeared much higher than those of other sugars. Maximum absorbance at 532 nm was obtained for control and glucose patties under the reaction conditions of 20 mM TBA with incubation at 94°C for 40 min (Figure 4.7.4).

Absorbance values of the different sugars observed between wavelengths from 400 nm to 600 nm are shown in Figure 4.7.5. In the absence of TBA, sugars did not produced any absorbance when incubated at either of the reaction conditions using incubation temperature at 94°C for 40 min or 40°C for 90 min. Figure 4.7.6 shows the absorbance values observed at a range of wavelength from 400 nm to 600 nm when sugars were treated with TBA. Irrespective of the sugars present in the reaction mixture, two distinct peaks were identified at 450 nm and at 532 nm.

4.5. Discussion

Measurement of thiobarbituric acid reacting substances (TBARS assay) is considered to be an easy, fast and reliable method for the estimation of lipid oxidation (Shlafer & Shepard, 1984). Sugars added to meat could interfere with the colorimetric readings in the TBARS assay that are used for measuring the lipid oxidation (Guillen-Sans & Guzman-Chozas, 1998; Wang et al., 2002). The reaction principle in TBARS assay is that TBA will react with malonaldehyde and produce a red adduct that is measured colorimetrically at 532nm. Different types of sugars may react differently with TBA and could produce substances visible at multiple wavelengths that

overlap with the spectrum of the TBA-MDA adduct and could produce false results. How sugars react under various conditions as well as the extent of interference they produce is still not exactly known, but because they could interfere in the assay, it is quite important to determine whether addition of sugars to the pork patties is interfering in the TBARS assay leading to incorrect results.

The sequence of development of colour in the TBARS assay had been studied and the development of yellow, orange and red pigment formation during the course of reaction documented (Guillen-Sans & Guzman-Chozas 1998). The yellow pigment will peak at 450 nm, the orange pigment at 495 nm and the red at 530 nm (Guillen-Sans & Guzman-Chozas, 1998). In the studies conducted by Wang et al. (2002) with sucrose added at 10%, a yellow colour was formed which had a maximum absorbance at 450 nm and the intensity of the coloration was high enough that the absorbance spectrum overlapped with the red colour absorption spectrum at 532 nm, allowing it to interfere with TBARS measurements at 532 nm. The reaction between TBA and MDA could take place in a wide temperature scale ranging from 20°C to 100°C (Witte et al., 1970) with the reaction slowed at low temperature (Wang et al., 2002). According to Wang et al. (2002) the intensity of the yellow colour formed was dependent on the incubation temperature with the yellow colour evident when the incubation temperature was above 50°C, but it ceased to be relevant at an incubation temperature of 40°C. There was intense yellow colouration when the incubation temperature was increased and the tail of the absorption spectrum from the yellow colour overlapped with the peak of the absorption spectrum of the pink color when the incubation temperature was 100°C. This most likely was the reason for Wang et al. (2002) to suggest that the optimum incubation temperature in order to avoid interference from sugars, especially at high concentrations, was 40°C.

Based on the testing done in this study on the procedures outlined by McDonald & Hultin (1987) & Nielsen et al. (1997) and Wang et al. (2002) using different reaction conditions, maximum absorbance at 532 nm was obtained using the reaction conditions outlined by McDonald & Hultin (1987) & Nielsen et al. (1997) using 20 mM TBA and an incubation temperature of 94°C for 40 min.

In the present study, however, the absorbance and interferences from sugars differed from those noted by Wang et al. (2002). When the samples were incubated at 94°C, there appeared to be a reduction in the yellow colour (absorbance at 450nm) as well as an increase in the red (pink) colour (absorbance at 532nm). This was evident even in the control samples as well as in the samples containing sugars (Figure 4.7.6). Also, Wang et al. (2002) stated that the yellow colour ceased to form at 40°C while in the present study it appeared to form more at 40ºC than when incubated at 94°C. The reason for the difference in the results at 40°C incubation is not clear and could be due to the high concentration of sugar (10%) used by Wang et al. (2002).

According to Guillen-Sans & Guzman-Chozas (1998), when the reaction mixture was incubated at 94°C for 10 to 50 min, the orange and red pigments increased and the yellow pigment reduced as the yellow pigment was heat labile after 60 min. Reduction of the yellow pigment (absorbance at 450 nm) was clearly demonstrated in the scans from the present study (Figure 4.7.6). Because we wanted to reduce the yellow coloration produced by sugar and also ensure that the yellow coloration produced did not overlap with the red (pink) absorption spectra at 532 nm, incubation at a high temperature (94°C) was tested. In Figure 4.7.4, which shows the spectral scans of xylose at different incubation times and TBA concentrations at 94°C, it was apparent that when high concentrations of TBA (40 and 60 mM) were incubated for 40 and 60 min, there was a shift in the peak at 530 nm over to the 500 nm range, which could have been due to the orange pigment. In the case of the control (Figure 4.7.4) and glucose (Figure 4.7.4) samples the optimum assay conditions appeared to be 20 mM for 40 min as there did not appear to be formation of the orange pigment and no shift of the peak was noted. In the case of the sucrose (Figure 4.7.4) samples, the shift was not as obvious as for xylose. This colour development pattern at high TBA concentration may be due to the fact that in the presence of an excess TBA to MDA there could be a blend of yellow, orange and red pigments obtained (Guillen-Sans & Guzman-Chozas, 1998). For optimum assay condition, red pigment formation has to be increased and yellow pigment formation has to be decreased, hence incubation at 94°C for 40 min appeared to be the ideal assay conditions.

