

**DEVELOPMENT AND EVALUATION OF OMEGA-3 FATTY ACIDS
ENRICHED CHICKEN FRANKFURTERS**

by

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DALHOUSIE UNIVERSITY
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ABSTRACT

Chemical, physical and sensory properties of omega-3 fatty acid enriched chicken frankfurters developed with flax oil and microencapsulated fish oil at 1.2%, 2.4% and 3.6% were evaluated. Four replicate batches of frankfurters were produced for texture profile analysis and TBARS for assessment of lipid oxidation over four weeks of refrigerated storage. Gas chromatograph analysis indicated that omega-3 fatty acid levels increased ($p < 0.05$) with flax and fish oils treatments resulting in a shift in omega-6/omega-3 with no increase in lipid oxidation over the storage period. The two highest levels of fish oil resulted in increased redness, hardness, gumminess and chewiness ($p < 0.05$) with the highest fish oil having the lowest rating for acceptability. 1.2 and 2.4% flax oil and 1.2% fish oil samples were softer and juicier than commercial frankfurters. Addition of oils high in omega-3 fatty acids to chicken-based frankfurters can result in product resistant to oxidation and acceptable to consumers.

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percent
ALA	Alpha linolenic acid
ANOVA	Analysis of Variance
CFIA	Canadian Food Inspection Agency
cm	Centimeter
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid Methyl Esters
g	Gram
GC	Gas Chromatography
hr	Hour
ISSFAL	International Society for the Study of Fatty Acids and Lipids
kg	Kilogram
LA	Linoleic acid
LC	Long chain
MDA	Malonaldehyde
mg	Milligram
min	Minutes
mL	Milliliter
MSM	Mechanically separated meat
MUFA	Monounsaturated Fatty acid
n-3	omega-3
n-6	omega-6

NaCl	Sodium Chloride
°C	Degree Celsius
PUFA	Polyunsaturated Fatty acid
RH	Relative Humidity
SEM	Standard Error of the Mean
SFA	Saturated Fatty acids
STPP	Sodium Tripolyphosphate
TBA	Thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Substances Assay
TEP	1,1,3,3 Tetraethoxy Propane
TPA	Texture Profile Analysis
UFA	Unsaturated Fatty acids

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CHAPTER 1

INTRODUCTION

Omega-3 fatty acids (n-3) are a group of polyunsaturated fatty acids (PUFA) which include α -linolenic acid (ALA, C18:3 n-3), its long chain metabolites eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C 22:6 n-3). Humans can synthesize EPA and DHA through desaturation and elongation of ALA (Gogus and Smith, 2010). However, this conversion has been found to be limited (Garg et al., 2006). There is increasing evidence on the importance of these essential fatty acids in relation to human health and disease prevention such as cardiovascular diseases, hypertension, diabetes, arthritis, other inflammatory diseases and autoimmune disorders (Calder, 2006; Gogus and Smith, 2010). Dietary recommendations for omega-3 fatty acids (2.2g of ALA/ day and 0.22g/day of EPA and DHA; International Society for the Study of Fatty acids and Lipids, 2004) can be obtained from the diet by the consumption of foods rich in these fatty acids (Gebauer et al., 2006).

Major sources of ALA include the seeds and oils of flaxseed, soybean and canola, with flaxseed containing 50-60% ALA (Moghadasian, 2008). EPA and DHA are obtained in the diet from aquatic and marine products only such as fish, shellfish, algae and their oils (Coates et al., 2009). However, fish consumption is low in the current North American diet and consequently low in EPA and DHA levels (Moghadasian, 2008). Most North Americans consume foods high in omega-6 PUFA which has resulted in 10 to 15 times more omega-6 than omega-3 fatty acids in the diet (Simopoulos, 2008). Increased knowledge of the health benefits of omega-3 fatty acids especially EPA and DHA has led to a growing demand for products rich in omega-3 fatty acids. These products represent

one of the fastest growing trends in the food industry which has opened up new era for functional foods. A wide variety of omega-3 fatty acid enriched foods are available to consumers today (Jacobsen, 2010).

Meat and meat products are excellent sources for fat, protein, essential amino acids, minerals and vitamins which are important components of the diet of individuals living in the developed countries (Weiss et al., 2010). The meat processing industry is driven by increasing consumers demand for healthier meat products which includes products with a reduced level of fat, cholesterol, sodium and nitrite, improved fatty acid composition, including omega-3 fatty acids enriched meat products (Jimenez-Colemenero et al., 2001). One approach to develop healthier fabricated meat products is through reformulation of meat products during processing (Jimenez-Colemenero, 2007). Modification of the fatty acid composition is mainly based on the replacement of animal fat with oils that meet dietary recommendations (Fernandez-Gines et al., 2005). Vegetable oils (flaxseed) and marine oils (fish oil) have been incorporated into meat products like sausages to increase their omega-3 PUFA content (Ansorena and Asitasaran, 2004; Pelsler et al., 2007; Caceres et al., 2008). Omega-3 fatty acid enriched meat products are highly susceptible to lipid oxidation because of their polyunsaturated nature. This can lead to product deterioration and development of rancid flavors which would result in consumer rejection (Lee et al., 2006). However, the problem of lipid oxidation can be overcome by the use of microencapsulated oils (Anwar et al., 2010). The microencapsulation process is the delivery of fish oil micro-droplets which are coated with materials which form a wall that minimize the exposure of the oil to oxygen (Kolanowski et al., 2007). The consumer

acceptance of omega-3 fatty acid enriched meat products by incorporation of oils into meat products depends on the sensory characteristics of the developed products.

When developing a product to be labeled as omega-3 fatty acid enriched it is important to consider the health claim regulations. European countries, Canada and USA have specific laws for the labeling of omega-3 fatty acid enriched products. For instance, in Canada a minimum level of 300mg of omega-3 PUFA per 100g of meat is required to label the product as a source of omega-3 fatty acids (Health Canada, 2003). In the USA, the health claim for omega-3 PUFA enriched foods, especially for EPA and DHA have been developed.

Meat products like sausages are mainly made from beef and pork. Meat from poultry can also be used to make acceptable comminuted meat products (Park et al., 1987). In 2010, consumption of red meats decreased while there was an increase in the consumption of chicken meat among Canadians with per capita amount of 31.5 kg/person (Canada Poultry Annual, 2010). The increased popularity of chicken meat has led to the development of chicken-based products like frankfurters. Moreover, poultry sausages have the potential to offer consumers a product with reduced fat content and with acceptable sensory characteristics (Gomez-Gonzalez et al., 1991). Many studies carried out in the past to develop omega-3 fatty acids enriched meat products included fermented sausages and bologna sausages of beef and pork origin. However, there is lack of information on the enrichment of omega-3 fatty acids in poultry products. The aim of this research study was to develop omega-3 fatty acids enriched chicken frankfurters by incorporation of flax oil and microencapsulated fish oil. The study would provide insight

regarding the interaction of meat proteins from chicken with vegetable and marine oils and information on the sensory characteristics of products.

CHAPTER 2

LITERATURE REVIEW

2.1 Omega-3 Fatty Acids

Fatty acids are the basic structural components of fats and oils present in foods. Based on the presence of double bonds in their structure they are classified into saturated fatty acids (no double bond), monounsaturated fatty acids (MUFA) (single double bond) and polyunsaturated fatty acids (PUFA) (more than 1 double bond) (Surette, 2008). PUFAs are grouped into omega-3 (n-3) and omega-6 (n-6) based on the presence of the first double bond from the terminal methyl carbon. Linoleic acid (LA; 18:2 n-6) and α -linolenic acid (ALA; 18:3 n-3) are the precursors of other omega-6 and omega-3 PUFAs, respectively, and are considered essential fatty acids (EFA) (Gogus and Smith, 2010). In humans, ALA is converted to eicosapentaenoic acid (EPA; 20:5 n-3) and docosohexaenoic acid (DHA; 22:6 n-3) which are considered long chain n-3 PUFA (Williams and Burdge, 2006). ALA is present in high amounts in various vegetable sources such as flaxseed, canola, and soy oils whereas fish oil and other marine food products such as algae are a good source of EPA and DHA (Moghadasian, 2008).

2.1.1 Flax Oil

Flax oil or linseed oil is obtained from flax which is a sub-tropical annual crop grown mainly in Canada, Argentina, India, USA and China (Kochhar, 2002). Most flax is grown for the production of flax oil. Canada is the largest producer and exporter of flaxseed, with a production of 0.93 million tonnes in 2009 (Flax council of Canada, 2010). Flax oil from common flax varieties contains around 60% ALA (Duguid, 2009). The high levels of ALA predisposes the oil to undergo oxidation quickly so edible flax oils have

been developed to lower the ALA down to 2% (Duguid, 2009). Flax oil, though a rich source of ALA (omega-3 fatty acid) is not commonly used as food oil (Kris-Etherton et al., 2000). The nutritional value of omega-3 fatty acids in flaxseed oil have gained importance in recent years and most of the intake of flaxseed and oils by humans comes in the form of capsules as a dietary supplement (Kochhar, 2002).

2.1.2 Fish Oil

Fish such as tuna, anchovy, trout and salmon are primary sources of EPA and DHA (Surette, 2008). Adequate intakes of EPA and DHA can be met by the consumption of two portions of fish per week (Gebauer, 2006). Due to low fish consumption in many developed countries, fish supplementation in the form of fish oil capsules is needed to meet the dietary requirements (Kolanowski, 2005). Another way to obtain EPA and DHA from the diet is by the incorporation of fish oil into food products (Rymer and Givens, 2005, Jacobsen et al., 2008). Food products enriched with omega-3 fatty acids by the addition of fish oil are currently available in the market (Jacobsen, 2010). Some examples are omega-3 milk (Parmalat, USA), Danino yogurt and Becel, DHA enriched (Canada) and Supajus DHA enriched orange drink (The Natural Fruit & Beverage Co., UK).

Crude fish oil is obtained by extraction of fish with food grade ethanol and subjected to various processes like filtration, neutralization, bleaching and distillation to obtain fish oil capsules or supplements (Kolanowski, 2005). Fish oil capsules that are available in the market vary widely in EPA and DHA, most commonly providing approximately 180 mg of EPA and 120 mg of DHA per one-gram capsule (Gebauer, 2006). The taste and smell of fish oil in the natural state is not liked by many consumers. Moreover, the high oxidative susceptibility nature of fish oil leads to the development of

fishy and rancid off-flavors in food products enriched with fish oil (Jacobsen, 2010). The problem of lipid oxidation in fish oil can be minimized or reduced by the use of microencapsulation (Anwar et al., 2010).

Microencapsulation has been defined as “the technology of packaging solid, liquid, and gaseous materials in small capsules that release their contents at controlled rates over prolonged periods of time” (Champagne and Fustier, 2007). Microencapsulated oil consists of a core which contains the fish oil, surrounded by a wall which prevents the diffusion of the core (Gharsallaoui, 2007). The wall is made of a number of materials like gelatin or soy protein and the microencapsulation is commonly achieved by a number of techniques, including spray drying method (Anwar et al., 2010).

2.1.3 Health Benefits of Omega-3 Fatty Acids

The potential health benefits of omega-3 fatty acids have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, brain development, diabetes, cancer, arthritis, inflammatory, autoimmune and neurological disorders (Simopoulos, 2000; Gogus and Smith, 2010).

Pioneering studies regarding the effect of omega-3 fatty acids on human health were carried out by Bang et al. (1971) with Greenland Eskimos. They showed the importance of omega-3 fatty acids in reducing cardiovascular disease. Long chain omega-3 polyunsaturated fatty acids (LC PUFA n-3), specifically EPA and DHA have been found to reduce the risk of cardiovascular disease by reducing the total serum cholesterol and serum triglycerides (Wang et al., 2006). Studies have shown that the inclusion of EPA and DHA at doses of 3g/day reduces the risk of cardiovascular diseases by decreasing plasma triacylglycerols, blood pressure and platelet aggregation (Breslow,

2006). Reduction in serum triacylglycerols (TAG) by dietary omega-3 PUFA has been supported by research studies in rats as well (Dasgupta and Bhattacharya, 2007). Intake of EPA and DHA were found to lower the risk of ischemic heart disease (Lemaitre et al, 2003) and cardiac arrest in humans (Bhatnagar and Durrington, 2003). The reduction of TAG by LC n-3 PUFA could be due to decreased hepatic synthesis of very low density lipoprotein (VLDL) by inhibition of various enzymes (Chan and Cho, 2009). Addition of EPA and DHA to cultured cardiomyocytes of neonatal rats inhibited the induction of tachyarrhythmia (Leaf et al., 2003).

DHA has been found to play a role in cognitive functions and also may protect against Alzheimer's disease (van Gelder et al., 2007). The consumption of LC n-3 PUFA, especially DHA is important during pregnancy in women since it is essential for the proper development of eyes, growth and function of brain and nerve tissue in infants (Cheatham et al., 2006; Innis, 2007). Several studies have determined the protective effect of dietary EPA and DHA against cancers by animal experiments (Berquin et al., 2008). Dietary supplementation of tumor bearing mice with LC n-3 PUFA have been found to slow down cancer of the colon, mammary gland and prostate (Hardman, 2004). A meta analysis study by Theodoratou et al. (2007) revealed the positive effect of LC n-3 PUFA on significant decrease in colorectal cancer. One of the mechanisms of cancer prevention by omega-3 PUFAs is by inhibition of production of various proteins that cause cell proliferation and tumor formation (Larsson et al., 2004). Inflammatory diseases such as rheumatoid arthritis and Crohn's diseases could be treated with LC n-3 PUFAs because of their anti-inflammatory properties by inhibition of prostaglandins (PGE₂), interleukin 1(IL -1) and tumor necrosis factor (TNF α) (Ferruci et al., 2006). The

consumption of omega-3 fatty acids has been found to have beneficial effects in age related memory loss as supported by a study in rats (Dyall et al., 2010). Overall, omega-3 fatty acids have been found to play a protective role in the prevention of various diseases, especially cardiovascular disease.

2.1.4 Dietary Recommendations for Omega-3 Fatty Acids

Omega-3 fatty acids could be considered as the basic components of daily nutrition due to their beneficial effects. Dietary recommendations for omega-3 fatty acids have been made by health authorities in different countries (Gogus and Smith, 2010). Dietary guidelines of the UK recommends average daily intake of 0.2g/day of EPA and DHA (Ruxton and Derbyshire, 2009). Daily intake of 0.5 to 1.0 g of EPA and DHA has been recommended by American Heart Association (Lichtenstein et al., 2006) and by The American Dietetic Association of Canada (Kris-Etherton and Innis, 2007). The International Society for the Study of Fatty acids and Lipids (ISSFAL, 2004) recommended 2.2 g/day of ALA and 650 mg of EPA plus DHA per day with a minimum of 220 mg of EPA and DHA per day. Recommendations have been made for the ratio of n-6: n-3 to be 2.5:1 to 5:1 based on the beneficial effect on disease conditions (Simopoulos, 2008). This ratio is important because high intake of omega-6 interferes with the metabolic pathway of conversion of ALA to EPA and DHA and results in production of more eicosanoids from arachidonic acid such as prostaglandins (PGE₂), thromboxanes and leukotrienes among others. These eicosanoids leads to formation of thrombus, allergic and inflammatory disorders. Thus a diet rich in n-6 fatty acids increases the risk of bleeding disorders and decreases the benefits of ALA (Simopoulos, 2002).

2.1.5 Omega-3 Fatty Acid Enriched Products

The current intake of omega-3 fatty acids in a typical Western diet is lower than the recommended level and the intake of PUFAs consists primarily of omega-6 fatty acids (Gebauer et al., 2006; Simopoulos, 2008). The present Western diet is estimated to have 10 to 15 times higher intake of omega-6 than omega-3 fatty acids (Simopoulos, 2008). The low intake of omega-3 fatty acids and increasing scientific evidence of the beneficial effects of EPA and DHA has led to introduction of omega-3 fatty acids enriched foods in the market (Jacobsen, 2010). Currently, functional foods containing omega-3 lipids is one of the fastest growing food product categories with around 1300 omega-3 fatty acids enriched products launched in 2007 in the USA and Europe (Daniells, 2008). These products ranged from breads to milk, juices, salad dressings, chocolates, yogurt, drinks, spreads, meal bars, margarines, mayonnaise, butter and eggs. It is predicted that by 2012, EPA/DHA fortified foods will represent 78% of all the omega-3 fatty acids enriched foods in the USA (Heller, 2009).

2.1.6 Omega-3 Fatty Acid Enriched Meat Products

Meat and meat products are the focal point in the diet of developed countries (Fernandez-Gines et al., 2005). Meat is a major source of saturated fatty acids and conventional meat products have an n-6: n-3 ratio of higher than 15 (Reglero et al., 2008). Therefore, meat products could benefit from the addition of omega-3 PUFAs. The primary approach to enrich meat with omega-3 fatty acids is by incorporation of omega-3 sources such as flaxseed and or oil and fish meal and or oil in the diet of animals. This strategy has been reported by several researchers in pigs (Nuernberg et al., 2005), lamb (Elmore et al., 2005) and poultry (Komprada et al., 2005). Meat products such as

sausages prepared from these animals have been found to be enriched with omega-3 fatty acids. Sausages made from pigs fed ALA (Enser et al., 2000) and chicken frankfurters from chickens fed fish oil at 2-4% (Juern-Horng et al., 2002) had increased levels of n-3 fatty acids.

Another approach for omega-3 PUFA enrichment of meat products is by introduction of omega-3 oils as an ingredient. The modification of the ratio of fatty acids in meat products could be achieved by replacement of animal fat with vegetable oils as vegetable oils are a rich source of PUFAs (Jimenez-Colmenero, 2007). Also, inclusion of oils such as fish oil results in omega-3 enrichment of meat products.