The present study focussed on the absorbance values obtained when the meat samples containing sugars were subjected to the experiments based on the procedures outlined by McDonald & Hultin (1987) & Nielsen et al. (1997) and Wang et al. (2002) under different reaction conditions outlined above. The scope and validity of the results illustrated were based on the absorbance values obtained from pooled experimental samples. Hence experimental variation within filtrate and within assay was not captured as duplicate samples were not included in each assay. As a result, there was insufficient replication for statistical analysis. However, the actual TBARS results of the cooked meat samples conducted with appropriate statistically sound experimental design is reported in section 3.4.2.3. Also, due to the large number of treatments (16) it further necessitated performance of this trial to assess the interference of sugars in TBARS assay on samples without taking into consideration the dietary vitamin E and flax levels. TBARS assays showed that sugars had an overriding effect over the dietary vitamin E and flax treatments and was statistically significant as reported in section 3.4.2.3.

The primary focus of the experiments performed was to check whether any interference was obtained in the TBARS assay readings from having the sugars present in the samples. Sugars could interfere in the TBARS assay readings as evident from the literature, but in a complex reaction system such as the TBARS assay, how they react is uncertain. Previous literature (Baumgartner et al., 1975; Wang et al., 2002) indicated that sugars react with TBA and could produce substances that have absorption bands located around 450 nm. However previous studies also showed that as the concentration of sugar increased, a shift of the absorption curves to the 530 nm range and thus overestimated the TBARS assay readings was observed. In the present experiment however there was no indication of such interference from sugars added at 2% levels to meat grinds as observed from the absorbance graphs at 532 nm and hence the reaction procedure outlined by McDonald & Hultin (1987) & Nielsen et al. (1997) for the estimation of TBARS needed no modification.

4.6. Conclusions

Based on all these results, it was concluded that the assay conditions proposed by McDonald & Hultin (1987) and Nielsen et al. (1997) could be

used to estimate the TBARS in this study where 2% sugars were added to meat as interference from sugars in the TBARS measurements did not appear to be occurring.

4.7. Figures

Figure 4.7.1 Absorption scans of cooked meat patties at different incubation time (60, 90, 120, 150 min), temperature (40° C) and concentration of TBA (60 & 80mM).

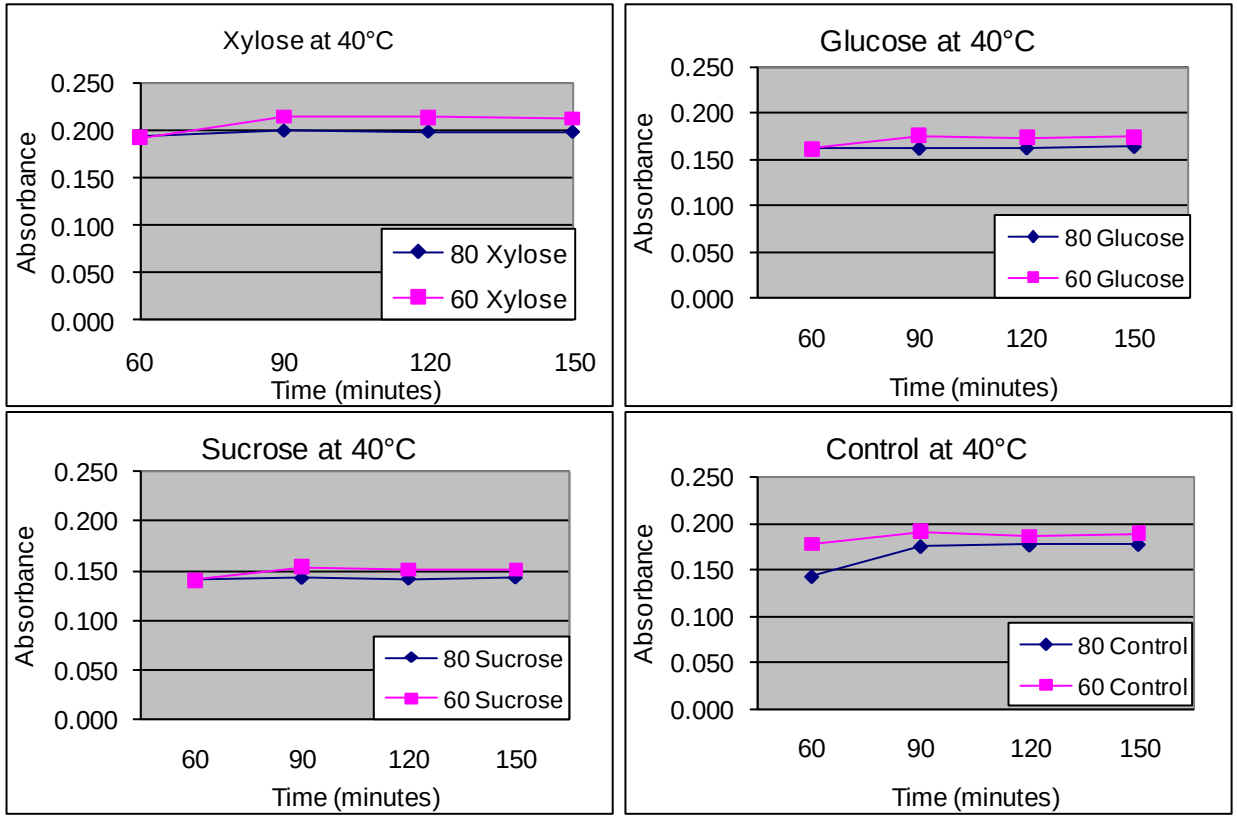


Figure 4.7.2 Absorption scans of warmed over meat patties at different incubation time (60, 90,120,150,240 min), temperature (40° C) and concentration of TBA (20, 40 & 60mM)

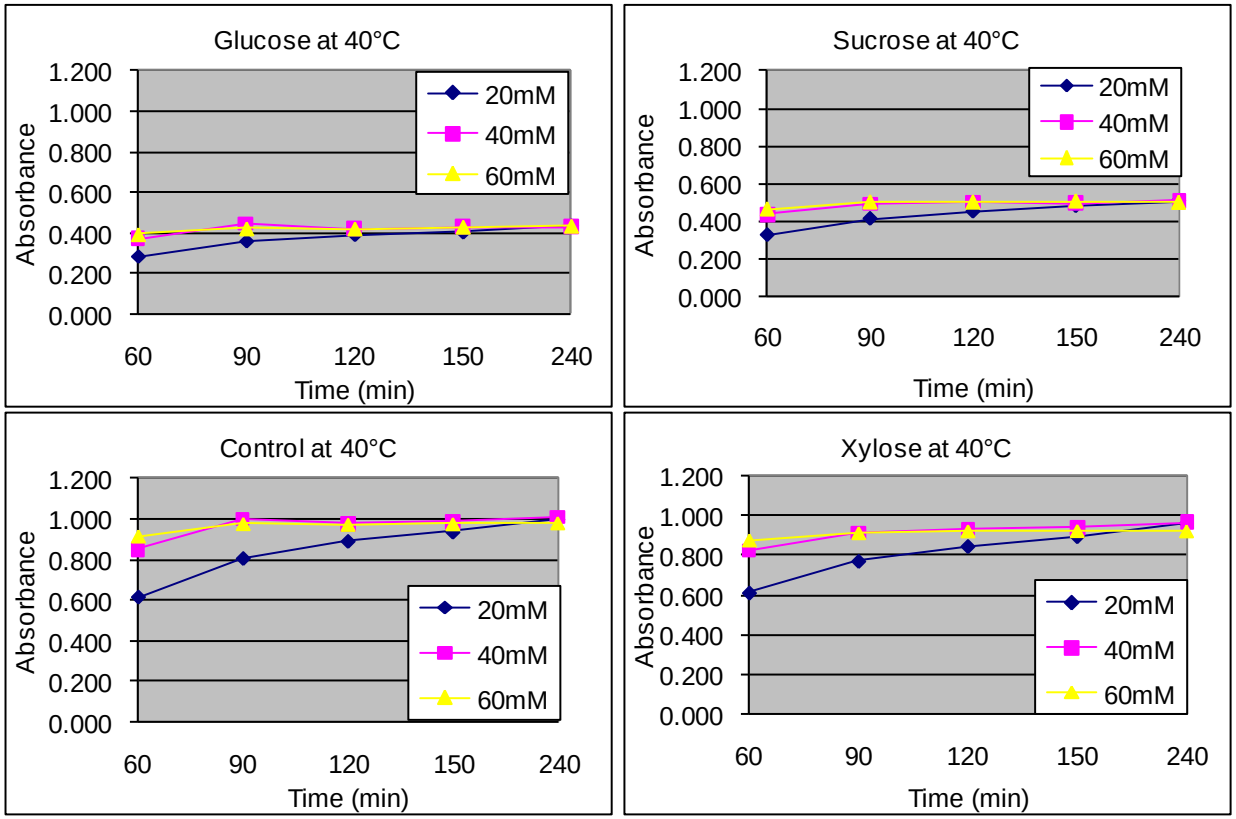


Figure 4.7.3 Absorption scans of warmed over meat patties at different incubation time (20, 40, 60 min), temperature (94° C) and concentration of TBA (20, 40 & 60mM)

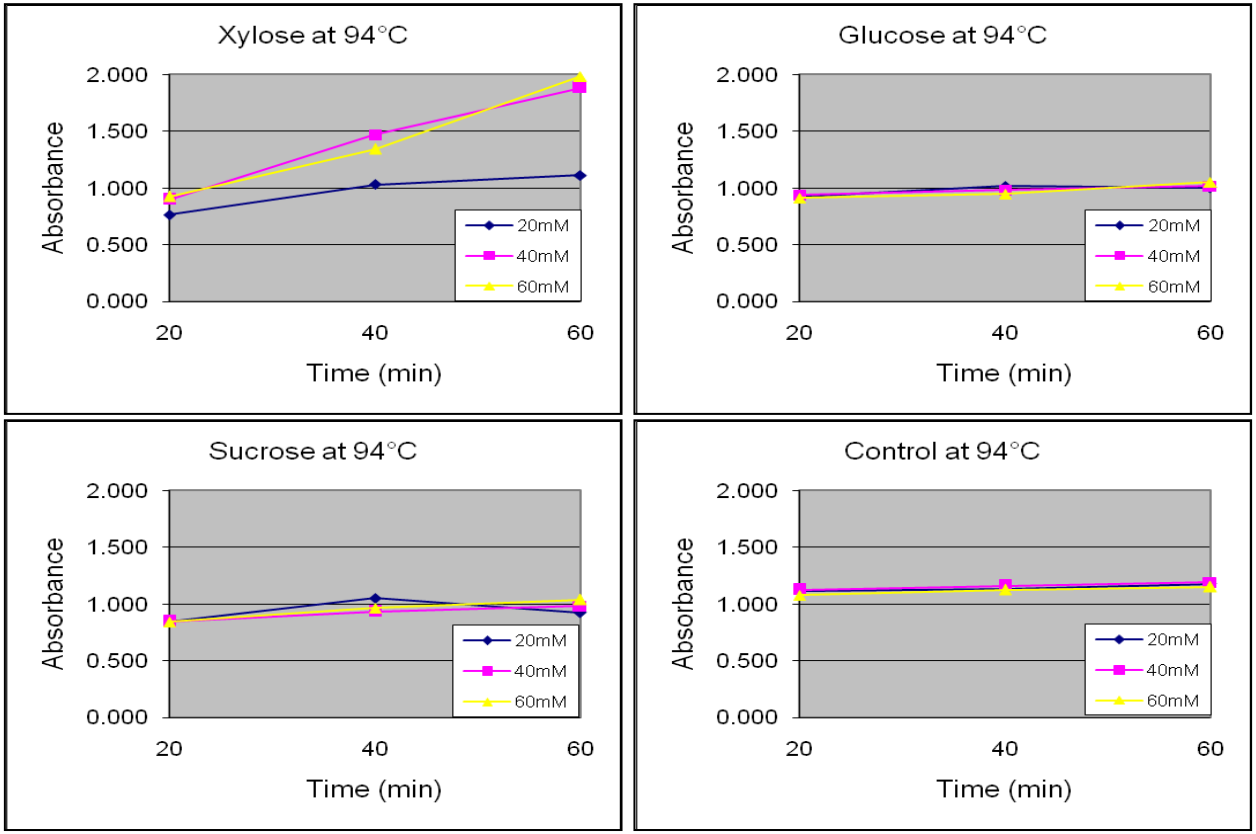


Figure 4.7.4 Absorption scans of warmed over meat (from 400nm to 600nm) patties at different incubation time (20, 40, 60 min), temperature (94°C) and concentration of TBA (20, 40 & 60mM)