Many research studies have been carried out on the incorporation of vegetable oils to meat products to increase PUFA levels, improve PUFA/SFA ratio and reduce cholesterol level. Olive oil has been most commonly used to modify the fatty acid profile in meat products because of its high MUFA content and lower ratio of SFA/MUFA (Bloukas et al., 1997; Muguerza et al., 2002, Severini et al., 2003; Ansorena and Astiasaran, 2004 and Jimenez-Colmenero et al., 2010). Corn oil and cottonseed oil have been used because of a high concentration of linoleic acid (> 56% of total fatty acids) (Ambrosiadis et al., 1996; Vural et al., 2004). Palm oil has been added to frankfurters because it is cholesterol free and also contains antioxidants (Tan et al., 2006). Canola oils have been used to increase the PUFA/SFA ratio in fermented sausages (Pelser et al., 2007). Flaxseed oil has been incorporated to meat products to increase omega-3 PUFA levels (Ansorena and Astiasaran, 2004; Makala, 2007; Pelser et al., 2007). Direct addition of flaxseed oil and in encapsulated form at 3-6% of the final product has been performed in Dutch style fermented sausages (Pelser et al., 2007). These sausages had increased

levels of PUFA (18-25%) in comparison to control sausages (13.59%). Also, the ratio of n-6: n-3 was reduced from 11.30 (control) to 1.05. Literature available on the use of flaxseed oil with reference to enrichment of meat products is uncommon.

Omega-3 enrichment of meat products with fish oil has been the focus of a number of studies. Park et al. (1989) prepared frankfurters with 5% fish oil and sensory panelist found their flavor and aroma undesirable. Whereas, incorporation of fish oil at 1-6% after emulsification (Caceres et al., 2008) or with the use of encapsulation technology (Valencia et al., 2006 and Pelsler et al., 2007) resulted in increased EPA and DHA content in fermented sausages without affecting their sensory properties. Enriched dry fermented sausages were produced with a ratio of n-6: n-3 that was reduced 5 times (Valencia et al., 2006). The use of other oil sources including squid oil at 5% in chicken sausages (Andres et al., 2009) and algal oil in fresh pork sausages (Lee et al., 2006) have resulted in increased level of omega-3 fatty acids.

2.2 Sausages

The term sausage is derived from the Latin word “salsus” meaning salt, referring to salted, seasoned, chopped meat product. They are usually cylindrical in shape, mainly because sausage mixtures have traditionally been encased in animal intestines or stomachs (Pearson and Gillett, 1996). Historically sausages are one of the oldest forms of processed foods. There are hundreds of different sausage products available to consumers today. Many of these sausages originated in Europe and were made in United States to meet the demand of ethnic groups. Sausages are classified into fresh sausages, fermented sausages, semi dry and dry sausages and cooked sausages based on the preservation methods (Toldra and Reig, 2007).

2.3 Frankfurters

Frankfurters are emulsion type cooked sausages which are very popular and highly consumed meat product in many countries (Özvural and Vural, 2008). Frankfurters which are commonly referred as “Wieners” or “Hotdogs” are usually made from beef or pork or a combination and are flavored with spices and smoke application (Gonzalez-Vinas et al., 2004). They are also made from other meats, including chicken or turkey. The word frankfurter originated from Frankfurt, Germany, where pork sausages originated (National Hotdog and Sausage Council, 2010). The type of meat used in frankfurters varies with the geographical location and the availability of meat. Where frankfurters from Europe are made with pork and beef mixtures; frankfurters from South Africa and Malaysia are made primarily with mechanically deboned chicken meat (Feiner, 2006). In Canada, frankfurters available in the market are made with all beef or pork or mechanically separated chicken meat.

Sausages like frankfurters are economical because of the use of meat by-products like skin and mechanically separated meat (MSM). MSM or mechanically deboned poultry meat (MDPM) is widely used for the preparation of frankfurters because of the low cost of this ingredient (Feiner, 2006). Frankfurters prepared with chicken have a healthy image because of the low fat content (18-22%) compared to frankfurters prepared with red meat (25-30% fat) (USDA, 2009).

The meat processing industry is the largest sector of food manufacturing industry in Canada with export of \$21.4 billion in 2007 (Canada Meat Council, 2008). The export of poultry and further processed poultry meat products in 2008 was 109 million kg and 7.8 million kg, respectively (Canada Meat Council, 2008). In the US market in 2009,

consumers spent more than \$1.6 billion on frankfurters and sausages (National Hotdog and Sausage Council, 2010).

2.3.1 Nutrient Value of Chicken Frankfurters

Protein and fat are the major components in frankfurters. The composition of a typical chicken frankfurter is given in Table 2.1. As per Canadian regulations, the minimum protein content in frankfurters should be 9.5% and fat should not be more than 40% (CFIA, 2003). Chicken frankfurters are found to be a good source of PUFA compared to beef and pork frankfurters due to the high content of PUFA in chicken meat and contain less saturated fatty acids (Andrés et al., 2006).

Table 2.1 Nutrient value of chicken frankfurters

Nutrient (%)	Chicken Frankfurter
Moisture	62.5
Carbohydrate	2.74
Energy(kcal)	223
Protein	15.51
Total fat	16.19
Ash	3.06
Saturated fat	3.86
MUFA	5.93
PUFA	3.87
Alpha- linolenic acid (18:3 n-3)	0.33
Eicosapentaenoic acid(20:5 n-3)	0.003
Docosahexaenoic acid(22:6 n-3)	0.003

Health Canada (2010)

2.3.2 Ingredients in the Formulation of Frankfurters

Selection of ingredients is important for the production of frankfurters of uniform standard quality. All frankfurters are formulated with lean meat and fat as main ingredients. Other non meat ingredients include salt, polyphosphates, binders (milk powder, corn starch), ice, spices and nitrite. Lean meat and fat can originate from various sources such as pork, beef, chicken etc. which forms the protein and fat components of the mixture. MDPM is incorporated into poultry meat products for its functional properties. Pork fat is commonly used in the preparation of cooked sausages like frankfurters. Pork skin or chicken skin is also widely used as they add firmness to products (Feiner, 2006). Skin contains 35% connective tissue which is mostly collagen. When heated, collagen turns into gelatin which contributes to firmness and texture in meat products (Babji et al., 1998).

Water is added to sausages like frankfurters to activate or solubilize muscle protein which is critical for meat emulsion stability. Water is also required to disperse salt, nitrite and other ingredients. Ice is commonly used to counteract the heating effects of the high cutting and shearing forces generated by the knives on the bowl cutter and to keep the temperature of the sausage mass down. Thus, ice aids in obtaining a homogeneous emulsion by activating the maximum amount of protein (Feiner, 2006). The amount of water to be added is limited to the extent that amount of added water plus fat content in frankfurters cannot exceed 40% (CFIA, 2003).

Salt (NaCl) is an essential ingredient added to frankfurter sausages. Salt is added at the beginning of comminution process and acts synergistically with phosphates (Feiner, 2006). Salt serves to flavor the product, lower water activity and increase ionic strength.

Increased ionic strength retards microbial growth, assists with the solubilization of meat proteins and acts synergistically with sodium nitrite to prevent the growth of *Clostridium botulinum* (Keeton, 2001). Phosphate in the form of sodium or potassium tripolyphosphate is added to frankfurter sausages for the following purposes: increase water holding capacity of muscle proteins, preserve juiciness and increase product yield. Phosphates chelate heavy metal ions and therefore slow down the process of oxidation; and phosphate along with salt solubilizes muscle protein (Findlay and Barbut, 1992). It also preserves the color of cured product and enhances flavor (Keeton, 2001). As per USDA and Canadian regulations the amount of added phosphate should not exceed 0.5% of the finished product (USDA, 1999). Curing salt which is made of 6.25% sodium or potassium nitrite and 93.75% salt (NaCl) is added to cure products like frankfurters.

The pink color and cured flavor of the frankfurters is due to the reaction of nitrite with myoglobin (Pegg and Shahidi, 2000). Nitrite also acts as an antioxidant and prevents the growth of *Clostridium botulinum*. The permissible limit of nitrite should be according to the regulation which is not to be more than 200 ppm of the frankfurter (USDA- FSIS, 1995). Ascorbic acid, sodium ascorbate and erythorbate are added as cure accelerators which speed up the formation of cured color and also act as antioxidants (Feiner, 2006).

Non-meat ingredients are used as binders, fillers and extenders in frankfurter formulations to reduce the cost and shrinkage during cooking. Proteins such as milk protein (Xiong, 2009), soy (Castro et al., 2007), egg (Trespacios and Pla, 2009) and gelatin are added to increase water holding capacity and to stabilize emulsions. Milk proteins exhibit greater heat stability than meat proteins, thus they are used to stabilize the meat emulsion (Xiong, 2009). Carbohydrate substitutes which include cellulose,

starches, maltodextrins, dextrans, hydrocolloids or gums are also used as binders (Keeton, 1994; Lin and Huang, 2008; Cierach et al., 2009; Jiménez-Colmenero et al., 2009). These are generally used to improve cooking yield, enhance water-holding ability, reduce formulation costs, modify texture and improve freezing stability. Starches are the most widely used carbohydrates which can serve as fat replacer and contribute to the firm texture of the product. Starch from potato, corn, wheat and tapioca are commonly used (Keeton, 2001). As per the Canadian Meat Inspection Act (1997), the use of binders in meat products should be within the limits as products like frankfurters should contain a minimum 9.5% meat protein and 11% total protein. So, care should be taken to meet minimum protein requirements if non-meat binders are used.

Spices which include parsley, black pepper, garlic, nutmeg, and mustard, among others are used in sausages to impart flavor and enhance aroma of the finished product. Spices such as paprika contribute to red color of the finished product. Spices are also added for their antimicrobial and antioxidant effects (Brown, 2009).

A large number of cooked sausages like frankfurters are filled into casings. Casings used could be natural or synthetic, either edible or inedible. Natural casings are derived from the submucosa layer of the gastrointestinal tract of cattle, swine and sheep. The characteristics of natural casings are that they cohere well to the filled meat mass and shrink during cooking (Feiner, 2006). Synthetic casings which include cellulose casings and collagen casings are widely used in the production of frankfurters, cooked ham and mortadella sausages as they are more uniform in size and stronger than the natural casings. Cellulose casings are generally non edible whereas collagen casings made from beef may be edible or non-edible (Pearson and Gillett, 1996).

2.3.3 Production

Production of frankfurters involves many steps. The processing of frankfurters starts with the grinding of meat and fat chunks through a grinder plate to obtain uniform particles. The size of the openings in the grinder plate determines the size of the meat particles prior to mixing and chopping. Next, the meat particles are transferred to a bowl chopper where emulsification takes place. A bowl chopper is a machine which has a circular, rotating bowl and knife blades are attached perpendicular to the bowl. This aids in cutting and shearing of the meat mixture to a fine paste. Initially, the meat is cut in the bowl chopper at low speed of around 1000-1500 rev/min and then all the functional ingredients salt, phosphates, nitrite and spices are added followed by water and ice (Feiner, 2006). The salt and phosphate will start to solubilize the meat proteins into a fine paste. Half of the water and ice is added initially to maintain the temperature to near 0°C and chopping is continued until the muscles are homogenized and until the temperature of the batter reaches 4°C. After solubilization of proteins the remaining water, fat and binders are added to the chopper under vacuum and homogenization is continued until the final temperature reaches 10 to 11.7°C in poultry frankfurters, whereas, the temperature for pork sausage is 15.6 to 17.8°C and 21.1 to 22.2°C for beef (Keeton, 2001).

The sausage batter is filled into the casings using a stuffer under vacuum. Vacuum is applied during filling to remove any air pockets from entering into the casings, which may cause air pockets in the final product. The speed of filling depends on the diameter of casings to be used. The diameter of the casings will determine the diameter of the filling horn or pipe used. The batter enters the casings in a continuous feed and forms a

long sausages tube which is cut or linked to a desired length. Linking is done either by automated machines or by hand. Frankfurters are processed thermally by smoking in a smokehouse chamber which imparts color, appearance, taste, flavor and shelf life to the product (Ahmed et al., 1990). After cooking, the frankfurters are cooled; the casing is removed and the product is stored at refrigeration temperature.

2.4 Meat Emulsions

An emulsion consists of two immiscible liquids where one of the liquids is dispersed as fine droplet (dispersed phase) within the other liquid which forms the continuous phase (McClements, 2005). According, to this definition meat batters are not a true emulsion since they do not contain two liquid phases. However, chopped meat mixture is generally referred to as a “meat emulsion” because the suspension of fat globules dispersed within a continuous protein and water network suggests a similar structure to that of an emulsion (Hansen, 1960). A meat emulsion is a multiphase system formed by the comminution of meat, fat, salt and other ingredients (Varnam and Sutherland, 1995). Meat products such as frankfurters and bologna are examples of meat emulsions. In an emulsion, proteins are present in three different phases: the protein matrix, the aqueous phase and the interfacial film (IPF) around fat globules (Gordon et al., 1992). Meat proteins represent the major functional ingredients which serve as the natural emulsifying agent in a meat emulsion (Álvarez et al., 2007). The stability of a meat emulsion is affected by both the type and amount of protein in these phases (Montejano et al., 1984; Gordon et al., 1992).

2.4.1 Meat Proteins

Muscle proteins are often classified into three groups based on their solubility: myofibrillar proteins, sarcoplasmic proteins and the stromal proteins. The myofibrillar proteins which are salt soluble proteins (1% salt concentration) mainly consist of actin and myosin (Barbut, 1995). Myosin plays an important role in fat emulsification and water holding capacity of products like sausages (Xiong, 2000). The sarcoplasmic proteins which include myoglobin and other heme pigments are water soluble. Myoglobin is very important for meat color but plays only a minor role in meat protein functionality (Smith, 2001). The stromal proteins which are insoluble in water and salt include collagen, elastin and reticulin. Collagen is the major stromal protein which is abundant in poultry skin (Smith, 2001). Collagen when present at high levels in poultry formulations may interfere with the functionality of myofibrillar proteins (Smith, 2001). Collagen is converted to gelatin when cooked at high temperatures and so a high level of collagen can be detrimental to meat emulsion stability because of protein matrix degradation (Ladwig et al., 1989).

2.4.2 Meat Emulsion Theory

The stability of a meat emulsion is explained by two theories: the emulsion theory and the physical entrapment theory. The emulsion theory proposes the formation of an interfacial protein film (IPF) around the fat globules in the meat emulsion (Hansen, 1960). Myosin is the major protein that contributes to the mass of IPF. The emulsifying property of myosin plays a key role in stabilization of the emulsion (Varnam and Sutherland, 1995). Addition of salt during chopping of meat, changes the conformational structure of myofibrillar protein molecules by increasing the hydrophobicity of their

surface (Voutsinas et al., 1983). The heavy mereomyosin molecules, subunits of myosin having high hydrophobic surface area, orient towards the fat globules and light mereomyosin being hydrophilic, faces the aqueous phase. This results in the formation of a covering layer of myosin around the fat globules (Figure 2.1) (Jones, 1984). During cooking, the IPF undergoes changes during which IPF is penetrated by small pores and the exudation fat from these pores maintains the integrity of the IPF (Varnam and Sutherland, 1995).

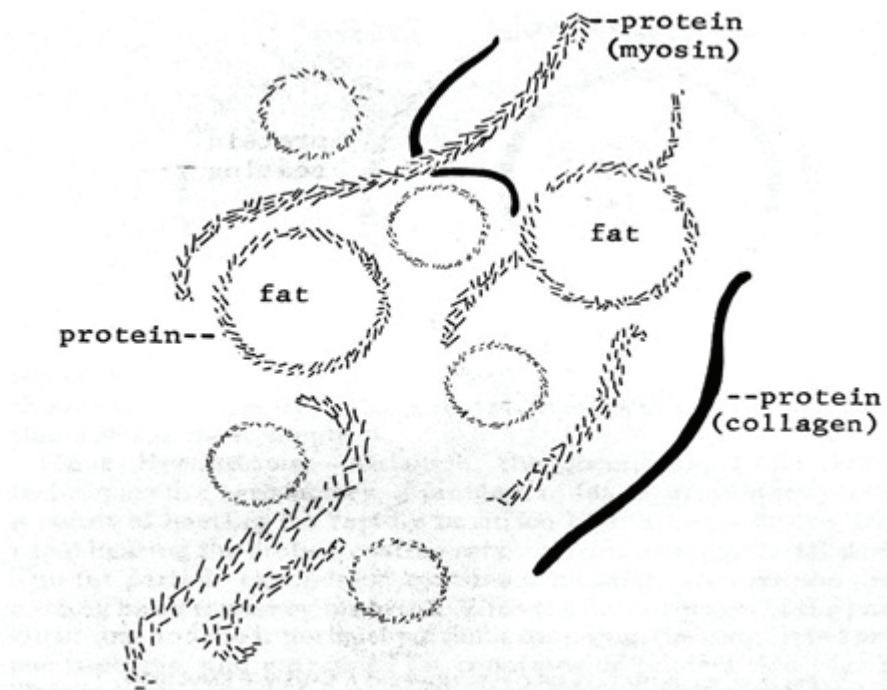


Figure 2.1 Schematic representation of meat emulsion showing solubilized protein and fat globules coated with protein (Source Pearson and Gillet, 1996).

The physical entrapment theory proposes that the fat within the meat batter is entrapped within the three dimensional protein matrix (Lee, 1985). Proteins coagulate during thermal processing and/or cooking, resulting in the formation of a gel-like structure, by binding together the batter structural units, e.g. the muscle myofibrils and

the emulsified fat particles and contributes to the development of the final product texture (Barbut, 1995; Xiong, 1999).

It is believed that both mechanisms may be involved in the formation of a meat emulsion (Barbut, 1995; Varnam and Sutherland, 1995) as numerous changes take place during the production of comminuted meat products like frankfurters. Thus, meat emulsion stabilization is the combination of formation of an IPF by the proteins and the formation of a gel matrix which restricts the movement of fat (Acton et al., 1983; Gordon and Barbut, 1997).