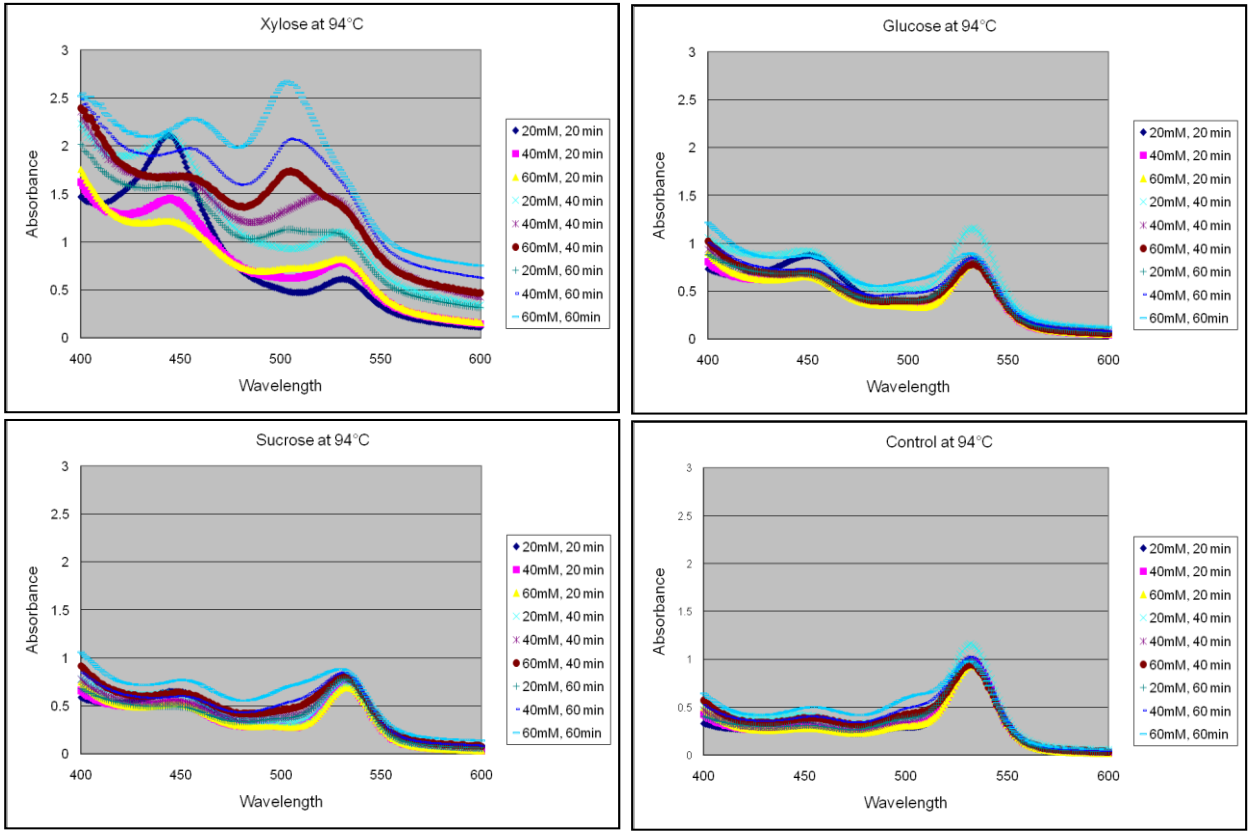


Figure 4.7.5 Absorption scan of sugars without TBA

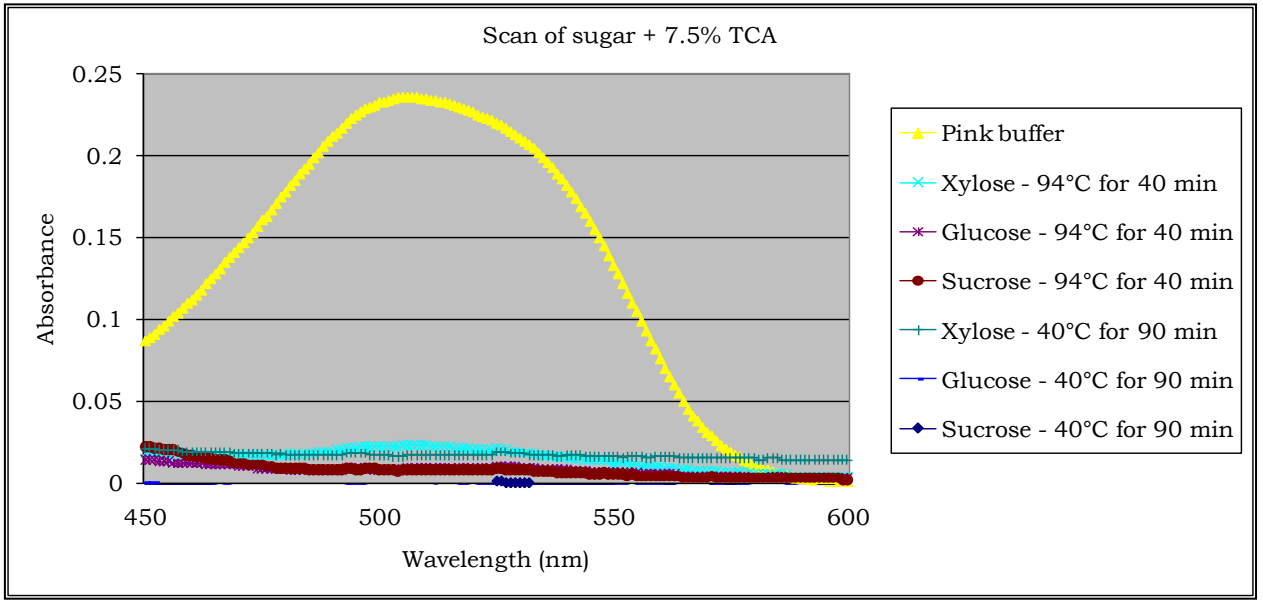
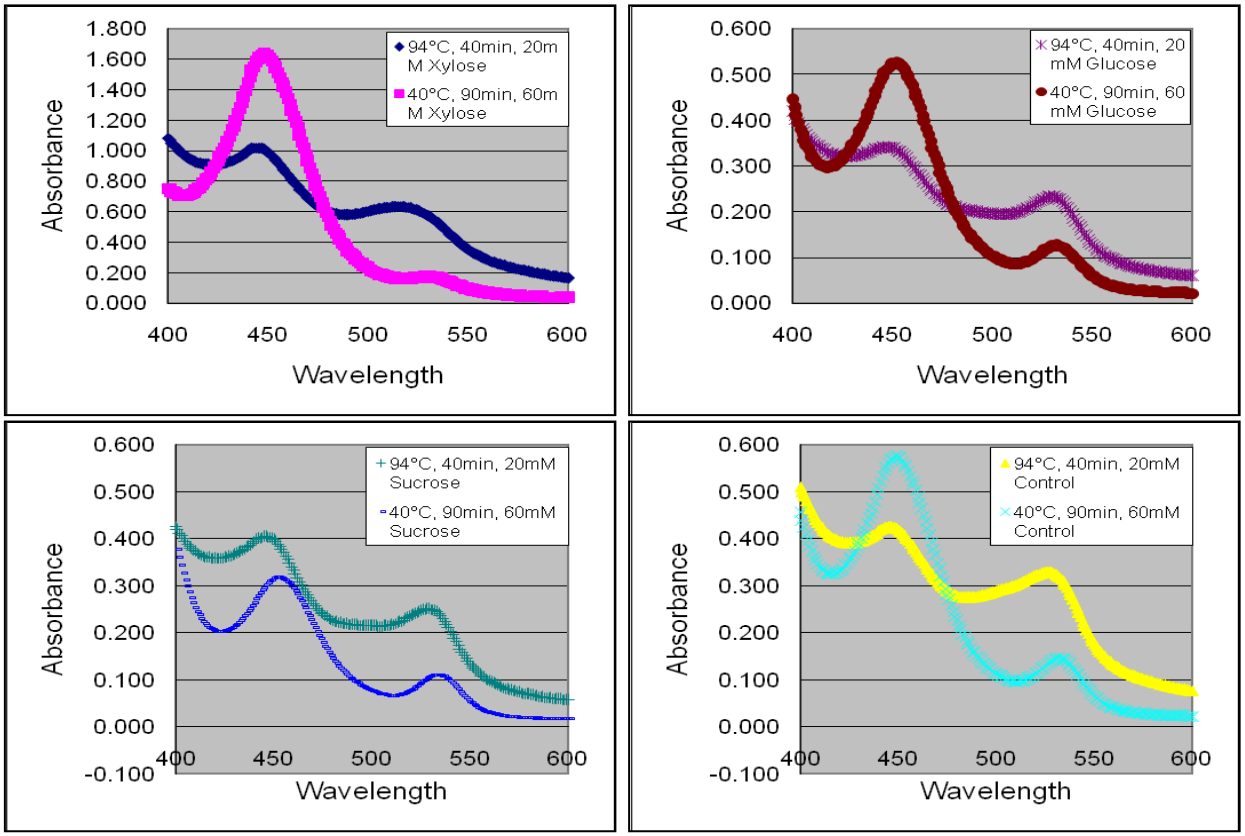


Figure 4.7.6 Absorption scans of sugars with TBA



4.8. Bibliography

Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnelt, C. H., Kuan, J. W., James Jr, C., et al. (1987). Chemical and instrumental analyses of Warmed-Over flavour in beef. *Journal of Food Science, 52*(5), 1163-1168.

Asakawa, T., Nomura, Y., & Matsushita, S. (1975). A modified TBA test for the determination of lipid oxidation. *Journal of Japan Oil Chemists' Society [Yukagaku], 24*(7), 481-482.

Baumgartner, W. A., Baker, N., Hill, V. A., & Wright, E. T. (1975). Novel interference in thiobarbituric acid assay for lipid peroxidation. *Lipids, 10*(5), 309-311.

Biggs, D. A., & Bryant, L. R. (1953). The thiobarbituric acid test for butterfat oxidation. *Canadian Journal of Food Technology, 31*, 138-145.

Du, Z., & Bramlage, W. J. (1992). Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *Journal of Agricultural and Food Chemistry, 40*(9), 1566-1570.

Guillen-Sans, R., & Guzman-Chozas, M. (1998). The thiobarbituric acid (TBA) reaction in foods: A review. *Critical Reviews in Food Science and Nutrition, 38*(4), 315-350.

Hoyland, D. V., & Taylor, A. J. (1991). A review of the methodology of the 2-thiobarbituric acid test. *Food Chemistry, 40*(3), 271-291.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine, 9*(6), 515-540.

Keeney, M., & Doan, F. J. (1951). Studies on oxidized milk fat. III. chemical and organoleptic properties of volatile material obtained by fractionation with various solvents and girard's Reagent. *Journal of Dairy Science, 34*(8), 728-734.

McDonald, R. E., & Hultin, H. O. (1987). Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. *Journal of Food Science, 52*(1), 15-21.

Melton, S. L. (1983). Methodology for following lipid oxidation in muscle foods. *Food Technology, 37*(7), 105-111.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Nielsen, J. H., Sørensen, B., Skibsted, L. H., & Bertelsen, G. (1997). Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Science, 46*(2), 191-197.

Patton, S., & Kurtz, G. W. (1951). 2-thiobarbituric acid as a reagent for detecting milk fat Oxidation. *Journal of Dairy Science, 34*(7), 669-674.

Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal of Agricultural and Food Chemistry, 37*(5), 1309-1313.

Raharjo, S., Sofos, J. N., & Schmidt, G. R. (1993). Solid-Phase acid extraction improves thiobarbituric acid method to determine lipid oxidation. *Journal of Food Science, 58*(4), 921-924.

Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science, 66*(9), 1483-1488.

Shlafer, M., & Shepard, B. M. (1984). A method to reduce interference by sucrose in the detection of thiobarbituric acid-reactive substances. *Analytical Biochemistry, 137*(2), 269-276.

Sinnhuber, R. O., Yu, T. C., & Yu, T. C. (1958). Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *Journal of Food Science, 23*(6), 626-634.

Tarladgis, B. G., Pearson, A. M., & Jun, L. R. (1964). Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II.—formation of the tba-malonaldehyde complex without acid-heat treatment. *Journal of the Science of Food and Agriculture, 15*(9), 602-607.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society, 37*(1), 44-48.

Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A., & Phillips, B. (2002). Modified extraction method for determining 2-thiobarbituric acid values in meat with increased specificity and simplicity. *Journal of Food Science, 67*(8), 2833-2836.

Wertheim, J. H., & Proctor, B. E. (1956). Radiation preservation of milk and milk products. III. the thiobarbituric acid test as a means of evaluating radiation-induced changes in Milk. *Journal of Dairy Science, 39*(4), 391-401.

Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science, 35*(5), 582-585.

Yu, T. C., & Sinnhuber, R. O. (1962). Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction. *Food Technology, 16*(6), 115-117.

CHAPTER -5

SUMMARY AND GENERAL CONCLUSION

5.1. General Summary

Omega-3 enrichment of pork and pork products opens a potential avenue for stimulating the pork industry. Due to the health benefits attributed to the consumption of *n*-3 fatty acids, considerable importance has been gained for this approach, which ensures sustainable supply of these fatty acids to human beings. The pig, being a monogastric animal, has the natural advantage over other livestock species like beef as fatty acid composition can be altered directly via diet and so the fatty acid content of muscle responds quickly to increased dietary concentrations (Rosenvold & Andersen, 2003; Van Elswyk, 1993). Flaxseed, linseed, canola, algae, and fishmeal are sources of *n*-3 fatty acids in animal feeds (Bečková & Václavková, 2010; Corino et al., 2008; HertzmanLeif, 1988; Kouba et al., 2003; Matthews et al., 2000; Musella et al., 2009; Realini et al., 2010; Specht-Overholt et al., 1997). Much knowledge has been gained in the field of *n*-3 enrichment of pork by adopting different feeding strategies and studying their implications on the eating quality of meat. Significant effort has been made to characterize the *n*-3 enrichment so that a standardized feeding strategy may be identified for attaining optimum *n*-3 fatty acid levels in pork.

Despite the fact that *n*-3 fatty acids are beneficial to human beings in preventing a wide range of diseases, the scientific community is still working to formulate a dietary requirement for different human subjects and dietary recommendations vary. The International Society for the Study of Fatty Acids and Lipids recommended a daily intake of 2,220 mg of α-linolenic acid (LNA) and 650 mg of (eicosapentaenoic acid + docosahexaenoic acid) EPA + DHA, with a minimum of 220 mg of both EPA and DHA for adults (Simopoulos et al., 1999) while the Society also recommended that in the case of pregnant and lactating women a minimum supplementation of 300mg/day of EPA and DHA was required to meet the increased requirements. The UK Department of Health recommended a total long chain polyunsaturated fatty acids (LCPUFA)

intake for an adult of 200mg/day whereas the American Heart Association and European society of Cardiology recommended 100mg/day of LCPUFA for adults (Kris-Etherton et al., 2003; Van de Werf et al., 2003). Omega-3 enriched foods have been characterized as 'functional foods' as they are believed to have medicinal properties apart from supplying nutrients to the body (Heasman & Mellentin, 2001). In Canada the requirement for *n*-3 enriched foods to be labelled as a source of *n*-3 is that it should provide 300mg of *n*-3 fatty acids per 100g of serving (CFIA & Health Canada, 2009).

Enrichment of *n*-3 fatty acids also necessitates adequate antioxidant supplementation to prevent fatty acid oxidation in foods; therefore, increasing dietary antioxidant levels in pig diets is a common practice in feeding strategies aimed to prevent lipid oxidation and increase *n*-3 fatty acid content in pork. The optimum levels of antioxidants that are needed to prevent lipid oxidation at *n*-3 levels that meet or exceed the Health Canada labeling requirements are often difficult to generalize because the activity of antioxidant varies with its chemical reactivity, physical location, reactivity with other components present in food, the processing conditions applied to foods and also the endogenous components present in meat could act as either pro-oxidants or antioxidants, which could also affect the activity of antioxidants and its level needed (Decker et al., 2005). Different types of antioxidants are commonly used in swine ration, but the most common is vitamin E used in the form of α-tocopheryl acetate. Even though high levels of *n*-3 fatty acids can be attained in pork by dietary manipulation, they may still lead to increased lipid oxidation in the pork product as increased levels of antioxidant supplementation may not prevent or reduce oxidation to a level that limits consequent off flavour generation due to lipid oxidation.