The properties of a meat emulsion depends on factors such as temperature, salt concentration for the extraction of meat proteins, size of fat droplets, pH, added milk or vegetable proteins and conditions of chopping (Zayas,1997). The reduction of particle size during chopping process and the amount of extracted proteins are important for stable meat emulsions which in turn have impact on the final product yield and quality (Jones and Mandigo, 1982). Larger fat and water separation will occur during cooking in unstable emulsions which will reduce the yield and the quality of the product (Álvarez et al., 2007)

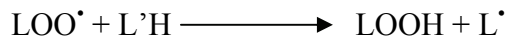
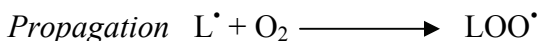
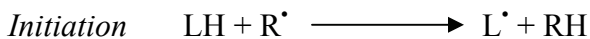
2.5 Lipid Oxidation

2.5.1 Lipid Oxidation in Meat Products

Lipid oxidation is one of the major causes of quality deterioration that affects the acceptance of meat and meat products (Ladikos and Lougovois, 1990; Baggio and Bragagnolo, 2006). Omega-3 fatty acid enriched meat products are highly susceptible to lipid oxidation because of the presence of a high number of unsaturated fatty acids with

double bonds (Olsen et al., 2005 and Jacobsen et al., 2008). Lipid oxidation has adverse effects on color, flavor and nutritive value of meat products (Gray and Pearson, 1987; Olsen et al., 2005) and also produces toxic compounds such as free radicals and reactive aldehydes (Fernández et al., 1997; Jacobsen, 1999; Jacobsen et al., 2008).

The most common mechanism by which the PUFA oxidizes is by autooxidation. The autooxidation reaction proceeds through a free radical mechanism involving three phases: initiation, propagation and termination (Raharjo and Sofos, 1993; Monahan, 2000). Oxidation in muscle foods starts with the initiation process at the highly unsaturated phospholipid fraction in subcellular membranes (Gray and Pearson, 1987). Initiation occurs when a hydrogen atom is extracted from the fatty acid molecule to form a lipid radical (L^\bullet). Propagation involves the reaction of the lipid radical (L^\bullet) with molecular oxygen to form a lipid peroxy radical (LOO^\bullet). This reaction is followed by extraction of another hydrogen atom from the unsaturated fatty acids and the chain reaction propagates. Lipid peroxides formed during the propagation process are unstable and are cleaved by reduction in the presence of trace elements to give a range of new free radicals and other non- radical compounds including alkoxy and hydroxyl radicals. These radicals are capable of propagation of further oxidation until stable compounds are formed at the termination phase.



Lipid oxidation in meat and meat products is affected by various internal and external factors. The internal factors include the total lipid content, fatty acid composition, types and amounts of iron present (Decker and Welch, 1990), natural antioxidants present in meat (α -tocopherol) and antioxidant enzymes (catalase, superoxide dismutase) (Renerre et al., 1996; Rojas and Brewer, 2007). The external factors include, processing procedures (heating, mincing, mixing) (Lee et al., 2006), cooking and storage temperature and additives used (Novelli et al., 1998). The use of substances with pro-oxidant and antioxidant activity such as salt, nitrite and phosphate in the processing of sausages also influences the oxidation process (Rhee, 1999).

2.5.2 Methods to Determine Lipid Oxidation in Meat Products

The oxidative status of meat products can be assessed on the basis of primary oxidation through the measurement of peroxide value or secondary oxidation through thiobarbituric acid reactive substances (TBARS), measurement as malonaldehyde (MDA) equivalents or cholesterol oxidation products (Grau et al., 2001). Lipid hydroperoxides formed during lipid oxidation are the primary products and aldehydes volatile compounds and cholesterol oxidation products are the primary or/and secondary products of lipid oxidation. The methods to detect lipid oxidation in meat products are classified as: detection of primary or secondary oxidation products; spectroscopy; fluorescence, chromatography and conjugated diene method (Fernández et al., 1997). However, sensory analysis is often used to detect oxidative off-flavors by taste or smell in order to decide if a lipid-containing food is suitable for consumption (Jacobsen, 1999). The ferrous oxidation-xylenol orange method is used to determine the primary oxidation product (Shantha and Decker, 1994; Grau et al., 2000).

2.5.3 Thiobarbituric Reactive Substances Assay (TBARS)

TBARS test is the most commonly used method to detect the secondary oxidation products such as MDA equivalents in meat and meat products (Raharjo and Sofos, 1993; Gomes et al., 2003). MDA is the breakdown product formed from oxidized PUFA (Shahidi and Hong, 1991; Fernández et al., 1997). The substances such as aldehydes, ketones, organic acids, esters, amides, amino acids, oxidized proteins, pyridines, pyrimidines, and vitamins which are not MDA but react with TBA are called TBA reactive substances (TBARS) (Guillen-Sans and Guzman-Chozas, 1998). TBARS assay is based on the reaction of one molecule of MDA with two molecules of TBA to form a pink complex which absorbs light at 532 nm (Ulu, 2004). The results are commonly expressed in milligrams of malonaldehyde equivalents per kilogram of meat (Pikul, et al., 1989). Different methods of assaying include: the distillation method (Tarladgis et al., 1960), an aqueous extraction method (Raharjo et al., 1993) and the spectrofluorometric method (Williams et al., 1983). Several modifications of the TBA test have been performed with meat products such as the addition of sulfanilamide for cured meat samples as nitrites interferes with the test (Shahidi et al., 1985), incorporation of an antioxidant in the distillation step and use of HPLC (Csallany et al., 1984). When residual nitrite is not present, or if it is present at a concentration of less than 100 ppm, the added sulfanilamide may lead to an underestimation of the TBA values (Fernández et al., 1997). Correlation of TBA with sensory evaluation to detect rancidity has been reported for chicken meat (Salih et al., 1987) and flavor deterioration in cooked meat (Poste et al., 1986).

TBARS assay has been used to evaluate lipid oxidation in sausages prepared with oils. For instance, Pelsler et al., (2007) studied lipid oxidation of fermented sausages prepared by addition of canola oil and flax oil at 3-6% and encapsulated flax oil and fish oil at 4.5%. They reported that the TBARS values of the sausages with canola oil and encapsulated oils were similar to that of the control, whereas the flax oil treated sausages had increased lipid oxidation after 12 weeks of storage at 7°C. Bishop et al. (1993) found that replacement of pork fat with pre-emulsified corn oil at 67% in bologna did not affect the lipid oxidation process. The TBARS value of chicken sausages prepared with 5% squid oil with synthetic vitamin E was between 0.20 to 0.50 mg/kg of MDA equivalents after 90 days of storage in the dark at 4°C (Andres et al., 2009). Park et al. (1989) also found low TBA values of 0.45 mg MDA equivalents/kg after 12 weeks of vacuum storage of low fat frankfurters prepared with high oleic acid sunflower oil. TBARS values of more than 2 mg of MDA equivalents/kg and 3 mg/kg have been found to be associated with rancid taste in beef burgers (Georgantelis et al., 2007) and in omega-3 fatty acid enriched fermented sausages (Cáceres et al., 2008) respectively. Melton (1983) reported that the TBA values of 0.3-1.0 mg/kg in beef or pork, 1.0 to 2.0 mg/kg in chicken and more than 3.0 mg/kg in turkey meat were associated with oxidized flavors.

2.6 Instrumental Measurement of Color

Color is an important quality attribute of meat products which has a major influence on consumer acceptability (Hutchings, 1999). Consumers prefer bright-red color for fresh meats, brown-gray color for cooked meats and pink color for cured meats (Jo et al., 2000). Consumers choose meat products based on the visual appearance (Barbut, 2002a). Color measurement as a quality parameter is used for analysis of quality changes as a

result of food processing and storage. The use of sensory panels to measure color is complex, time-consuming, expensive and subject to error because of the complex process of color perception in humans (Ansorena et al., 1997). Therefore instrumentation based on the spectral characteristics and color co-ordinates of samples is often used for measurement of color (Ansorena et al., 1997).

The visual appearance of color by human eyes is the result of reflected light coming back from the object (Barbut, 2002b). Visible light is found between 380 and 750nm in the electromagnetic spectrum. When light strikes an object it is reflected, absorbed or transmitted. The reflected light determines the color of the object which depends upon the light source, amount of light, the observer's angle of view, object size and background differences (Figura and Teixeira, 2007).

The methods used for color measurement are classified into: visual; spectral photometry and reflectance colorimetry, where reflectance colorimetry is the most commonly used method (Barbut, 2002b). Hue, lightness and saturation are commonly used to describe the perception of color (Commission Internationale de l'Éclairage (CIE), 1987). Many color scales or schemes such as Munsell, Hunter and CIE are used for color specifications. The most commonly used method for color assessment in the food industry is Hunter L*, a* and b* system which is based on food colorimetry. The Hunter L*, a*, b* system measures the degree of lightness (L*), redness (a*) and yellowness (b*). Lightness is measured on a scale of 0-100 where L*= 0 represents black and L*=100 being white. A positive value of a* indicates redness whereas negative value indicates greenness. The positive and negative values of b* indicates yellow and blue, respectively (Hunter Lab, 1999). Most color measurements of food products

recommend the use of the average of 2 readings per sample by rotating the sample at 90° (Giese, 2000). The use of scanning color equipment which utilizes the actual light source similar to the source that employed in stores will give better results (Barbut, 2002b). D65 (daylight) illuminant and standard observer angle of 10° is commonly used for the evaluation of color of sausages (Ansorena et al., 1997; Muguerza et al., 2002).

The color of sausages mainly depends on the myoglobin content of the meat used (Toldra and Reig, 2007). For instance, the use of chicken breast in preparation of sausages results in light product color due to low myoglobin content. However, use of leg meat results in darker color (Barbut, 2002b). The typical pink color of cooked sausages like frankfurters is due to the formation of nitrosylferrohaemochrome from nitrosomyoglobin during the heating process (> 65°C) (Toldra and Reig, 2007). Nitrosomyoglobin (reddish color) is formed by the reaction of added nitrites with myoglobin. During the heating process, the globin part of the meat pigment denatures from the iron and surrounds the heme moiety (Pegg and Shahidi, 2000). The processing steps such as mincing, mixing and cooking together with the addition of additives and spices influence the final color of sausages (Pérez-Álvarez and Fernández-López, 2008).

The effect of replacement of fat with various vegetable oils (olive oil, corn oil, flax oil, hazelnut oil, palm oil, canola oil, etc.) and fish oils on color of a variety of sausages has been studied extensively by meat researchers (Bishop et al., 1993; Bloukas and Paneras, 1993; Bloukas et al., 1997; Tan et al., 2001; Muguerza et al., 2002; Severini et al., 2003; Pelsler et al., 2007; Cáceres et al., 2008; Hur et al., 2008; Yıldız-Turp and Serdaroğlu, 2008; Andres et al., 2009). However, there is lack of literature on the color evaluation of chicken frankfurters related to the replacement of fat with oils. Only few

studies are found in the literature related to replacement of fat with fish oil (Andres et al., 2009) and palm oil (Tan et al., 2006) in chicken sausages/frankfurters.

2.7 Instrumental Evaluation of Texture

The texture of food is an important quality attribute which influences the acceptance of food by consumers (Herrero et al., 2007). Many instrumental methods have been developed for the determination of texture in food (Bourne, 2002b; Kilcast, 2004). Determination of textural parameters is based on compression, shear, penetration, tension and torsion tests (Barbut, 2002b). Nowadays, the most commonly used method is texture profile analysis (TPA), which is based on the compression test. This test imitates the conditions to which the food is chewed in the mouth or cut on the plate (Bourne, 1978).

2.7.1 Texture Profile Analysis (TPA)

TPA was first developed by Friedman et al. (1963) of General Foods (GF) used a texturometer to measure TPA parameters. The GF-TPA was first applied to meats by Szczesniak et al. (1963). The TPA test is based on the compression of a cylindrical sample in two cycles. In the first cycle, the sample is compressed to a certain predetermined deformation and then compressed further in the second cycle (Bourne, 1978). The definitions of TPA parameters developed by Szczesniak et al. (1963) were modified by Bourne (1978). The testing conditions for TPA such as sample dimension, deformation level and compression rate are found to vary in texture measurements of meat products. Mittal et al. (1992) reviewed the testing conditions for texture of meat products and found that specimen length (L) varied from 10 to 20 mm, diameter (D) from 13 to 73 mm and D/L ratio from 1 to 4. The compression ratio varied from 50 to 85% and cross-head speed from 5 to 200 mm/min. The testing conditions for frankfurter type

products have been found to vary in the works of Sofos et al. (1997), Keeton et al. (1984) and Correia and Mittal (1991). Reporting of testing conditions is important to obtain reliable data and TPA parameters are more comparable when performed by standard procedures (Barbut, 2002b). Texture determination of foods by TPA method has often been found to correlate with the textural characteristics by sensory evaluation (Bourne, 2002). For the sausages prepared by replacement of animal fat with vegetable oils TPA method has commonly been used to determine the textural parameters (Park et al., 1989; Bishop et al., 1993; Muguerza et al., 2002; Tan et al., 2006; Pelsler et al., 2007; Jiménez-Colmenero et al., 2010) and fish oils (Park et al., 1989; Pelsler et al., 2007; Cáceres et al., 2008; Andres et al., 2009). Park et al. (1989) used the TPA method as described by Bourne (1978) to measure the texture of beef sausages prepared with 5% fish oil. They found that the addition of fish oil increased the firmness and springiness of sausages which also correlated with the sensory evaluation. Cáceres et al. (2008) also found that the addition of pre-emulsified fish oil at 5-6% to bologna sausage resulted in increased firmness because of the addition of caseinates during pre-emulsification. Pelsler et al. (2007) found that the addition of encapsulated fish oil at 4.5% in fermented sausages was found to have higher hardness than the control whereas addition of flax oil at 6% resulted in lower firmness than the control.

2.7.2 Shear Test

There are other instrumental methods to determine texture of food besides TPA that provide additional information on the textural properties of the meat products (Herrero et al., 2008). Shear test is a commonly used method to determine the toughness of meat and meat products (Flores and Toldrá, 1993). The use of shear tests to determine tenderness

had been found to correlate well with the sensory hardness for chicken breasts (Xiong et al., 2006). Warner-Bratzler (W.B.) is one of the most commonly used shear tests (Bratzler, 1949). This test employs a single blade to cut the meat sample. The result provides the peak force required to shear the sample otherwise known as the W.B shear force. The result for the W.B shear test depends on various factors which include uniformity of sample size, presence of connective tissues and fat, sample temperature and speed of shearing (Zhang and Mittal, 1993). Another method used to measure texture is the Allo-Kramer test (AKS) (Kramer et al., 1951). This test uses a cell which consists of 10-13 blades guided into a square box to compress and shear the sample. Cáceres et al. (2008) used AKS test to evaluate the texture of omega-3 fatty acid enriched bologna sausage and found that the addition of fish oil at 6% resulted in increased shear work. Thus the use of shear test was found to afford results similar to the TPA test.

2.8 Sensory Evaluation

Sensory evaluation of food has been defined as a scientific method used to evoke, measure, analyze and interpret responses to products as perceived through the senses of sight, touch, smell, taste, and hearing (Lawless and Heymann, 1998). Sensory analysis provides a better understanding of consumer perception of food products (Matulis et al., 1994). The development of new food products by modification of ingredients or processing conditions, cost reduction and quality control, often employs sensory evaluation techniques to determine the acceptability of food (Barbut, 2002b). Sensory evaluation methods are classified into discriminative tests, descriptive and affective methods (Lawless and Heymann, 1998). Discriminative tests are often used to determine the existing differences between products and affective method focuses on the consumer

evaluation of products. Among these methods, descriptive test is the most useful method for providing an in depth sensory properties of meat products (Hayes, 2009).

Descriptive sensory analysis involves the detection and description of both the qualitative and quantitative sensory aspects of food by trained panels (Meilgaard et al., 1991). The qualitative attributes of products which include aroma, appearance, flavor, texture, after taste and sound properties of the products are detected first by the panels and then quantified to facilitate the description of the perceived attributes of the products (Murray et al., 2001). Descriptive methods are most commonly used for product development, shelf life testing and consumer perception of products (Lawless and Heymann, 1998). The panels used in descriptive sensory methods play the role of measuring or testing devices to measure specific attributes of food. The panelists are screened with initial tests, trained to familiarize the test procedures and used to evaluate the attributes of interest of the product (Meilgaard et al., 1991). A scaling system is used to measure the responses of the panels (Meilgaard et al., 1991).

Different methods of descriptive analysis used are: the flavor profile method (FPM) (Cairncross and Sjostrom, 1950), the texture profile method (TPM) (Brandt et al., 1963), quantitative descriptive analysisTM (QDA) (Stone et al., 1974), the spectrumTM method (Meilgaard et al., 1991), quantitative flavor profiling (QFP) (Stampanoni, 1993), free-choice profiling (FCP) (Williams and Langron, 1984) and generic descriptive analysis (Lawless and Heymann, 1998). FPM is used to detect flavor of the food while TPM is used to evaluate the texture. In the spectrum method the perceived intensities are recorded in relation to universal scale that is constant for all products. QFP focuses on the descriptive attributes of flavor only and FCP is developed to evaluate the products based

on consumers' perception of products. The generic descriptive analysis is the most commonly employed method which uses the combination of different methods (Murray et al., 2001). Descriptive sensory methods have been used in the evaluation of frankfurters prepared with vegetable oils by trained and untrained panels (Paneras et al., 1998); (Bloukas and Paneras, 1993; Jiménez-Colmenero et al., 2010; Tan et al., 2006). Descriptive sensory method has been employed in studies of fermented sausages prepared with hazelnut oil (Yıldız-Turp and Serdaroğlu, 2008), olive oil (Bloukas et al., 1997), flax oil (Pelser et al., 2007) and fish oil (Pelser et al., 2007; Cáceres et al., 2008; Andres et al., 2009). Cáceres et al. (2008) employed descriptive sensory method using a non-structure 10 cm scale to evaluate the effect of omega-3 fatty acid enriched bologna sausages with fish oil (up to 6%). They found that addition of fish oil did not affect the odor and overall acceptability.