5.2. Summary of results

In the present study high levels of flax (10% for 6 weeks) were fed to pigs along with high levels of vitamin E (400mg/kg) to determine if high levels of vitamin E are able to offer sufficient protection against lipid oxidation. Implications of the dietary administration of high levels of flax and vitamin E on the performance of the animals as well as on the carcass characteristics have been studied and were not shown to deleteriously affect animal performance parameters or carcass characteristics in this study, which

is in agreement with previous research (Corino et al., 2008; Guillevic et al., 2009; Nuernberg et al., 2005; Realini et al., 2010). The superior quality of Canadian pork has been attained through careful breeding and animal husbandry strategies. It is important, therefore, that all efforts towards *n*-3 enrichment should not compromise other physicochemical properties that have been attained over years of research.

Lipid oxidation and associated off flavour generation is a major problem encountered during *n*-3 enrichment in pork. Off flavour generated during cooking and reheating cooked meats is a serious problem causing considerable consumer dissatisfaction with *n*-3 enriched meat. Meat flavour is produced by volatile compounds that are generated when meat is cooked. Maillard reaction and thermally induced lipid oxidation are major pathways in generating flavour components (Meinert et al., 2009). The Maillard reaction occurs between amino compounds and reducing sugars and is one of the most important reactions producing flavour and browning in cooked foods (Mottram, 1998). Off flavour generation is most pronounced when meat is cooked and then re-heated. In order to promote Maillard reactions in meat, this study added the food grade sugars xylose, glucose and sucrose at the level of 2% to *n*-3 enriched pork meat patties to improve their oxidative stability and sensory characteristics particularly after re-heating. The various sugars reacted differently and produced different levels of oxidative stability in cooked and reheated cooked meat patties. Thiobarbituric acid reactive substances (TBARS) assay of patties that were fresh or frozen for 8, 12 or 16 weeks showed that addition of sugars improved oxidative stability after 12 and 16 weeks of frozen storage under vacuum ($P>0.05$). Sugars interacted with dietary vitamin E and flax treatments and improved oxidative stability in cooked samples that had high vitamin E (400mg/kg) and no flax ($P=0.0006$). Addition of sugars (glucose and sucrose) improved the oxidative stability of all meat patties over refrigerated storage and provided oxidative stability in the high Vitamin E/No flax treatment only after cooking.

Flavour is considered to be an important attribute determining consumer acceptability (Mottram, 1998) and hence sensory characterization of the meat patties in this study was performed using free choice profiling. Eight panellists were used to describe the aroma, texture and flavour of meat patties

that were freshly cooked and re-heated after 2 days refrigeration. Panellists described the aroma of meat patties that contained glucose and sucrose as pork meaty, roasted pork, browned and oily. Meat patties were described as having a moist, juicy and soft texture when they contained sucrose and glucose. Sweet and caramelized flavour notes apart from pork meaty and roasted pork flavours were detected in meat patties that contained sucrose. Meat patties that contained xylose were described as spongy and fibrous with cardboard, fishy and warmed-over flavours and burnt, rancid and warmed over aromas. Addition of sugars produced distinctive sensory characteristics irrespective of the source grind fatty acid or α-tocopherol composition.

5.3. Implications to meat industry

Omega-3 enrichment of meat offers a step towards value addition to the meat industry. There is considerable demand for the *n*-3 enriched food products. There are *n*-3 enriched food products like eggs, milk and juices available in the market. Omega-3 enrichment of meat is a step towards popularizing the consumption of *n*-3 fatty acids to a wide extent as meat is always a major item in our daily food menu. This research focused on an important issue related to the *n*-3 enrichment of meat; that of improving the oxidative stability and sensory characteristics of *n*-3 enriched pork meat by addition of sugars. Unlike in other *n*-3 enriched foods, *n*-3 enriched meat has additional issues associated with oxidation and off flavour generation especially when it is cooked and reheated after refrigerated storage, a phenomenon often called warmed over flavour (WOF). WOF generation in meat is a complex phenomenon and the bulk of research presently done in this field addresses controlling off flavour generation in meat. Oxidation and generation of off-flavours in meat are related to one another and hence controlling oxidation will help in controlling off-flavours. Flavour is produced by volatile compounds and modification of volatiles produced would help in preventing the generation of off-flavours. Maillard reaction products would impart anti-oxidative action also. Present research with the addition of sugars improved oxidative stability and the addition of sugars produced distinct sensory characteristics irrespective of the levels of *n*-3 fatty acid and antioxidant levels in the pork patties. This could be used as a beginning towards exploring the vast opportunity of using the sugars in meat processing

industry during *n*-3 enrichment and thereby modifying the flavour and reducing lipid oxidation. It could also be summarized that addition of sugars might be overriding the effects of *n*-3 fatty acid oxidation due to its involvement in the Maillard reaction.

5.4. Limitations

This study involved the participation of different agencies like Agriculture and Agri-Food Canada (Lacombe), the Northern Alberta Institute of Technology and the University of Alberta. The animals were raised at the Agriculture and Agri-Food Canada Swine Unit in Lacombe and, as reported in the thesis, a subset of treatments was chosen for this study. This reduced the number of replications, which increased the chance of the occurrence of a type II (or 2) error. Also, rearing of the animals at the AAFC Swine Unit in Lacombe limited the direct participation in animal rearing activities and animal performance data collection, and so these data were collected from AAFC, Lacombe, subsequently for reporting in the thesis.

Meat grinds and patties were made at the Retail Meat cutting facility at the Northern Alberta Institute of Technology as the addition of sugars to meat was not permitted as per the CFIA guidelines in place at AAFC, Lacombe. This necessitated freezing of the pooled mince samples for transport to NAIT and then thawing them prior to making the patties and then freezing the patties for transport to the University of Alberta for the sensory analysis. Repeated freezing and thawing can increase the purge or drip loss and can therefore produce a dry product; however, all mince was treated similarly so any effects would have been consistent throughout the products tested.

The thiobarbituric acid reacting substances assay, even though it is a common method for quickly assessing lipid oxidation, has its own limitations due to its lack of specificity and sensitivity as identified and reported in this thesis. However the matter of interference from sugars in the TBARS assay has also been investigated as reported in thesis, although due to the limitations in the availability of the samples, neither a statistical analysis nor a second method of assessing lipid oxidation was possible and hence the study of the relationship between TBARS and lipid oxidation was restricted to the way it is reported here. Despite the shortcoming of the TBARS assay, however, the most meaningful measure of product oxidation was with the sensory panel, as

the human palate is the most sensitive measure of sensory attributes, and attributes that have been associated in the literature with oxidation like ‘cardboardy’, ‘stale’, ‘fishy’, and ‘rancid’ were noted.

For the sensory study, the large number of treatments (16 treatments replicated thrice equals to 48) was a complication, as each member of the sensory panel could taste only a maximum of 8 samples per session and this increased the duration of the study. This also led to the use of a free choice profiling approach within an incomplete block design for the sensory study as there were limitations in the availability of the panellists for conducting a trained sensory panel study. Preparing a trained sensory panel can require months of training and the limited shelf-life of the pork patties prompted the use of a sensory panel that required few training sessions in order to limit the weeks over which the sessions were conducted.

5.5. Scope of future research

The present study opens avenues for further research. The study findings show that sugars reacted differently in their ability to offer oxidative stability in the meat products. Also they differed in their reaction in the fresh, frozen, cooked and warmed over meat patties. This could probably be due to their involvement in the complex chemical reactions when meat is cooked. Also the reaction they produce may differ with inclusion concentration and this study examined their effect only at the 2% level. The way the sugars reacted in the sensory study also varied and the different sugars produced different sensory characteristics. That these sensory attributes were discernable by inexperienced panellists with limited training was interesting and further study with a trained panel may provide additional insight. Because the addition of sugars offered oxidative stability, it would also be interesting to identify the reaction products and characterize the compounds formed during cooking. The cooking temperature and type of cooking might also have an effect in the generation of the flavour volatile compounds and hence that could also be investigated.

5.6. Bibliography

Bečková, R., & Václavková, E. (2010). The effect of linseed diet on carcass value traits and fatty acid composition in muscle and fat tissue of fattening pigs. *Czech Journal of Animal Science., 55*(8), 313-320.

CFIA & Health-Canada (2009). Guide to Food Labelling and Advertising. CanadianFood Inspection Agency: http://www.inspection.gc.ca/english/fssa/labeti/guide/ch7be.shtml. Retrieved on February 21, 2011

Corino, C., Musella, M., & Mourot, J. (2008). Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of liveweight. *Journal of Animal Science, 86*(8), 1850.

Decker, E. A., Warner, K., Richards, M. P., & Shahidi, F. (2005). Measuring antioxidant effectiveness in food. *J.Agric.Food Chem, 53*(10), 4303-4310.

Guillevic, M., Kouba, M., & Mourot, J. (2009). Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of french fresh and cooked pork meats. *Meat Science, 81*(4), 612-618.

Heasman, M., & Mellentin, J. (2001). *The functional foods revolution: Healthy people, healthy profits?*. Earthscan Publications Ltd., London., UK.

HertzmanLeif, C. (1988). Influence of fishmeal, rape-seed, and rape-seed meal in feed on the fatty acid composition and storage stability of porcine body fat. *Meat Science, 23*(1), 37-53.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., & Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science, 81*(8), 1967-1979.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(2), e20-e30.

Matthews, K. R., Homer, D. B., Thies, F., & Calder, P. C. (2000). Effect of whole linseed (linum usitatissimum) in the diet of finishing pigs on growth performance and on the qualityand fatty acid composition of various tissues. *British Journal of Nutrition, 83*(06), 637-643.

Meinert, L., Schäfer, A., Bjergegaard, C., Aaslyng, M. D., & Bredie, W. L. P. (2009). Comparison of glucose, glucose 6-phosphate, ribose, and mannose as flavour precursors in pork; the effect of monosaccharide addition on flavour generation. *Meat Science, 81*(3), 419-425.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Musella, M., Cannata, S., Rossi, R., Mourot, J., Baldini, P., & Corino, C. (2009). Omega-3 polyunsaturated fatty acid from extruded linseed influences the fatty acid composition and sensory characteristics of dry-cured ham from heavy pigs. *Journal of Animal Science, 87*(11), 3578.

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G., et al. (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science, 70*(1), 63-74.

Realini, C. E., Duran-Montgé, P., Lizardo, R., Gispert, M., Oliver, M. A., & Esteve-Garcia, E. (2010). Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition. *Meat Science, 85*(4), 606-612.

Rosenvold, K., & Andersen, H. J. (2003). Factors of significance for pork quality--a review. *Meat Science, 64*(3), 219-237.

Simopoulos, A. P., Leaf, A., & Salem Jr, N. (1999). Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Journal of the American College of Nutrition, 18*(5), 487-489.

Specht-Overholt, S., Romans, J., Marchello, M., Izard, R., Crews, M., Simon, D., et al. (1997). Fatty acid composition of commercially manufactured omega-3 enriched pork products, haddock, and mackerel. *Journal of Animal Science, 75*(9), 2335-2343.

Van de Werf, F., Ardissino, D., Betriu, A., Cokkinos, D. V., Falk, E., Fox, K. A. A., et al. (2003). Management of acute myocardial infarction in patients presenting with ST-segment elevation. *European Heart Journal, 24*(1), 28-66.

Van elswyk, M. E. (1993). Designer foods: Manipulating the fatty acid composition of meat and eggs for the health conscious consumer. *Nutrition Today, 28*(2), 21-28.