2.9 Summary

Omega-3 fatty acids are essential fatty acids which have many beneficial health effects for humans. The current intake of omega-3 in developed countries is below the dietary recommendations and there is a high ratio of n-6: n-3. This ratio is important because high intake of omega-6 interferes with the metabolic pathway of ALA and results in production of more eicosanoids from arachidonic acid such as prostaglandins, thromboxanes, leukotrienes etc. High levels of eicosanoids lead to the formation of thrombus, allergic and inflammatory disorders. To meet the increasing demand for omega-3 enriched foods, several products enriched with omega-3 fatty acids have been introduced to the market. Meat being a center piece of the diet in Western countries is a natural choice for enrichment with omega-3 fatty acids. Most of the research related to

omega-3 enrichment in meat products has been carried out with pork and beef sausages. There is limited literature available on the incorporation of oils into chicken products. Moreover, incorporation of flax oil into sausages is very limited. The replacement of animal fat with encapsulated fish oil in chicken sausages has not been previously reported. Incorporation of oils into chicken frankfurters would provide knowledge about the interaction of oils with proteins in a chicken meat emulsion. Production of chicken frankfurters enriched with omega-3 fatty acids requires the evaluation of the textural and sensory attributes for their acceptance in the market place.

2.10 Objectives and Hypothesis

The main objective of this research project was to develop an omega-3 fatty acid enriched chicken frankfurter acceptable to consumers. The sub objectives to accomplish the main objective were:

1. To incorporate flax oil or microencapsulated fish oil as a source of omega-3 fatty acids.
2. To study the chemical composition, fatty acid profile and lipid oxidation of omega-3 fatty acid enriched chicken frankfurters.
3. To evaluate the effect of omega-3 fatty acids on color and texture of chicken frankfurters.
4. To study the acceptability of omega-3 fatty acid enriched chicken frankfurters by sensory evaluation.

It was hypothesized that the incorporation of flax and microencapsulated fish oil into chicken frankfurters would result in changes to the fatty acid profile so that the

product meets the specifications of an omega-3 fatty acid enriched food. Moreover, the omega-3 enrichment of chicken frankfurters would not negatively impact the chemical composition, fatty acid profile, texture, flavor and overall acceptability. The addition of oils which are high in PUFAs would make the product more susceptible to oxidation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Chicken Frankfurters Enriched with Omega-3 Fatty Acids

3.1.1 Materials

Boneless chicken thigh meat, mechanically separated chicken meat (MSM), chicken skin, flax oil and microencapsulated fish oil were used as raw materials. Commercial flax oil, cold pressed (Omega nutrition®, Canada) and microencapsulated fish oil powder of Ocean Nutrition Canada Ltd. were used as lipid sources.

3.1.2 Preparation of Chicken Fat from Skin

Chicken fat contains high a proportion of unsaturated fatty acids and less cholesterol than the fat from red meats. Chicken fat can be obtained by rendering of chicken skin which is byproduct of poultry industry. Before the day of preparation of frankfurters, chicken fat was rendered by placing 4 kg of chicken skin in a conventional oven at 121°C for 1 hr. Cooled to room temperature for 30 min and kept at 4°C for 3 to 4 hr. When cooled, fat was stored at 4°C. After removing the fat, the bottom layer had gel like consistency which was separated as gelatin and stored at 4°C.

3.1.3 Chicken Frankfurter Manufacture

Boneless chicken thigh meat and mechanically separated chicken meat (MSM) were thawed at 4°C for 24 hr before the day of preparation of frankfurters. A 4 kg batch of chicken frankfurters for each treatment was prepared, following the procedure as presented in the Figure 3.1. A total of seven treatments were prepared and each batch was replicated four different times. The formulations of chicken frankfurters are shown in the

Table 3.1. The seven treatments were flax oil at three levels (1.2, 2.4, and 3.6%), microencapsulated fish oil powder at three levels (1.2, 2.4, and 3.6%) and control. The control treatment was produced with an additional 3.5% chicken fat which was substituted partially or entirely with flax oil or fish oil. Oil levels were chosen based upon the level needed to enable product to be labeled as omega-3 fatty acid enriched according to CFIA regulations. A level of 1.2% would provide approximately 300 mg of omega-3 fatty acids per 100 g in the final product which meets the minimum specifications for labeling the product as omega-3 enriched. More extreme levels were chosen to study the effect of incorporation of omega-3 fatty acids at higher levels on the oxidative stability of PUFAs, texture and sensory properties of chicken frankfurters. Extracted gelatin from chicken skin was added to make up for the fish gelatin content (40%) present in the encapsulated fish oil.

Boneless chicken thigh meat and chicken skin were ground separately using a plate with 3 mm holes (Hobart Meat grinder, Model 4246S, Ohio, USA). The required quantities of all the ingredients and meat formulation were mixed in a bowl chopper (Mainca, Model CM 21, Barcelona, Spain) in the following order. Half the quantity of chicken thigh meat and MSM was first chopped for several revolutions of the bowl with STPP dissolved in water. The spices were added with half of the ice and chopped to a fine paste during which the temperature remained at 0°C. Then the remaining meat, MSM, binders (milk powder, corn starch), skin, fat, gelatin, oil and /or chicken fat and the remaining ice were added. The mixture was chopped to the endpoint temperature of 4°C to create a stable emulsion.

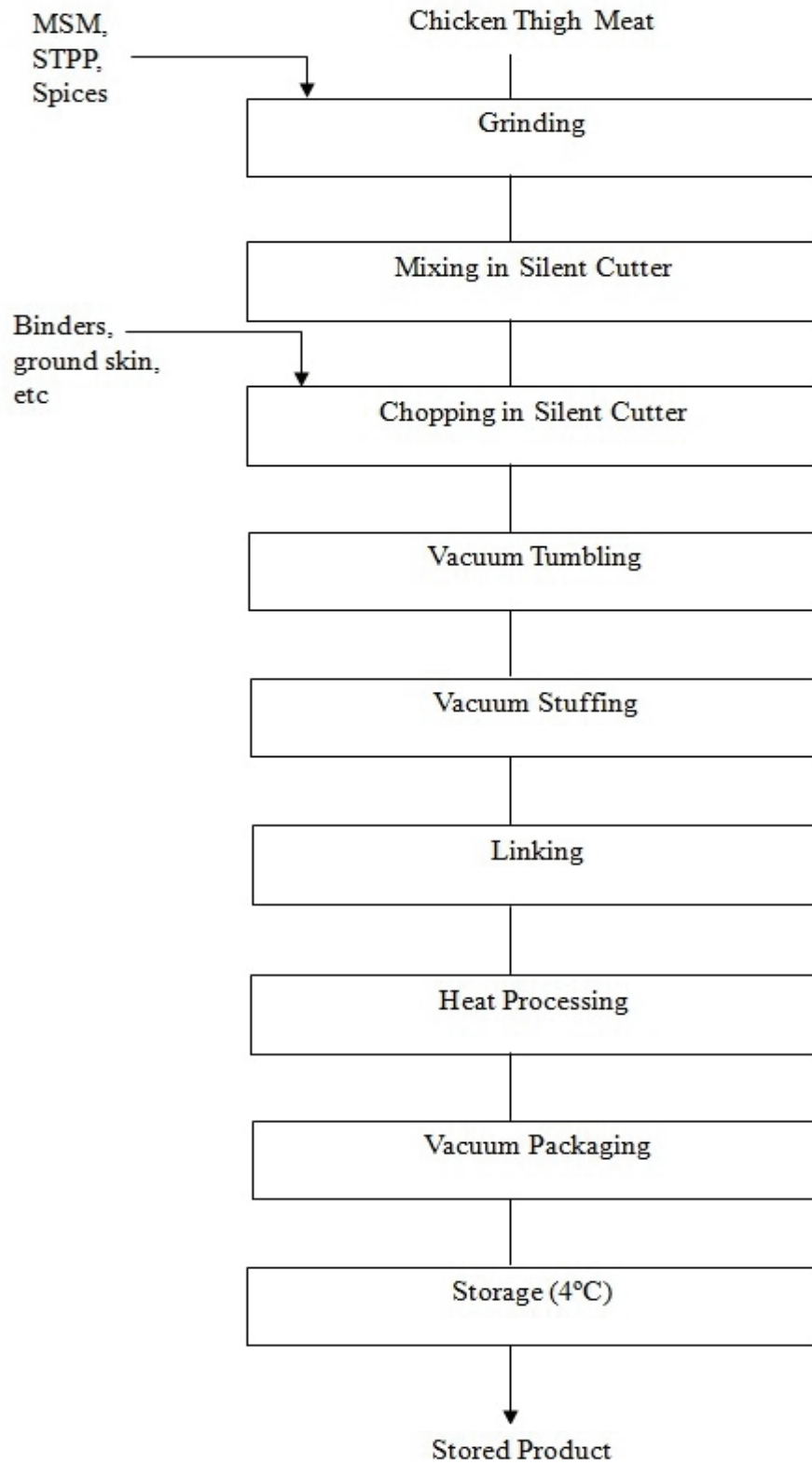


Figure 3.1 Flow diagram of frankfurter preparation

Table 3.1 Chicken frankfurter formulation

Ingredients (g)	Flax oil (1.2%)	Flax oil (2.4%)	Flax oil (3.6%)	Fish oil (1.2%)	Fish oil (2.4%)	Fish oil (3.6%)	Control
Chicken fat	96	48	-	96	48	-	144
Oil	48	96	144	80	160	240	-
Chicken gelatin	144	144	144	112	80	48	144
Chicken thigh meat	1148	1148	1148	1148	1148	1148	1148
Mechanically separated meat	800	800	800	800	800	800	800
Chicken skin	928	928	928	928	928	928	928
Water/Ice	720	720	720	720	720	720	720
Salt	62	62	62	62	62	62	62
Prague powder (6.25% Sodium nitrite)	12	12	12	12	12	12	12
Sodium erythorbate	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Sodium tripolyphosphate	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Garlic powder	8	8	8	8	8	8	8
Black pepper	4	4	4	4	4	4	4
Mustard powder	10	10	10	10	10	10	10
Skim milk powder	52	52	52	52	52	52	52
Corn starch	100	100	100	100	100	100	100
Total	4144	4144	4144	4144	4144	4144	4144

The mixture was vacuum tumbled (Lumbar Ideal Inc., Model LU25A, Quebec, Canada) at 20 psi pressure for 10 min to remove air pockets and stuffed (Mainca, Model EM20, Barcelona, Spain) into 22 x 84 mm cellulose casings. Sausages (Frankfurters) were hand linked into single 40g portions and stored at 4°C overnight. The next day the weight of the frankfurters was recorded before thermal processing.

The frankfurters were cooked with application of natural smoke (Enviro-Park Microprocessor MP 1000, Model CVU-200E, Oregon, USA) to a final core temperature of 74°C. Cooking involved 7 stages programmed into the oven (Table 3.2). The last stage was showering off the product with cold water. After processing, the frankfurters were weighed to calculate the cook yield. Individual frankfurters were randomly selected for each treatment, vacuum packaged (Lumbar Ideal Inc., Model T2-19, Quebec, Canada) four frankfurters to a package and stored at 4°C.

Table 3.2 Processing conditions for chicken frankfurters

Stage	Time (min)	Oven Temperature (°C)	Core Temperature (°C)	% RH
1	15	54		
2	15	54		
3	30	60		
4	15	68	58	48
5	15	68	60	48
6	15	82	74	48
7	10	Shower		99

3.2 Sampling

Four chicken frankfurters from each treatment were removed from refrigerated storage for the analysis of color, texture and lipid oxidation following storage for 0, 10,

20 and 30 days at 4°C. Fatty acid analysis and proximate analysis were performed on immediately frozen frankfurters.

3.3 Experimental Design and Statistical Analysis

A completely randomized block design with 7 treatments and 4 storage periods as main effects was used. For each of four batches of chicken meat there were seven treatments including, three levels of flax oil, three levels of encapsulated fish oil and control. The batch of meat was used as block and considered as a random variable. Four chicken frankfurters per treatment were randomly assigned to each storage time (0, 10, 20 and 30 days). The response variables measured were proximate composition (crude fat, protein and moisture), fatty acid profile, lipid oxidation, color, texture and sensory evaluation. All the parameters were analyzed by ANOVA with the Proc Mixed procedure of SAS version 9.1 (Littell et al., 1996; SAS institute Inc., 2003).

The data for color, texture and lipid oxidation were subjected to repeated measure analysis. If significant main effects or interactions were found, the Tukey test was used to compare differences among the least square means at $P \leq 0.05$. Also, if a significant difference was found in the main effect, orthogonal contrasts were performed to compare the oil treatments (flax vs fish, flax vs control and fish vs control). Any data that did not follow a normal distribution was transformed before analysis. The null (H_0) and alternative (H_A) hypothesis for individual response variables are indicated as:

H_0 : there were no differences among oil treatments ($H_0: \mu = 0$)

H_A : there were differences among oil treatments ($H_A: \mu \neq 0$)

The statistical model for this design is represented as:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijk}$$

Where Y is the response variable, μ is the overall mean, α_i is the effect of block ($i=1-4$), β_j is the effect of treatments ($j=1-7$) and γ_k is the effect of storage days ($k = 1-4$), $(\beta\gamma)_{jk}$ is the effect of a two-way interaction between treatments and days and ε_{ijk} is the random effect of error.

The data for proximate composition and fatty acid analysis were subjected to one-way ANOVA according to the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where, Y is the response variable, μ is overall mean, α_i is the effect of oil treatments, β_j is the effect of block and ε_{ij} is the residual error.

CHAPTER 4

CHEMICAL ANALYSIS OF OMEGA-3 FATTY ACID ENRICHED CHICKEN FRANKFURTERS

4.1 Abstract

Omega -3 fatty acids are essential nutrients for human health and the fortification of meat products with these fatty acids is an emerging practice to meet the demand for omega-3 enriched products. The partial replacement of animal fats with oils rich in omega-3 fatty acids would produce healthier meat products. Chicken frankfurters were manufactured by replacing chicken fat with either flax oil or microencapsulated fish oil at 1.2%, 2.4% and 3.6% of the batter. The frankfurters were cooked to endpoint temperature of 74°C, vacuum packed and stored at 4°C for 30 days in the dark. The chemical composition and fatty acid profile of the frankfurters were determined. Lipid oxidation of these frankfurters was monitored following 10, 20 and 30 days of refrigerated storage. No differences were detected in moisture and fat content between the control and oil added frankfurters ($p < 0.05$). Protein content in the 3.6% microencapsulated fish oil treatment ($14.34\% \pm 0.46$) was higher than the control ($11.62\% \pm 0.46$). Omega-3 PUFA content of all the treatments was significantly higher than the control except in 1.2% microencapsulated fish oil. High omega-3 PUFA content in flax oil treatments (843 to 1740 mg/100g of frankfurter) was mainly due to increased amount of α -linolenic acid compared to the control (364.5mg/100g of product). Eicosapentaenoic acid and docosahexaenoic acid were the main source of omega-3 in microencapsulated fish oil treatments. EPA and DHA content increased with increasing levels of microencapsulated fish oil ranging from 137.42 to 361.76 mg/100g of product and 102.32 to 258.46 mg/100g of product, respectively. Omega-6/omega-3 decreased with increasing levels of flax oil (1.69 to 3.67) and microencapsulated fish oil (2.55 to 4.79) compared to control (9.31). PUFA/SFA ratio increased with increasing levels of flax oil while there was no difference in the ratio in microencapsulated fish oil treated frankfurters. There was no difference in lipid oxidation between oil added frankfurters and control as determined by thiobarbituric reactive substances assay. During storage for 30 days at 4°C an increase in TBARS was observed on day 30 (3.47 ± 0.11 mg/kg of product) compared to day 10 (3.06 ± 0.11 mg/kg of product). The oil added frankfurters can be considered as technologically viable omega-3 fatty acid enriched food.

Keywords: omega-3, chicken frankfurter, microencapsulated fish oil, lipid oxidation, EPA, DHA

4.2 Introduction

Frankfurters are very popular and frequently consumed meat products with high consumption in many countries (Jimenez-Colmenero et al., 2010). In frankfurters 20-25% of the overall composition is derived from animal fat which often contains a high amount of saturated fatty acids (Ordonez et al., 2001). The intake of saturated fatty acids has been found to increase low density lipoprotein (LDL) cholesterol which is associated with diet related diseases like cardiovascular disease (CVD) (Decker and Park, 2010). The consumption of polyunsaturated fatty acids (PUFA), especially omega-3 fatty acids may reduce the incidence of CVD. Long chain omega-3 polyunsaturated fatty acids (LC ω -3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA) are prime examples of these (Wang et al., 2006). The lack of omega-3 PUFA in the Western diet and increased consumption of omega-6 have resulted in a ratio of omega-6 to omega-3 that is 10-15: 1 instead of 1- 4:1 which has been demonstrated to be more healthful for humans (Simopoulos, 2008). Therefore, manipulations that reduce saturated fatty acids and increase in omega-3 fatty acids in high fat meat products like frankfurters could become very marketable products for health conscious consumers.

The fatty acid profile of frankfurters can be altered with processing strategies that replace animal fat with more healthful oils (Jimenez-Colmenero, 2010). The addition of carefully selected oils can reduce the saturated fatty acid content and increase the PUFA content (omega-3) (Jimenez-Colmenero, 2007). A variety of plant derived oils such as olive, flaxseed, canola, etc. and marine derived fish oil and algal oils have been used to modify the fatty acid profile of beef and pork frankfurters and bologna sausages (Jimenez-Colmenero, 2007). A number of studies have been carried out in the past to

modify the fatty acid profile of meat products especially by addition of olive oil (Bloukas et al., 1997; Muguerza et al., 2002 Jimenez-Colmenero et al., 2010; Lopez-Lopez et al., 2009). The use of flaxseed oil in meat products is limited because of its high oxidizing nature (Pelser et al., 2007). Flaxseed oil is a rich source of the omega-3 fatty acid (50-60% ALA) while fish oil is rich in EPA and DHA (Moghadasian, 2008). Incorporation of these oils into frankfurters, a widely consumed product would result in omega-3PUFA enrichment of frankfurters that would appeal to health conscious people. Frankfurters that are commercially available is made of, mainly, of mechanically separated meat (MSM) because of its cheap cost. However, the use of MSM is associated with increased hardness and more prone to lipid oxidation because of high content of heme pigments. Enrichment of meat products with omega-3 PUFA also increases the susceptibility of the product for lipid oxidation. This problem of lipid oxidation in frankfurters by incorporation of omega-3 fatty acids can be overcome by the use of encapsulated oils (Jacobsen, 2010).

Microencapsulation is defined as a process by which liquid droplets, solid particles or gaseous compounds are entrapped into thin films of a food grade microencapsulating agent (Gharasalloui et al., 2007). Microencapsulation of oils delivers the oil in the form of powder thus protecting the oil from lipid oxidation and also increases the bioavailability of omega-3 fatty acids (Jacobsen, 2010). The availability of encapsulated fish oils makes it easier to deliver the omega-3 fatty acids into meat product like frankfurters (Decker and Park, 2010).

In order to increase the omega-3 fatty acid content in chicken frankfurters, flax oil and microencapsulated fish oil were incorporated into frankfurter formulation at 1.2%,

2.4% and 3.6% by replacing chicken fat. There is no available literature on the use of flax oil and microencapsulated fish oil in chicken frankfurters. Therefore, the present study characterizes the chemical composition, fatty acid profile and lipid oxidation of the oil added frankfurters.

4.3 Materials and Methods

4.3.1 Chemical Composition

Moisture, protein and fat content of chicken frankfurters prepared with the addition of flax oil and microencapsulated fish oil were analyzed. Moisture content of the chicken frankfurters before storage was determined as follows: 2 chicken frankfurters from each treatment were ground to fine particles by using tabletop meat grinder (Waring Pro, Ontario, Canada). The ground chicken frankfurters were weighed (approximately 20g) and then frozen at -20° C. The frozen chicken frankfurters were then freeze-dried at -20° C for 24 hr and then weighed to calculate dry matter using the AOACS (2005) method 935.29. Moisture content was calculated by subtracting % dry matter from 100. Protein content was determined in quadruplicate using a Leco Nitrogen analyzer (Leco Corporation, St Joseph, MI) according to the AOACS (2005) method 990.03. Fat % or ether extract % was determined on freeze dried samples in duplicates using soxhlet fat extraction apparatus with petroleum ether as the solvent by the AOACS (2005) method 982.23. All the chemical composition determinations were done in quadruplicate.

4.3.2 Determination of Fatty Acid Profile

Extraction of lipids from raw ingredients (chicken thigh meat, MSM, chicken fat, flax oil and microencapsulated fish oil powder) and frankfurters was carried out

according to the modified procedure of Folch et al. (1957) using chloroform/methanol/water (8:4:3,v/v/v) as the extraction solvent. The lipids were extracted from 0.5g samples after homogenization (Polytron, Switzerland) at medium speed with 5ml of 2:1 (v/v) chloroform/ methanol (C/M) and vacuum filtered. Three mg of internal standard (C23:0) (Sigma) dissolved in 2:1 (v/v) C/M and 0.7% sodium chloride were added to each milliliter of the filtrate. The layers containing lipid phase (lower phase) was separated from non- lipid phase (upper phase) after centrifugation at 1000 rpm for 15 min at 4°C.

The lower phase containing lipids was filtered after rinsing with chloroform 3 times. Fatty acids (FA) were evaporated using nitrogen and then converted to FA methyl esters (FAME) with acidified methanol (Hilditch reagent). The FAMEs were dissolved in hexane to a concentration of 50 mg/mL and stored at -20°C until analysis.

Fatty acid analysis of FAMEs was performed by gas chromatography (GC) using a Perkin Elmer Autosystem II Capillary GC equipped with a flexible fused silica column (30m × 0.25mm ID) coated with 50% cyanopropyl polysiloxane (0.25µm film thickness; J and W DB-23; Folsom, CA) and flame ionization detector. Helium was used as the carrier gas and the gas line was equipped with an oxygen scrubber. The GC was set up with the following temperature program: 153°C for 2 min, ramp at 2.3°C/min, hold at 174°C for 0.2 min and hold at 220°C for 3 min after ramping at 2.5°C/min. Up to 66 FAMEs were identified and individual FAME was reported as weight percent of the total for raw ingredients. Each fatty acid was reported as weight per 100g of chicken frankfurter. Fatty acid analysis was carried out on two frankfurters from each treatment in duplicate for a total of 56 samples.

4.3.3 Determination of Lipid Oxidation

Lipid oxidation of chicken frankfurters during storage for 30 days was determined by TBARS assay according to a modified method of Juncher et al. (2000). TBARS test was carried out by homogenization (Polytron, Switzerland) of a 5 ± 0.2 g sample with 8.75 mL of 7.5% trichloroacetic acid and 1.25mL sulphanilamide at approximately 15000 rpm for 1 min. The homogenized samples were centrifuged at $500 \times g$ for 10 min at $4^{\circ}C$ and the supernatant was removed and mixed with 5mL of 0.02M thiobarbituric acid (TBA). All the steps were carried out at $4^{\circ}C$ by placing the samples on ice. The samples were then heated at $95-100^{\circ}C$ for 30 min. After cooling for 5 min on ice bath, the samples were centrifuged at $500 \times g$ for 2 min. The absorbance of the samples was measured at 532nm and 600nm in a 96 well microplate using reader. Malonaldehyde (MDA) standards were prepared using 1, 1, 3, 3-tetraethoxypropane (TEP) at a concentration of 0.0001mM to 0.05mM. TBARS results were expressed as mg of MDA equivalents/kg of the product. TBARS assay was carried out on four chicken frankfurters from each treatment for storage days 0, 10, 20 and 30.

4.4 Results and Discussion

4.4.1 Chemical Composition

Frankfurters treatments were formulated to be similar in all aspects of proximate composition such that only difference between treatments would be fatty acid profiles. The proximate composition of chicken frankfurters prepared by addition of oils is reported in Table 4.2. There was no difference ($p>0.05$) in the moisture content for all formulations.

Table 4.1 Chemical composition of chicken frankfurters

Treatments	Moisture %	Fat %	Protein%
Flax oil 1.2%	58.63a	21.42a	12.73abc
Flax oil 2.4%	59.42a	20.8a	12.35bc
Flax oil 3.6%	58.55a	20.37a	13.05abc
Microencapsulated fish oil powder 1.2%	58.15a	20.12a	13.09abc
Microencapsulated fish oil powder 2.4%	55.26a	21.5a	13.69ab
Microencapsulated fish oil powder 3.6%	55.26a	20.75a	14.34a
Control	57.06a	21.27a	11.62c
SEM	1.08	0.60	0.46
ANOVA	P-Value	P-Value	P-Value
Treatments	0.072	0.60	0.001

SEM- Standard error of mean

Means with different letters in the same column are significantly different ($p < 0.05$).

The replacement of fat with flax oil and microencapsulated fish oil powder did not alter the fat content ($p > 0.05$). Protein content of cooked chicken frankfurters was close to the target level of 14%. The protein content of chicken frankfurters with flax oil was not different from that of the control samples ($p > 0.05$). Frankfurters with microencapsulated fish oil at 2.4% and 3.6% levels were found to have higher ($p < 0.05$) protein values than the control. All chicken frankfurters were formulated with the same muscle protein content and chicken gelatin was added to all the treatments to make up the protein content in fish oil powder due to microencapsulation. Added chicken gelatin had water in it. Gelatin is added to meat products like sausages to increase the protein content and also to improve the texture (Prabhu, 2003). The higher protein values for 2.4% and 3.6% microencapsulated fish oil powder treated frankfurters could be due to the fish gelatin

present in the microencapsulated fish oil. Added chicken gelatin was hydrated while gelatin in fish powder was dry. This was likely to lower level of protein in non fish oil treatments. Pelsler et al. (2007) reported that the addition of flax oil at 3 to 6% and encapsulated fish oil at 4.5% did not affect the protein content of fermented pork sausages.*

4.4.2 Fatty acid Composition

Fatty acid composition of raw ingredients used for the preparation of chicken frankfurters showed high amount of PUFA in flax oil (65.23%) and 40.10% in microencapsulated fish oil (Table 4.2). Alpha-linolenic acid is the major source of omega-3 PUFA in flax oil. The microencapsulated fish oil powder contained 40.17g PUFA/100g of fatty acid in which the long chain omega-3 fatty acids were 17.82g EPA/100g and 12.65g DHA/100g fatty acids making this powder an excellent source of omega-3 fatty acids to be incorporated into frankfurter formulation. MUFA content was higher in chicken sources (45%) while MUFA content in flax oil and fish oil was less than the chicken sources.

Results of the fatty acid profiles of the finished product reflected the profile of the raw ingredients used. The results of the 3 main categories of fatty acids, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are reported in Table 4.3.

Table 4.2 Fatty acid composition of ingredients used for the formulation of chicken frankfurters (% Fatty acid of total methyl esters)

Fatty acid	Chicken meat	MSM	Chicken fat	Flax oil	Microencapsulated fish oil powder
Lauric C12:0	0.03	0.03	0.03	0.00	0.11
Myristic C14:0	0.55	0.50	0.48	0.09	7.04
Myristoleic C14:1 n-5	0.18	0.15	0.14	0.02	0.05
Physeteric C14:1 n-9	0.03	0.02	0.02	0.00	0.14
Pentadecylic C 15:0	0.08	0.07	0.07	0.03	0.44
Palmitic C16:0	21.11	21.00	20.99	6.92	17.74
Palmitoleic C16:1	5.24	4.60	4.71	0.54	7.68
Margaric C17:0	0.15	0.13	0.12	0.06	0.5
Stearic C18:0	6.21	6.15	5.56	3.78	3.35
Oleic C18:1n-9	39.08	38.12	39.00	21.56	8.62
Vaccenic C18:1n-7	2.10	1.92	1.81	0.81	2.56
Linoleic C18:2 n-6	18.23	20.65	21.51	15.99	1.11
γ Linolenic C18:3 n-6	0.20	0.24	0.24	0.04	0.34
α Linolenic C18:3 n-3	1.58	2.03	2.33	48.88	0.60
Stearonic C18:4 n-3	0.05	0.06	0.07	0.04	2.51
Arachidic C20:0	0.09	0.08	0.08	0.14	0.25
Gadoleic C20:1 n-11	0.08	0.07	0.07	0.05	0.12
Gondoic C20:1 n-9	0.36	0.34	0.32	0.18	0.88
Eicosadienoic C20:2 n-6	0.28	0.26	0.18	0.09	0.24
Dihomo \square linolenic C20:3 n-6	0.26	0.26	0.16	0.05	0.17
Arachidonic C20:4 n-6	1.02	0.82	0.25	0.04	0.81
Eicosatrienoic C20:3 n -3	0.03	0.03	0.02	0.06	0.06
Eicosatetraenoic C20:4 n -3	0.02	0.02	0.02	-	0.66
EPA C20:5 n -3	0.07	0.08	0.05	0.01	17.82
Behenic C22:0	0.04	0.04	0.02	0.12	0.11
Cetoleic C22:1 n-11	0.01	0.01	-	-	0.52
Erucic C22:1 n-9	0.03	0.03	0.02	0.02	0.17

Fatty acid	Chicken meat	MSM	Chicken fat	Flax oil	Microencapsulated fish oil powder
Docosadienoic C22:2 n-6	0.04	0.02	0.02	-	0.01
Heneicosapentaenoic C21:5 n-3	-	0.01	-	-	0.72
Docosatetraenoic C22:4 n-6	0.22	0.21	0.06	0.01	0.08
Osbond C22:5 n-6	0.05	0.05	0.01	-	0.3
DPA C22:5 n-3	0.17	0.15	0.06	0.01	1.68
DHA C22:6 n-3	0.12	0.11	0.04	0.00	12.65
Nervonic acid C24:1	0.04	0.03	0.01	0.01	0.50
Total Omega-3 PUFA	2.06	2.51	2.60	49.01	36.88
Total Omega -6 PUFA	20.30	22.51	22.43	16.22	3.29
∑SFA	28.03	27.80	27.15	11.05	28.59
∑PUFA	22.36	25.02	25.03	65.23	40.17
∑MUFA	46.15	46.67	45.55	22.93	19.97
Other	3.46	2.51	2.27	0.79	11.27

MSM- Mechanically separated chicken meat

EPA- eicosapentaenoic acid, DHA- docosohexaenoic acid

SFA- Saturated fatty acids, ∑SFA= 12:0+ 14:0 + 16:0 + 18:0 + 20:0 +22:0

PUFA- Polyunsaturated fatty acids. Calculated as omega-6 + omega-3

MUFA- Monounsaturated fatty acids. Calculated as 14:1 n-9 +14:1 n-5 +18:1 n-9 +18:1 n-7+20:1 n-11+20:1 n-9+22:1 n-11+22:1 n-9 +24:1 n-9

Total omega-3 calculated as 18:3 n-3 +18:4 n-3 + 20:3 n-3 + 20:4 n-3+ 20:5 n-3 +21:5 n-3 +22:4 n-3 +22:5 n-3+ 22:6 n-3

Total omega-6 calculated as 18:2 n-6+ 18:3 n-6+20:2n-6+ 20:3 n-6+22:2 n-6+22:4 n-6+22:5 n-6

Other- 14:1 n-7+15:0+16:1 n-11+16:1 n-5+16:2 n-4+16:3 n-4+16:4 n-1+17+17:1+18:1 n-11+18:1 n-5+18:2 n-7+18:2 n-4+18:3 n-4+18:4 n-1

Overall, level of SFA and MUFA was not altered by the incorporation of flax oil and microencapsulated fish oil to chicken frankfurters (Table 4.3). PUFA content of chicken frankfurters prepared with microencapsulated fish oil powder was not different compared to control when incorporated at 3.6%.

Table 4.3 Fatty acid composition of chicken frankfurters prepared with oils (mg of fatty acids/ 100g of product)

Fatty acid	Flax oil 1.2%	Flax oil 2.4%	Flax oil 3.6%	Fish oil powder 1.2%	Fish oil powder 2.4%	Fish oil powder 3.6%	Control	SEM
Lauric C12:0	3.80cd	3.39d	3.36d	4.56bc	5.24ab	5.58a	3.99cd	0.21
Myristic C14:0	69.14d	62.88d	62.84d	119.96c	165.99b	204.92a	74.85d	5.66
Myristoleic C14:1 n-5	21.1ab	19.07b	18.53b	21.26ab	20.89ab	20.24ab	22.99a	0.77
Physeteric C14:1 n-9	2.63d	2.62d	2.57d	4.02bc	4.89ab	5.51a	3.32cd	0.20
Pentadecylic C15:0	10.15c	9.38c	9.37c	13.22b	15.94a	18.1a	10.82bc	0.56
Palmitic C16:0	2986.41ab	2719.4ab	2694.4b	3029.8ab	3048ab	3008.6ab	3196.1a	122.85
Margaric C17:0	17.4b	17.33b	17.6b	22.63ab	25.7a	28.31a	16.42b	1.69
Stearic C18:0	838.14a	780.35a	795.7a	821.0a	811.67a	793.98a	874.51a	37.72
Oleic C18:1 n-9	5751.54a	5389.08a	5368.07a	5576.35a	5459.71a	5235.81a	6096.76a	265.72
Vaccenoic C18:1 n-7	273.66a	240.25a	252.06a	280.63a	290.57a	262.46a	284.53a	18.4
Linoleic C18:2 n-6	2924.52a	2731.55ab	2785.58ab	2783.46ab	2609.91ab	2446.53b	3047.01a	383.04
γ Linolenic C18:3 n-6	31.30ab	28.34b	27.87b	34.67a	35.47a	35.98a	34.93a	1.29
α Linolenic C18:3 n-3	802.35c	1240.88b	1688.56a	304.39e	278.29e	263.55e	315.56d	10.0
Stearonic C18:4n-3	8.21d	7.66d	8.04d	27.54c	44.55b	59.0a	9.40d	1.56
Arachidic C20:0	12.7b	12.7b	13.8ab	13.49b	14.88ab	16.33a	13.1b	0.6
Gadoleic C20:1 n-11	9.21b	10.09ab	10.51ab	9.84ab	12.12a	9.22b	7.95b	0.6

Fatty acid	Flax oil 1.2%	Flax oil 2.4%	Flax oil 3.6%	Fish oil powder 1.2%	Fish oil powder 2.4%	Fish oil powder 3.6%	Control	SEM
Gondoic C20:1 n-9	57.37bc	53.18c	54.51bc	62.47abc	67.57ab	71.01a	61.24abc	2.2
Eicosadienoic C20:2n-6	27.65ab	25.16b	26.41ab	29.75ab	30.52a	30.81a	30.69a	1.01
Dihomo γ Linolenic C20:3 n-6	24.82ab	23.06b	24.54ab	25.92ab	26.1ab	26.05ab	27.42a	0.86
Arachidonic C20:4 n-6	61.59bc	57.12d	58.69c	65.55bcd	70.75ab	74.76a	66.79abcd	1.91
Eicosatrienoic C20:3 n-3	3.8ab	3.64b	4.97a	3.65b	3.97ab	4.25ab	3.63b	0.28
Eicosatetraenoic C20:4 n-3	2.51d	2.09d	2.41d	7.71c	12.14b	16.53a	2.68d	0.39
Behenic C22:0	6.01b	6.23ab	7.93a	5.16bc	6.27ab	6.55ab	4.18c	0.4
Cetoleic C22:1 n-11	0.49d	0.52d	0.84d	5.85c	10.00b	14.67a	0.78d	0.64
Erucic C22:1n-9	25.91ab	23.87b	25.27ab	27.84ab	29.10a	28.77ab	27.39ab	1.08
Docosadienoic C22:2n-6	2.96a	2.92a	2.40a	2.74a	2.6a	2.06a	2.63a	0.33
Heineicosapentaenoic C21:5n-3	0.00d	0.07d	0.17d	6.19c	10.51b	15.57a	0.26d	0.35
Docosatetraenoic C22:4n-6	15.02ab	13.47b	14.39ab	16.08a	15.64ab	15.99a	15.99a	0.53
Osbond C22:5n-6	3.62d	2.21d	3.31d	5.61c	8.01b	9.33a	4.07d	0.26
Docosapentaenoic C22:5n-3	11.56d	10.52d	11.39d	25.07c	35.56b	45.26a	12.82d	1.15
Nervonic C24:1	3.95d	3.51d	4.23d	9.22c	11.41b	14.27a	4.36d	0.46

Fatty acid	Flax oil 1.2%	Flax oil 2.4%	Flax oil 3.6%	Fish oil powder 1.2%	Fish oil powder 2.4%	Fish oil powder 3.6%	Control	SEM
SFA	3945.7a	3614.6a	3606.78a	4029.96a	4093.76a	4082.37a	4194.27a	164.39
PUFA	3934.31b	4166.04ab	4683.7a	3579.21b	3630.9b	3667.6b	3593.37b	472.67
MUFA	6145.85a	5717.15a	5736.58a	5997.48a	5906.24a	5661.34a	6509.32a	282.5
Total Omega-6	3091.46ab	2884.96ab	2943.39ab	2966.34ab	2802.58ab	2646.50b	3230.08a	397.56
Total Omega-3	843.12c	1281.61b	1740.68a	617.4cd	834.9c	1029.45bc	364.5d	86.38
α - Linolenic (ALA) C18:3 n-3	802.35c	1240.88b	1688.56a	304.39e	278.29e	263.55e	315.56d	10.0
Eicosapentaenoic (EPA) C20:5n-3	6.49d	7.69d	11.14d	137.42c	258.09b	361.76a	7.95d	9.43
Docosahexaenoic (DHA) C22:6n-3	7.78d	10.91d	13.24d	102.32c	187.37b	258.46a	10.91d	6.69
Omega-6: omega-3	3.67bc	2.25cd	1.69d	4.79b	3.32c	2.55cd	9.31a	0.34
PUFA:SFA	0.99c	1.15b	1.31a	0.88d	0.89d	0.90d	0.85d	0.01
UFA:SFA	2.55c	2.73b	2.89a	2.38de	2.33e	2.28f	2.41d	0.009

SFA- Saturated fatty acids. Calculated as 12:0 + 14:0 + 15:0 + 16:0 +17:0 + 18:0 + 20:0 + 22:0

PUFA-Polyunsaturated fatty acids. Calculated as omega-6 +omega-3

MUFA- Monounsaturated fatty acids. Calculated as 14:1 n-9 +14:1 n-5 +18:1 n-9 +18:1 n-7+20:1 n-11+20:1 n-9+22:1 n-11+22:1 n-9 +24:1

UFA-Unsaturated fatty acids

Total omega-3 calculated as 18:3 n-3 +18:4 n-3 + 20:3 n-3 + 20:4 n-3+ 20:5 n-3 +21:5 n-3 +22:4 n-3 +22:5 n-3+ 22:6 n-3

Total omega-6 calculated as 18:2 n-6+ 18:3 n-6+20:2n-6+ 20:3 n-6+22:2 n-6+22:4 n-6+22:5 n-6

PUFA content increased significantly when flax oil increased from 1.2% to 3.6% with increased amount of flax oil addition. This was caused by the high amount of linolenic acid (49%) in flax oil. Pelsler et al. (2007) found that PUFA content increased with increased levels (3% to 6%) of flax oil in fermented sausages. In our study, PUFA content of flax oil added frankfurters was mainly due to linolenic acid while, EPA and DHA were the major source of PUFA in microencapsulated fish oil powder added frankfurters.

Alpha-linolenic acid (ALA) was found to increase in frankfurters with the increasing levels of flax oil (ranging from 0.8 g to 1.7 g/100g). ALA was found to increase in flax oil treated frankfurters at 1.2%, 2.4% and 3.6% by 486 mg/100g, 925 mg/100g and 1376 mg/100g, respectively when compared to the control treatment. Considering, 50g of frankfurter as standard serving size, consumption of 1 chicken frankfurter treated with 3.6% flax oil would supply 36% of the dietary recommendation of 2.2 g/ day for ALA. Total omega-3 fatty acids were found to be more than minimum 430 mg/100g in all the treatments when compared to control except for the 1.2% fish oil treatment.

To label a product as a source of omega-3 in Canada, a minimum level of 300 mg of omega-3 PUFA per 100 g meat is necessary (Health Canada, 2003). The addition of flax oil to chicken frankfurters at any of the levels tested and the 2.4% and 3.6% fish oil treatments could be labeled as omega-3 fatty acid enriched. The total omega-6 PUFA was found to be lower ($P < 0.05$) for the 3.6% microencapsulated fish oil powder treatment compared to the control. This low level was mainly related to low linoleic acid content in 3.6% microencapsulated fish oil powder treatment.

EPA and DHA, long chain omega-3 PUFAs were found to increase with increasing levels of microencapsulated fish oil powder. EPA and DHA content in frankfurters with 1.2% microencapsulated fish oil powder were 137 mg/100g and 103 mg/100g, respectively. These increased by 87% and 163% in frankfurters with 2.4% and 3.6% microencapsulated fish oil powder, respectively, compared to the control. The recommended dietary requirement for each EPA and DHA is 220 mg/day (ISSFAL, 2004). Consumption of 50g of chicken frankfurter containing 1.2% microencapsulated fish oil would supply 31% of EPA and 23% DHA of this requirement. While, 2.4% fish oil would provide 58.6% of EPA and 42.6% of DHA and 3.6% microencapsulated fish oil would supply 82% of EPA and 58% of DHA of the minimum recommended dietary requirement.

With all the levels of fish oil, the optimum levels of 220 mg/day for EPA and DHA were achieved as per the dietary recommendations (ISSFAL, 2004). So, the microencapsulated fish oil powder incorporated frankfurters would be an excellent source of EPA and DHA, while flax oil added frankfurters, an excellent source of ALA. The amount of EPA and DHA in the final product depends on the omega-3 content of the ingredients used. Substantial amounts of EPA and DHA have been found in this study in fish oil added frankfurters.

Apart from the level of omega-3 in foods, the ratio of fatty acids especially PUFA/SFA and Omega-6/omega-3 are also important from nutritional point of view (Simopoulos, 2008). The ratio of PUFA/SFA significantly increases ($p < 0.05$) with the addition of flax oil to frankfurters while there was no change in the ratio with fish oil treatment (Table 4.3). The ratio of PUFA/SFA was increased by 16.5%, 35% and 54% in

flax oil treatments at 1.2%, 2% and 3.6% compared to the control treatment respectively. Similar results in relation to PUFA/SFA ratio have been found by Ansorena and Astiasaran (2004) in sausages modified with flax oil. Ratio of UFA/SFA was also found to increase ($p < 0.05$) in flax oil treatments (1.2%, 2.4% and 3.6%) by 5%, 13.2% and 19.9% compared to the control, respectively. The increased ratio in flax oil treatments is considered beneficial from a health point of view. Fish oil treatments show a decrease in the ratio of UFA/SFA in comparison to control except for fish oil at 1.2%.

Possibly the most important ratio to consider is omega-6/omega-3. This ratio decreased from 9.3 in the control treatment to 1.7- 3.7 in the frankfurters containing flax oil and to 2.6- 4.8 in the frankfurters containing fish oil powder. This ratio decreased by 81% and 72% with 3.6% flax oil and 3.6% fish oil from that of the control frankfurters, respectively. Among all the treatments 3.6% flax oil frankfurters had lowest ratio of 1.7 because of increased linolenic acid. A ratio of less than 4 is considered to be beneficial (Simopoulos, 2008) and was achieved by incorporation of oils (flax and microencapsulated fish oil powder) into frankfurters except the fish oil powder at 1.2% was too low to achieve this. A decrease in omega-6/omega-3 ratio has been reported by others when fish oil is added at 3-6% (Caceres et al., 2008) or flax oil at 0.6% (Makala et al., 2007) in bologna sausage. The addition of oils to chicken frankfurters not only resulted in enrichment of omega-3 fatty acid but also resulted in decreased omega-6/omega-3 ratio which is excellent for nutritional benefits.

4.4.3 Lipid Oxidation

Lipid oxidation in the chicken frankfurters was measured by TBARS assay. TBARS values of the frankfurters prepared with oils did not show any significant

difference with that of control (Table 4.4). There was no interaction between treatment formulations and duration of storage.

Table 4.4 Effect of omega-3 oils and storage days on lipid oxidation of chicken frankfurters

Treatments	TBARS values (MDA equivalents mg/kg)
Flax oil 1.2%	3.73a
Flax oil 2.4%	3.35a
Flax oil 3.6%	2.95a
Microencapsulated fish oil powder 1.2%	3.39a
Microencapsulated fish oil powder 2.4%	2.88a
Microencapsulated fish oil powder 3.6 %	3.00a
Control	3.78a
SEM	0.25
Storage days¹	
Day 0	3.37a
Day 10	3.06b
Day 20	3.28ab
Day 30	3.47a
SEM	0.11
ANOVA	P-Value
Treatment	0.087
Days	0.0001
Trt*days	0.98

SEM- Standard error of mean

Means with different letters in the same column are significantly different (p<0.05).

¹ Means of all treatments on storage days

TBARS values of the frankfurters with added flax oil ranged from 2.95 to 3.73 mg of MDA equivalents/kg and that of fish oil powder containing frankfurters were 2.88 to 3.39 mg/kg. The observed low values of TBARS fish oil powder treatment compared to that of control treatment may have been due to the antioxidative effect of sodium ascorbate and tocopherols present in the fish oil powder. These antioxidants were added to the fish oil powder during the microencapsulation process to stabilize the omega-3 PUFA and minimize oxidation. The low value of TBARS in microencapsulated fish oil frankfurters demonstrated that microencapsulation protected the oxidative sensitive lipids as was intended. Others have microencapsulated oils for inclusion in foods as nutrients and reported similar findings for oxidative stability (Pelser et al., 2007). Pelser et al. (2007) made fermented sausage with encapsulated fish oil at 4.5% and measured oxidation stability after 90 days of storage. They reported no change in TBARS values from control treatments.

Irrespective of treatments, TBARS values ranged from a minimum of 3.06 mg MDA equivalents/kg on day 10 to a maximum of 3.47mg MDA equivalents/kg which indicated an increase in lipid oxidation between day 10 and 30. The TBARS values increased from day 10 by 0.41 mg MDA equivalents /kg till day 30. Overall, there was no difference in lipid oxidation during the storage period of 1 month as the oxidation level on day 30 reached to the same level as on day 0. The stability of chicken frankfurters during 30 days of storage could be due to combined effect of the antioxidants (spices, nitrites) added to the frankfurters during processing; vacuum packaging and low storage temperature (Andres et al., 2009). The higher values on day 0 than day 10 could be due to lipid pro-oxidation nature of the cooking process. A similar result of stable product was

observed in squid oil added chicken sausages during storage period of 30 days at 4°C (Andres et al., 2009).

The TBARS values obtained in this study were higher than those reported for mortadella sausages with added fish oil (Caceres et al., 2008 or chicken sausages with added squid oil (Andres et al., 2009) and fermented sausages with added flax oil (Pelsler et al., 2007). TBARS values of 0.5 to 2 mg MDA equivalents /kg of meat (Gray and Pearson, 1987), 2 mg of MDA equivalents /kg in beef burgers (Georgantelis et al., 2007) and 3 mg MDA equivalents /kg in omega-3 fatty acid enriched fermented sausages have been associated with rancidity (Ansorena and Astiasaran, 2004). However, Fernandez-Gines et al. (2003) reported the absence of rancid taste in vacuum packed bologna sausages at TBARS level of 4-6 mg MDA equivalents /kg. The observed values in the current study could be due to combination of factors such as incorporation of mechanically separated meat which contains iron as pro-oxidant (Fernandez et al., 1997), rendered chicken fat and oils high in PUFAs (Cortinas et al., 2005).

4.5 Conclusion

Incorporation of flax oil and microencapsulated fish oil powder to chicken frankfurters was found to alter the fatty acid profile without having an impact on the proximate composition of chicken frankfurters. The claim of omega-3 enrichment (300mg/100g of product) can be achieved by incorporation of both flax oil (1.2% to 3.6%) and microencapsulated fish oil (2.4% and 3.6%). A good source of ALA, omega-3 fatty acid, in chicken frankfurter can be achieved by addition of flax oil. While, enrichment with microencapsulated fish oil proved to be a good source of long chain omega-3 fatty acids (EPA and DHA). The dietary requirement for ALA, EPA and DHA

can be met adequately by intake of omega-3 fatty acid enriched chicken frankfurters. The enriched frankfurters were also found to lower the ratio of omega-6 /omega-3 below the recommended level of 4. Omega-3 fatty acid enrichment of chicken frankfurters can be achieved by replacement of chicken fat with flax oil and microencapsulated fish oil without the problem of lipid oxidation.

CHAPTER 5

PHYSICAL CHARACTERISTICS OF OMEGA-3 FATTY ACID ENRICHED CHICKEN FRANKFURTERS*

5.1 Abstract

The present study was undertaken to evaluate the effect of incorporation of microencapsulated fish oil and flax oil on color and texture of chicken frankfurters. A total of seven treatments on 4 storage days (0, 10, 20 and 30) at 4°C were used for this study. Chicken frankfurters for all the treatments were prepared with boneless skin-on chicken thighs, mechanically separated chicken meat and chicken fat as the main meat ingredients. For test treatments, chicken fat was partially replaced with either flax oil or microencapsulated fish oil at 1.2%, 2.4% and 3.6% of the batter, whereas in control there was no addition of oil. Four replicates of each batch were prepared, vacuum packed and stored at 4°C for a month. A completely randomized design was used to study the effects of lipid source and storage time (0, 10, 20 and 30 days) on the physical parameters such as cook yield, color and texture from four samples. Replacement of chicken fat with oils did not affect ($p>0.05$) the cook yield which was found to be $82\% \pm 1.5\%$. The addition of oil to frankfurters did not affect ($p>0.05$) lightness (L^*) or yellowness (b^*) whereas the redness (a^*) was higher ($p<0.05$) in the treatment with 2.4% and 3.6% microencapsulated fish oil (2.21 ± 0.11 , 2.24 ± 0.11) than control (2.09 ± 0.11). There were color changes ($p<0.05$) in lightness, redness and yellowness due to storage time. The textural parameters such as hardness (kg), gumminess (kg) and chewiness (kg mm) were higher ($p<0.05$) in microencapsulated fish oil treatment at 2.4% and 3.6% than the flax oil and control. However, cohesiveness and springiness were not affected ($p>0.05$) by oil treatments. The textural parameters were also affected ($p<0.05$) during storage but these were consistent between oil treatments. The shear force (kg) was higher ($p<0.05$) in frankfurters with 2.4% and 3.6% microencapsulated fish oil (1.36 ± 0.04 , 1.33 ± 0.04) than the control (1.06 ± 0.04) for all storage times.

Keywords: omega-3, chicken frankfurter, microencapsulated fish oil, flax oil, color, texture

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5.2 Introduction

Color and texture are important organoleptic characteristics for consumers' acceptance of meat products (Ansorena et al., 1997). The textural property of frankfurters is largely influenced by the presence of animal fats (Keeton, 1994). Fats in meat products play vital functional roles stabilizing meat emulsions, reducing cooking loss and improving water holding capacity (Choi et al., 2009). Also, fats interact with other ingredients to develop texture (hardness and juiciness), flavor, mouthfeel, appearance and overall palatability (Giese, 1996).

The growing demand by consumers for healthier meat products has resulted in strategies to reduce the use of animal fat (Youssef and Barbut, 2009). One approach is to replace animal fat in meat formulations with various vegetable oils. Olive oil in low fat frankfurters or fermented pork sausages (Bloukas et al., 1997; Paneras, et al., 1998; Pappa et al., 2000; Muguerza et al., 2003), canola oil in Dutch style fermented sausages (Pelser et al., 2007), corn oil in low fat frankfurters (Paneras, and Bloukas, 1994) and palm oil in chicken frankfurters (Tan et al., 2001), are examples of this strategy. Oils which are major sources of omega-3 fatty acids such as flax oil and fish oil have also been used to replace pork fat in fermented sausages (Ansorena and Astiasaran, 2004; Valencia et al., 2008; Pelsler et al., 2007).

Differences in color, flavor and fatty acids content of oils may affect quality characteristics of products such as color and texture and also cooking yield (Youssef and Barbut, 2009). Incorporation of oils might cause technological problems in product preparation. Oil in the liquid form should be incorporated in such a way that oil droplets do not coalesce during product preparation which would result in unstable emulsion and

would result in unacceptable texture and high cook loss. The textural properties of frankfurters cannot be compromised for the objective of healthier meat production. The level and form of oil (encapsulated or pre emulsified form) to be incorporated are important for maintaining the product quality (Jimenez-Colmenero, 2007). Incorporation conditions vary according to the type of product and characteristics of the oil. For example, replacement or substitution of animal fat with vegetable oils (Paneras, and Bloukas, 1994; Bloukas et al., 1997; Ambrosiadis et al., 2003) and fish oils (Park et al., 1989;) in liquid form have resulted in low processing yield, darker color and firmer texture in meat products like frankfurters and fermented sausages. On the other hand, flax oil and fish oil have been incorporated into sausages in encapsulated form and pre-emulsified form without affecting the color and textural properties (Pelser et al., 2007; Caceres et al., 2008).

Many studies have been carried out on replacement of fat with oils in pork and beef sausages but there is a lack of literature on the study of effects of replacement of chicken fat with oils (flax oil and encapsulated fish oil) on the textural properties of chicken frankfurters. The objective of this study was to evaluate the physical properties (cook yield, color and texture) of chicken frankfurters prepared with the aim of producing omega-3 fatty acid enriched chicken frankfurters by replacing fat with flax or fish oils.

5.3 Methods

5.3.1 Cook Yield

Cook yield was determined in for four batches by weighing the product before and after thermal processing and calculated using the following formula:

$$\text{Cook yield \%} = \frac{\text{Wt. after thermal processing}}{\text{Wt. before processing}} \times 100$$

5.3.2 Sample Preparation for Color and Texture

Instrumental measurements of color and texture were performed on day 0, 10, 20 and 30. The frankfurters were warmed to room temperature for 1 hr and 4 samples from each treatment were used. After peeling off the casing each frankfurter was cut into 20 mm long pieces by a transverse cut.

5.3.3 Color Measurement

Internal color was measured at room temperature on the surface of transversal cuts with MiniScan XE plus (Hunter Associates Laboratory, Inc. Virginia, USA). System settings included a D65 light source at 10° observer angle to determine the CIE- L* a* b* parameters. The results were expressed in L*(lightness) (0= black, 100= white), a* (redness) (- = green, + = red) and b* (yellowness) (- = blue, + = yellow). Duplicate measurements were taken for each slice by rotating the sample at 45°. Values obtained for each treatment were the average of 16 measurements. The instrument was calibrated with a white tile before the measurement of color.

5.3.4 Texture Analysis

5.3.4.1 Texture Profile Analysis

Texture profile analysis (TPA) (Bourne, 1978) of chicken frankfurters was performed using a texture analyzer TA.XT Plus (Stable Microsystems, New York, USA) with integrated software by Texture Technologies Corp. The textural properties were evaluated at room temperature. Four 1 core samples of 20 mm thickness from each

treatment on each storage sampling day were compressed twice in upright position to 50% of their original height. In these experiments a crosshead speed of 2 mm/s with a 10 mm cylindrical probe and 5 kg load cell was used. A typical graph generated by texture analyzer is located in the Appendix I. The following parameters were determined as described by Bourne (1978)

Hardness: peak force of the first compression of the sample in kg (F1)

Cohesiveness: extent to which the sample could be deformed prior to rupture. It was measured as the ratio of area of work during the second compression to area of work of first compression.

Gumminess: force necessary to disintegrate a semisolid product for swallowing which was measured as Hardness x cohesiveness.

Springiness: ability of the product to restore its original form after the removal of compression force measured by the distance of the detected height of the product on the second compression divided by the first compression distance.

Chewiness: work required to masticate the sample for swallowing measured in kg (Springiness x gumminess).

5.3.4.2 Shear Force

Shear force (peak force) and work required to shear frankfurters were determined using the Warner-Bratzler blade attached to the Texture analyzer. Four cores of 20 mm were sheared on side from each treatment using a crosshead speed of 2 mm/s with 30 kg cell load.

5.4 Results and Discussion

5.4.1 Cook Yield

The cook yield of chicken frankfurters prepared with flax oil and fish oil were not different ($P=0.7431$) from that of the control (Table 5.1) which indicates that substitution of chicken fat with oils resulted in a similar cook yield. The cook yield of approximately 82% obtained in the study for all treatments is in agreement with the findings of Marquez, et al. (1989); Park et al. (1989); Bloukas and Paneras, 1993; Bloukas et al. (1997) when fat was replaced with vegetable oils in beef frankfurters. Park et al. (1989) reported that the high oleic acid sunflower oil added to sausages reduced processing yield to 82% compared to beef sausages without oil addition. The low processing yield of frankfurters in this study could be due to a lower fat to protein ratio (Mittal and Blaisdell, 1983).

Table 5.1 Effect of replacement of chicken fat with omega-3 rich oils on cook yield of chicken frankfurters

Treatments	Cook yield (%)
Flax oil (1.2%)	82.42a
Flax oil (2.4%)	81.90a
Flax oil (3.6%)	82.13a
Microencapsulated fish oil (1.2%)	82.52a
Microencapsulated fish oil (2.4%)	82.36a
Microencapsulated fish oil (3.6%)	82.71a
Control	81.82a
SEM	1.53
ANOVA	P-VALUE
Treatments (Trt)	0.74

SEM – Standard error of mean

Means with different letters in the same column are significantly different ($p<0.05$).

Andres et al. (2009) found a high cook yield of 97% in chicken sausages prepared with squid oil and beef tallow. Stability of the protein matrix in the emulsion affects the cooking loss. An increase in both the moisture and fat content has been found to reduce the cook yield (Claus et al, 1989). In most studies, replacement of fat has been accompanied with a change in the water content (Ahmed et al., 1990) but in our study the water content was kept constant as also revealed by moisture % estimation.

5.4.2 Color

5.4.2.1 Effect of Oil Addition on Color

Replacement of chicken fat with different levels of flax and encapsulated fish oils did not affect lightness (L^*) or yellowness (b^*) ($p>0.05$) of chicken frankfurters (Table 5.2). The lightness of chicken frankfurters was in the range of 39.89 ± 0.35 to 40.45 ± 0.35 . These results were in agreement with Bloukas and Paneras (1993) and Marquez et al. (1989) who found that replacement of fat with olive oil in pork sausage and peanut oil in beef frankfurters, respectively, did not affect the lightness or yellowness. Redness (a^*) of chicken frankfurters prepared with 2.4% and 3.6% encapsulated fish oil were found to be higher ($p=0.001$) than chicken frankfurters with 1.2% and 2.4% flax oil and control (Table 5.2). Many studies have reported variable results regarding the effect on color due to replacement of animal fat with both vegetable oils and fish oils in different meat products. For instance, replacement of pork backfat with olive oil at 10-20% in fermented sausages prepared with beef and pork meat were lighter and more yellow than the controls on day 0 and redness was not affected (Bloukas et al., 1997; Muguerza et al., 2002). Choi et al. (2009) found that the lightness and redness of cooked pork meat batter with different vegetable oils (olive oil, corn oil) were lower and yellowness was higher

Table 5.2 Effect of omega-3 oils and storage days on color of chicken frankfurters

Treatment	Lightness (L*) units	Redness (a*) units	Yellowness (b*) units
Flax oil (1.2%)	39.89a	2.06c	3.10a
Flax oil (2.4%)	40.10a	2.10bc	3.27a
Flax oil (3.6%)	40.37a	2.17abc	3.43a
Microencapsulated fish oil (1.2%)	39.94a	2.15abc	3.00a
Microencapsulated fish oil (2.4%)	40.28a	2.21a	3.15a
Microencapsulated fish oil (3.6%)	40.45a	2.24a	3.24a
Control	39.98a	2.09bc	2.98a
SEM	0.35	0.11	0.13
Storage days¹			
0	39.88±0.17b	2.11±0.11b	2.98±0.06b
10	40.38±0.13a	2.10±0.11b	3.22±0.05a
20	40.25±0.21a	2.21±0.11a	3.22±0.06a
30	40.08±0.10ab	2.16±0.11ab	3.26±0.04a
ANOVA	P-VALUE	P-VALUE	P-VALUE
Treatments	0.856	0.001	0.275
Days	0.004	0.019	<0.0001
trt*days	1.000	0.999	0.966

SEM – Standard error of mean

Means ± standard error

Means with different letters in the same column are significantly different (p<0.05).

¹Means of all treatments on storage days

than the control prepared with pork backfat. Pelsler et al. (2007) reported that 10-20% substitution of pork backfat with flax oil and encapsulated fish oil in fermented beef sausages did not affect the lightness and redness whereas it resulted in more yellowness than the control sausages due to the yellow color of the flax oil. Replacement of tallow in chicken sausages with squid oil (Andres et al., 2009) and replacement of pork backfat

with pre-emulsified fish oil at 1-6% in mortadella bologna sausage (Cáceres et al., 2008) resulted in higher lightness and lower redness than the control sausages.

The results obtained in the present study are comparable to the work of Pelsler et al. (2007) as flax oil at 3-6% and encapsulated fish oil at 4.5% were used which agreed with the findings in terms of lightness. The results obtained in the various works are different from each other which could be speculated to be due to the differences in the species/type of meat, type of sausage and the characteristics of the oils assayed. For instance, lightness value of the results obtained in this study (40 color units) was found to be lower in comparison to the values (82- 86 color units) found in the works of Andres et al.(2009) which could be due to the darker meat from chicken thighs used for the preparation of chicken frankfurters. The result obtained in this study confirms that the change of meat color by oil treatment may not be consistent with trends among meat products.

5.4.2.2 Effect of Storage Days on Color

Refrigerated storage time had a significant effect ($p < 0.05$) on lightness, redness and yellowness of the chicken frankfurters (Table 5.2). Lightness increased from day 0 to day 20, redness was highest on day 20 and yellowness increased by day 10 and remained stable during the remaining storage days. Similar result was found by Muguerza et al. (2002) in fermented sausages prepared with olive oil and stored for 28 days. The increase in redness and yellowness was consistent with the findings of Andres et al. (2009) and Papadima and Bloukas (1999). However, Cáceres et al. (2008) found no difference in the color of bologna type sausage prepared with fish oil during 90 days of storage. The increase in redness on day 20 could be due to formation of nitrosylmyoglobin which is formed by reaction of myoglobin with nitrites (Papadima and Bloukas, 1999) as nitrite

was added to the chicken frankfurters as a curing agent. After 20 days the redness value started to decrease which may be attributed to the oxidation of nitrosylmyoglobin.

5.4.3 Textural Properties

Replacement of chicken fat with oils influenced ($p < 0.05$) hardness, gumminess and chewiness of chicken frankfurters, while cohesiveness and springiness were not affected ($p > 0.05$) (Table 5.3). Hardness, gumminess and chewiness of flax oil treated chicken frankfurters were similar to the control, but hardness and gumminess increased with high level of flax oil inclusion compared to the control and there was no difference within the flax oil treatments. The results obtained in this study are in agreement to the work of Muguerza et al. (2002) who found no effect of replacement of 20% pork backfat with olive oil as evaluated by instrumental hardness measurements. Also, similar results in hardness and chewiness were found by Tan et al. (2006) when chicken fat was substituted with palm oil at 0.2% in chicken frankfurters. Pelsler et al. (2007) found that the addition of flax oil to fermented sausage at 4.5% of the final product caused a softer texture. Luruena- Martinez et al. (2004) also reported that the replacement of pork with olive oil at 5% in liquid form caused a significant decrease in hardness, gumminess and chewiness of frankfurters. A decrease in hardness with replacement of fat with extra virgin olive oil at 5-10% has been reported in fermented sausages by Bloukas et al. (1997); and Severini et al. (2003). An increase in hardness has been reported in beef frankfurters prepared by substitution of beef fat with olive oil at 10% (Paneras and Bloukas, 1994) and in meat batters with canola oil at 25% by Youssef and Barbut (2009). In general, a decrease in hardness would be expected if solid fat is replaced with liquid oil (Hur et al., 2008).

Table 5.3 Effect of omega-3 oils and storage on texture of chicken frankfurters

Treatment	Hardness (kg)	Cohesiveness (ratio)	Gumminess (kg)	Springiness (mm)	Chewiness (kg* mm)
Flax oil (1.2%)	0.505cd	0.87a	0.439cd	0.958a	0.42c
Flax oil (2.4%)	0.525bcd	0.865a	0.454bcd	0.958a	0.435bc
Flax oil (3.6%)	0.584bc	0.862a	0.503bc	0.958a	0.481bcd
MF (1.2%)	0.605b	0.866a	0.524b	0.957a	0.501b
MF (2.4%)	0.73a	0.874a	0.637a	0.96a	0.611a
MF (3.6%)	0.798a	0.87a	0.693a	0.958a	0.664a
Control	0.5d	0.865a	0.428d	0.958a	0.41cd
SEM	0.03	0.01	0.02	0.002	0.02
Storage days¹					
0	0.599bc	0.866ab	0.516b	0.955b	0.493bc
10	0.566c	0.871ab	0.492b	0.959a	0.472c
20	0.65a	0.873a	0.567a	0.959a	0.544a
30	0.612ab	0.86b	0.526b	0.959a	0.504b
SEM	0.02	0.01	0.02	0.001	0.02
ANOVA	P-VALUE	P-VALUE	P-VALUE	P-VALUE	P-VALUE
Treatments	<0.0001	0.496	<0.0001	0.435	<0.0001
Days	<0.0001	0.014	<0.0001	0.003	<0.0001
trt*days	0.246	0.74	0.302	0.478	0.288

SEM – Standard error of mean

MF – Microencapsulated Fish Oil

Means with different letters in the same column are significantly different (p<0.05).

¹Means of all treatments on storage days

However, the addition of flax oil at 1.2% and 2.4% levels in this study resulted in textural properties similar to the control. The increased hardness of frankfurters prepared with the highest level of flax oil (3.6%) could be due to influence on the fat physicochemical properties and rheology of the meat batter. Smaller fat globules in the emulsion prepared with 3.6% flax oil would require a larger surface area of the protein

membrane to surround the fat globules. This larger surface area would have resulted in more protein-protein interactions and increased the hardness of the frankfurters because of stronger bond of the fat globules with the protein matrix (Youssef and Barbut, 2010).

Addition of fish oil at 2.4% and 3.6% was found to increase hardness, gumminess and chewiness ($p < 0.05$) (Table 5.3). The values for hardness, gumminess and chewiness of 1.2% fish oil frankfurters were found to be higher than the control but similar to 2.4% and 3.6% flax oil treated frankfurters. Similar results were found by Cáceres et al. (2008) with increasing levels (1-6%) of pre-emulsified fish oil in bologna sausage. They also observed that the gumminess and chewiness also increased with hardness and same springiness for all levels of fish oil.

Pelser et al. (2007) showed that encapsulated fish oil and flax oil at 4.5% treated fermented sausages had increased hardness compared to control because of the high carbohydrate content in encapsulated oils. The changes in texture observed in the present study could be related to the high protein content in 2.4% and 3.6% fish oil frankfurters. The increased protein content would have resulted in formation of dense emulsion matrix and thus increasing hardness (Fernandez- Colmenero et al., 1995). The protein content has been found to be positively correlated with hardness (Youssef and Barbut, 2009). Gumminess and chewiness which are secondary parameters depends on the hardness (Caceres et al., 2005). Therefore, gumminess and chewiness also increased with fish oil addition.

Storage time significantly ($p < 0.05$) changed all the textural properties of the frankfurters (Table 5.3). Hardness, gumminess and chewiness significantly increased

from day 0 to day 20. The increase in hardness during storage at 4°C agrees with the findings of Andres et al. (2006; 2009) and Candogan and Kolsarici (2003) who also reported an increase in hardness of chicken sausages and low fat beef frankfurters, respectively. The increase in hardness was likely due to purge loss from the product during storage. When purge loss increases, the availability of water to act as plasticizer of the matrix is reduced resulting in increased hardness.

Results corresponding to shear force by Warner-Bratzler tests are shown in Table 5.4. The replacement of chicken fat with flax oil at all levels did not require more force (kg) to shear than the control. Shear force required for the fish oil (medium and high levels) treated frankfurters were higher ($p < 0.05$) than the control (Table 5.4). Shear work was not affected ($P > 0.05$) by the oil treatments. Results of this test for shear strength agreed with the results of texture profile analysis (hardness) which showed the same trend with oil treatments. Shear strength test is considered to simulate the masticability of a product as compression, cutting and shearing are simultaneously applied. These are the forces applied during mastication by humans (Huda et al., 2010). Caceres et al. (2008) reported that the shear strength increased with increasing levels of fish oil at 1-6% in fermented sausages. The shear force could be related to the firmness of gel formed with addition of high level of fish oil. Ambrosiadis et al. (1996) found that the addition of different vegetable oils (sunflower, cottonseed and corn oil) at 19.5% lowered the shear force in comparison to the control with 19.5% lard. The shear force could also be affected by pH, protein and water content of the frankfurters (Jin et al., 2007). Storage time for chicken frankfurters also had an effect on shear force ($p < 0.05$) which was similar to the hardness during storage on day 20.

Table 5.4 Effect of omega-3 oils on shear strength of chicken frankfurters

Treatment	Shear force (kg)	Shear work (kg*mm/s²)
Flax oil (1.2%)	1.16abc	2.37a
Flax oil (2.4%)	1.12bc	2.29a
Flax oil (3.6%)	1.21abc	2.36a
Microencapsulated fish oil (1.2%)	1.21abc	2.32a
Microencapsulated fish oil (2.4%)	1.36a	2.56a
Microencapsulated fish oil (3.6%)	1.33ab	2.42a
Control	1.06c	2.05a
SEM	0.05	0.15
Storage days¹		
0	1.24±0.05ab	2.45±0.12a
10	1.16±0.04b	2.24±0.12a
20	1.25±0.05a	2.39±0.12a
30	1.18±0.35ab	2.29±0.12a
ANOVA	P-VALUE	P-VALUE
Treatments	0.003	0.189
Days	0.024	0.068
trt*days	0.845	0.772
Contrast	P-VALUE	P-VALUE
Flax vs fish	0.002	0.342
Control vs flax	0.072	0.051
Control vs fish	0.0003	0.012

Means ± standard error

Means with different letters in the same column are significantly different (p<0.05).

¹Means of all treatments on storage days

5.5 Conclusion

Substitution of chicken fat with flax oil and fish oil did not affect the cook yield. The addition of flax oil at all the three levels (1.2%, 2.4% and 3.6%) did not affect the color, but textural parameters were affected by the high replacement of chicken fat with

flax oil. Replacement of chicken fat with highest level (3.6%) of fish oil was found to affect the color and textural properties of chicken frankfurters resulting in more red color and a harder texture. The form of oil incorporated to chicken frankfurters had influenced the physical properties of chicken frankfurters. The replacement of fat with fish oil at the highest level is not desirable as it resulted in harder texture whereas flax oil addition exhibited a great potential for substitution for chicken fat in frankfurters.

CHAPTER 6

SENSORY EVALUATION OF OMEGA-3 FATTY ACID ENRICHED CHICKEN FRANKFURTERS

6.1 Abstract

The following study was undertaken to evaluate the effect of omega-3 fatty acids enrichment of chicken frankfurters on sensory attributes. A total of 6 treatments of chicken frankfurters were prepared with chicken thigh meats with skin, mechanically separated meat and chicken fat as the main meat ingredients. For test treatments, chicken fat was partially replaced with either flax oil at 1.2% and 2.4% or microencapsulated fish oil at 1.2%, 2.4% and 3.6% of the batter, whereas in control there was no addition of oil. Each batch was cooked to an endpoint temperature of 74°C, vacuum packed and stored at 4°C until use for sensory evaluation. Sensory attributes included hardness and juiciness (texture), fishy flavor and overall acceptability of all treatments and a commercial brand. They were evaluated by a trained 16 member panel on two different days by the descriptive sensory method. A completely randomized block design was used to study the effect of treatments using the panelist as blocks. Hardness and juiciness of flax oil treatments and 1.2% microencapsulated fish oil treatments were not different from control and commercial frankfurters ($p < 0.05$). Microencapsulated fish oil treatments at 2.4% and 3.6% were found to be harder and less juicy than the control but had similar texture to the commercial brand. There was no detectable fishy flavor though there was significant difference ($p < 0.05$) between 1.2% and 3.6% microencapsulated oil treated frankfurters. The panelists rated all the treatments including commercial brand to be highly acceptable. However, overall acceptability of 3.6% microencapsulated fish oil treatment was found to be lower than the control due to differences in texture. Omega-3 enrichment of chicken frankfurters which meet labeling requirement can be achieved by incorporation of flax oil and microencapsulated fish oil without affecting the sensory properties.

Keywords: omega-3, chicken frankfurter, microencapsulated fish oil, sensory evaluation

6.2 Introduction

Meat products like frankfurters are considered to be very good sources of protein, essential minerals and vitamins (Weiss et al., 2010). However, the presence of high fat (30%) in meat products is viewed by the consumers as a potential risk factor for diseases such as cardiovascular disease, obesity, hypertension and cancer (Arihara, 2006). Therefore, there is a demand for more healthy meat products. These changes in consumer demand have resulted in the development of novel functional meat products (Arihara, 2006). Dietary recommendations for humans of reduced intake of saturated fat and increased awareness of the beneficial health effects of polyunsaturated fatty acids (PUFA) have led to an increased demand for omega-3 fatty acid enriched meat products (Valencia et al., 2008).

One of the approaches to increase the level of PUFA, especially omega-3 fatty acids, is the incorporation of these fatty acids in meat products (Jimenez-Comenero et al., 2007). This can be achieved by replacement of fat with oils such as flax oil and fish oil (Fernandez-Gines et al., 2005). Fat and its composition in frankfurters is largely for the texture and flavor and therefore for overall acceptability of the finished product (Ventanas et al., 2010). Ideally, the incorporation of oils should be accomplished without a negative impact on sensory properties. The addition of fish oil to meat products often imparts fishy flavor which can be overcome by use of microencapsulated fish oil (Jacobsen, 2010).

The sensory properties of omega-3 fatty acid enriched meat products should be assessed for the consumer acceptance before introduction into the market (Resurreccion, 2003). Descriptive sensory analysis is the most commonly used method to evaluate the

texture and flavor of meat products (Murray et al., 2001). Textural attributes by sensory evaluation employs the use of reference foods and standard rating scales developed by Szczesniak (1963) and mostly requires the use of a trained sensory panel (Kilcast,1999). Sensory evaluation of sausages prepared by addition of vegetable oils and fish oil has been the focus of research interest by a number of groups (Ansorena and Astiasaran, 2004; Pelser et al., 2007 and Valencia et al., 2008). There are examples of meat researchers that have produced omega-3 enrichment of bologna type sausages and fermented sausages by addition of flax oil and fish oil. They all reported that sensory attributes were not affected by addition of oils to sausages (Makala et al., 2007; Caceres et al., 2008; Pelser et al., 2007).

The objective of this study was to evaluate the sensory properties of omega-3 fatty acid enriched chicken frankfurters prepared by incorporation of flax oil and microencapsulated fish oil. It was hypothesized that omega-3 enrichment of chicken frankfurters would result in a high quality product that a sensory panel would find acceptable.

6.3 Methods

6.3.1 Preparation of Omega-3 fatty acid Enriched Chicken Frankfurters

Omega-3 fatty acid enriched chicken frankfurters were prepared following the formulations and processing procedures as described in Chapter 3. Prepared chicken frankfurters were stored at 4°C and were used for sensory evaluation within 10 days of preparation. Microbiological safety (Aerobic plate count, *E.coli* and *Staphylococcus*

aureus count) of omega-3 fatty acid enriched chicken frankfurters from each treatment was determined before using the frankfurters in sensory evaluation (Appendix III).

6.3.2 Sensory Evaluation by Trained Panel

Descriptive sensory analysis of omega-3 fatty acid enriched chicken frankfurters was carried out after recruitment, selection and training of panelists (Meilgaard et al., 1991).

6.3.2.1 Recruitment and Selection

Twenty potential panelists were recruited from students, staff and faculty of Nova Scotia Agricultural College who responded to posted announcements. All panelists were recruited on the basis of the following criteria: between 18 and 70 years of age, non-smoker, not allergic to any foods, were available and willing to participate during training and testing. Panelists were selected by a screening test after the completion and signing of consent forms approved by Human Research Ethics Board of Nova Scotia Agricultural College. Panelists were screened using the Duo-trio test (Meilgaard et al., 1991) for basic taste (saltiness), fishy flavor and texture (hardness and juiciness).

6.3.2.2 Training

Sixteen panelists (nine males and seven females) were selected and trained for 4 hr in two sessions (each session was for 2 hr) in a focus group setting. In the first training session, the panelists were introduced to the scaling system (15cm scale), terminology and description of the sensory attributes (hardness, juiciness and fishy flavor) of chicken frankfurters. The panelists were provided with a broad range of commercial foods which represented different intensities of hardness, juiciness and fishy flavor (Table 6.1).

Table 6.1 Attributes, definitions and reference samples used to evaluate omega-3 fatty acid enriched chicken frankfurters

Attributes	Definition/ Technique	Standard reference foods (scale value- 15cm long)
Texture Hardness	Force required to compress a sample between the molar teeth, measured by placing the food between the molar teeth and biting down evenly	Cooked egg white (2.5) Cheddar cheese “Kraft” (4.5) Stuffed olive (6.0) Chicken Frankfurter cooked Compliments brand (7.0)
Juiciness	Amount of juice released from the sample evaluated by chewing the samples for 2 to 5 chews with the molar	Planter almond (11.0) Cookie (1.8) Chicken Frankfurter cooked “Compliments brand”(4.0) Banana (4.7) Apple (9.7) Seedless grape (12.8)
Flavor Fishy	combined effects of the aromatics, tastes and chemical feelings stimulated by cooked fish in the mouth	Chicken Frankfurter cooked “Compliments brand”(2.4) Chicken nuggets “Compliments brand” cooked as per manufacturer’s instruction (3.0) Tilapia fish stick “Captain highliner”(7.3) Cod fish stick “Captain highliner” cooked as per manufacturer’s instruction (9.8)

The samples were provided to the panelists in increasing order of intensity during the first training session. A commercially available chicken frankfurter (Compliments brand) was also used during the training session to help the panelists familiarize themselves with the sensory attributes. In the second training session, the standard reference samples for each attribute were presented in random order. The panelists were trained to perform a descriptive test using a horizontal scale of 15 cm long line with two anchor points of 1.5 cm from each end. Reference samples were evaluated as a group to

assure that ratings of each panel were in the same range of other panelists. All the standard references were prepared according to Meilgaard et al. (1991) and for juiciness the reference foods was according to Ruiz de Huidobro et al. (2003). Reference standards for fishy flavor in this study were developed using commercially available foods. The definition and techniques for evaluation of the sensory attributes were as described by Szczesniak et al. (1963) and Meilgaard et al. (1991).

6.3.2.3 Descriptive Sensory Test

Sensory evaluation of omega-3 fatty acid enriched chicken frankfurters was carried out in the product evaluation lab of NSAC by 16 panelists. For the sensory test, chicken frankfurters from each treatment were prepared by steeping frankfurters in boiling water for 7 min after peeling off the casing. After draining the liquid, both the ends of the frankfurters were removed and the frankfurters were cut into 2.5 cm long pieces, wrapped with aluminum foil and held in a warm tray for no longer than 30 min. Warm 2.5 cm long pieces of seven chicken frankfurters were provided to each panelist in white plastic plates using one tray. Chicken frankfurters from six treatments including the control and one commercial frankfurter were used. The samples on the trays were arranged in balanced and randomized order so that each sample appeared in a given position an equal number of times. Random three digit numbers were assigned to each sample to minimize the expectation error of the panelist (Meilgaard et al., 1991). Panelists evaluated the following sensory attributes: hardness, juiciness, fishy flavor and overall acceptability by drawing a vertical line on a horizontal 15 cm long line with two anchor points of 1.5 cm from each end in the score sheet (Appendix II). Potable water and saltless crackers were also provided to clean the palate between samples. The test was carried out in individual

booths under fluorescent lights. The test was performed twice over 2 days (duplicate) by the same 16 panelists.

6.3.3 Experimental Design and Statistical Analysis

A completely randomized block design with 7 treatments of omega-3 fatty acid enriched chicken frankfurters as main effects was used. The treatments were randomly assigned to 16 panelists and the panelists were used as blocks. All the sensory parameters (hardness, juiciness, fishy flavor and overall acceptability) were analyzed by ANOVA with the Proc Mixed procedure of SAS version 9.1 (Littell et al., 1996; SAS institute Inc., 2003). The Tukey test was used to compare differences among the least square means at $P \leq 0.05$ when significance was found. The statistical model for this design is represented as:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where Y is the response variable, μ is the overall mean, α_i is the effect of treatments (1-7), β_j is the block effect ($j = 1-16$) and ε_{ij} is the random effect of error.

6.4 Results and Discussion

The effect of addition of oil to chicken frankfurters on the sensory properties is shown in Table 6.2. Adding flax oil (1.2% and 2.4%) or microencapsulated fish oil (1.2%) to chicken frankfurters resulted in a softer and a juicier product similar to the control ($p < 0.05$). The addition of microencapsulated fish oil at 2.4% and 3.6% resulted in harder and less juicy frankfurter than the control. This increased hardness could be attributed to high protein content in 2.4% and 3.6% microencapsulated fish oil treatments which is in line with the results of instrumental evaluation of texture. High protein

content has been found to increase the emulsion matrix and thus hardness of frankfurters (Jimenez-Colmenero et al., 1996). The high protein content can be speculated to be due to the addition of gelatin as microencapsulation material. The commercial brand of chicken frankfurter used in the study was rated by the panelists to be harder and less juicy similar to the two higher microencapsulated fish oil treatments. The control treatment was found to be less hard and juicier than the brand which suggested the high acceptability of the chicken frankfurters developed in this study. Panelists rated overall acceptability considering both texture and flavor. The incorporation of chicken thighs, MSM and skin in combination resulted in superior quality chicken frankfurters compared to commercial available ones.

Table 6.2 Sensory properties of chicken frankfurters prepared with omega-3 oils in comparison to a commercial brand

Treatments	Hardness	Juiciness	Fishy flavor	Overall acceptability
Flax oil (1.2%)	3.84b	6.87a	3.82ab	8.64ab
Flax oil (2.4%)	4.22b	6.99a	3.71ab	8.77ab
MF (1.2%)	4.54b	6.22a	3.19b	8.95ab
MF (2.4%)	5.87a	4.52b	3.68ab	8.10ab
MF (3.6%)	6.37a	3.85b	4.65a	6.82b
Control	3.74b	6.98a	3.97ab	9.75a
Commercial brand	6.20a	3.59b	3.11b	7.30ab
SEM	0.25	0.27	0.29	0.62
ANOVA	P-VALUE	P-VALUE	P-VALUE	P-VALUE
Treatment	<0.001	<0.001	0.008	0.02

Means with different letters in the same column are significantly different ($p < 0.05$).

Score ratings on 15 cm scale

SEM- Standard error of mean

MF – Microencapsulated Fish Oil

There was no significant difference in fishy flavor between oil treated frankfurters and the control ($p < 0.05$). Fishy flavor of chicken frankfurters prepared with microencapsulated fish oil at 3.6% was found to be significantly higher ($p < 0.05$) than the fish oil at 1.2%.

Overall acceptability score for all the chicken frankfurters was high. It was more than 7 on 15 cm scale for all treatments except 3.6% microencapsulated fish oil added frankfurters. The overall acceptability of 3.6% microencapsulated fish oil treatment was lower than the control ($p < 0.05$) which could be due to the previously mentioned textural attributes. Whereas, 2.4% microencapsulated fish oil treated frankfurters had higher hardness and lower juiciness than the control, it was considered acceptable by the panelists because of absence of fishy flavor. Finally, it is important to point out that the panelists considered oil added frankfurters to be acceptable independent of the amount of oil added except in 3.6% fish oil added frankfurter.

One of the limiting factors for the use of fish oil in foods can be the fishy taste. This has been reported by Park et al. (1989) with 5% fish oil addition in beef frankfurters and in omega-3 fatty acid enriched fermented sausages with 3.3% fish oil (Valencia et al., 2006). This can be overcome by the use of microencapsulated fish oil which is delivered in powder form. During the microencapsulation process, fish oil is created in microdroplets and is coated with a material which forms the wall thus minimizing the exposure of oil to oxygen (Kolanowski et al., 2007; Jacobsen, 2010). Others have incorporated microencapsulated fish oil in meat products and found no detrimental effect on flavor (Pelser et al., 2007). Microencapsulation of fish oil is considered an easy and effective way to deliver long chain omega-3 fatty acids into meat products (Kolanowski

et al., 2007). In the present study, addition of flax oil (1.2% - 2.4%) and microencapsulated fish (1.2% - 2.4%) to enrich chicken frankfurters resulted in a product as acceptable as that of control. Makala et al. (2007) found that addition of flax oil to bologna pork sausage at 0.6% of the final product did not change sensory attributes compared to the control. The enrichment of omega-3 fatty acids in chicken frankfurters can be performed with 2.4% microencapsulated fish oil without affecting texture, flavor and overall acceptability. The result of sensory evaluation for hardness confirmed the result of instrumental hardness measurement as observed by Bloukas et al. (1997).

6.5 Conclusion

Omega-3 fatty acid enrichment of chicken frankfurters can be achieved by incorporation of flax oil or microencapsulated fish oil at 1.2% and 2.4% without affecting the texture, flavor and overall acceptability. Addition of microencapsulated fish oil at 3.6% to chicken frankfurters resulted in a hard and less juicy texture and noticeable fishy flavor contributing to low overall acceptability. Omega-3 fatty acid enriched chicken frankfurters were found to have better texture and overall acceptability than the commercially available chicken frankfurters.

CHAPTER 7

CONCLUSION

7.1 Summary and Conclusion

The objective of this research was to develop chicken frankfurters enriched with omega-3 fatty acids. Omega-3 fatty acid enriched chicken frankfurters may contribute to a more healthful diet. Fat content in frankfurters is generally up to 30% and the presence of saturated fats in these products is a main concern for consumers as saturated fats are associated with increased risk of cardiovascular disease. Reformulation of meat products is one strategy to develop healthier meat products. This can be accomplished by replacement of fat with oils rich in omega-3 fatty acids which would lead to a healthier fatty acid profile in the final product which would have more appeal to the consumers. However, addition of oil should not have any detrimental effect on the quality attributes which includes color, texture and sensory properties (texture and flavor) as consumers often relate these attributes to overall quality. In this study, replacement of chicken fat in frankfurters with flax oil and microencapsulated fish oil powder at 1.2%, 2.4% and 3.6% was carried out.

The findings of this study revealed that the fatty acid composition of chicken frankfurters was modified by addition of oil without affecting the moisture and fat content. Whereas, there was minor differences in the protein content with protein content being higher in 3.6% microencapsulated fish oil powder treatment. Omega-3 fatty acids content in oil added frankfurters were higher than the control treatment by minimum 470 mg/100g of frankfurter. This increased level was found to meet the labeling requirement for omega-3 fatty acid enriched products (300 mg/100g). α -Linolenic acid was the major

omega-3 fatty acid in flax oil added frankfurters and EPA and DHA were the main source of omega-3 in fish oil treatments. Omega-3 fatty acid enrichment of chicken frankfurters can be achieved with minimum level of flax oil (1.2%) or microencapsulated fish oil powder at 2.4%. Also, the ratio of omega-6: omega-3 was modified to the recommended ratio of 4 in all the oil-added frankfurters compared to 9 in the control. There was no measureable lipid oxidation in the manufactured chicken frankfurters including as the products were stable during 30 days of storage at 4°C.

Color of omega-3 fatty acid enriched chicken frankfurters resembled that of control except there were an increase in redness in 3.6% fish oil added frankfurters. During storage period of 30 days lightness and redness increased from day 10 while yellowness increased on day 20. The textural attributes (hardness, gumminess and chewiness) were not modified by the addition of flax oil to chicken frankfurters and resembled that of control. Whereas, the microencapsulated fish oil incorporation at 2.4 % and 3.6% were found to be more hard, gummy and chewy than the control. Hardness, gumminess and chewiness increased at day 20 during storage period.

The sensory evaluation of oil added chicken frankfurters also revealed similar characteristics of hardness, juiciness, fishy flavor and overall acceptability of flax oil added frankfurters with that of control. Addition of microencapsulated fish oil powder at 2.4% and 3.6% level was found to be harder and less juicy than the control. However, the panelist rated 2.4% to be highly acceptable while acceptability of 3.6% fish oil treatment was lower than control. Addition of microencapsulated fish oil powder did not result in fishy flavor.

Overall, the study provided an insight on the textural and sensory characteristics of chicken frankfurters prepared by replacing chicken fat with flax and microencapsulated fish oils. According to the results of the present research it can be established that it is possible to develop chicken frankfurters enriched with omega-3 fatty acids and with a favorable omega-6/omega-3 ratio without the problem of lipid oxidation and loss of sensory quality by replacing chicken fat with flax oil. Addition of flax oil at a minimum level of 1.2% is sufficient enough to enrich chicken frankfurters with omega-3 fatty acids especially ALA. Further increased levels of flax oil more than 1.2% would increase the cost of production of chicken frankfurters. On the other hand, addition of microencapsulated fish oil at 2.4% was found to be optimum for enrichment of chicken frankfurters with long chain omega-3 fatty acids, EPA and DHA. Replacement of chicken fat with 3.6% microencapsulated fish oil resulted in increased EPA and DHA amount, but the developed product was affected for its texture and measured sensory attributes. Microencapsulation of fish oil protected the omega-3 fatty acids from lipid oxidation which resulted in a stable product because of the presence of antioxidants added to fish oil. Consumption of fish oil incorporated frankfurters would be a good alternative source of omega-3 fatty acids especially EPA and DHA for young children and people who may not consume fish. Thus it is possible to replace part of chicken fat with omega-3 rich oils to give a healthy image to chicken frankfurters.

7.2 Future Prospective of Research

In the present study, the observed effects of microencapsulated fish oil powder on texture in frankfurters could not be related to effect of microencapsulation. The study of interaction of proteins and added oils in meat emulsion at the microstructure level could

generate information on the emulsion stability. Inclusion of fish oil treatment would give a clear idea on the observed differences between flax oil and microencapsulated fish oil in relation to textural properties. Analysis of lipid oxidation products by gas chromatography would provide an insight on the type of oxidation products in the oil added frankfurters.

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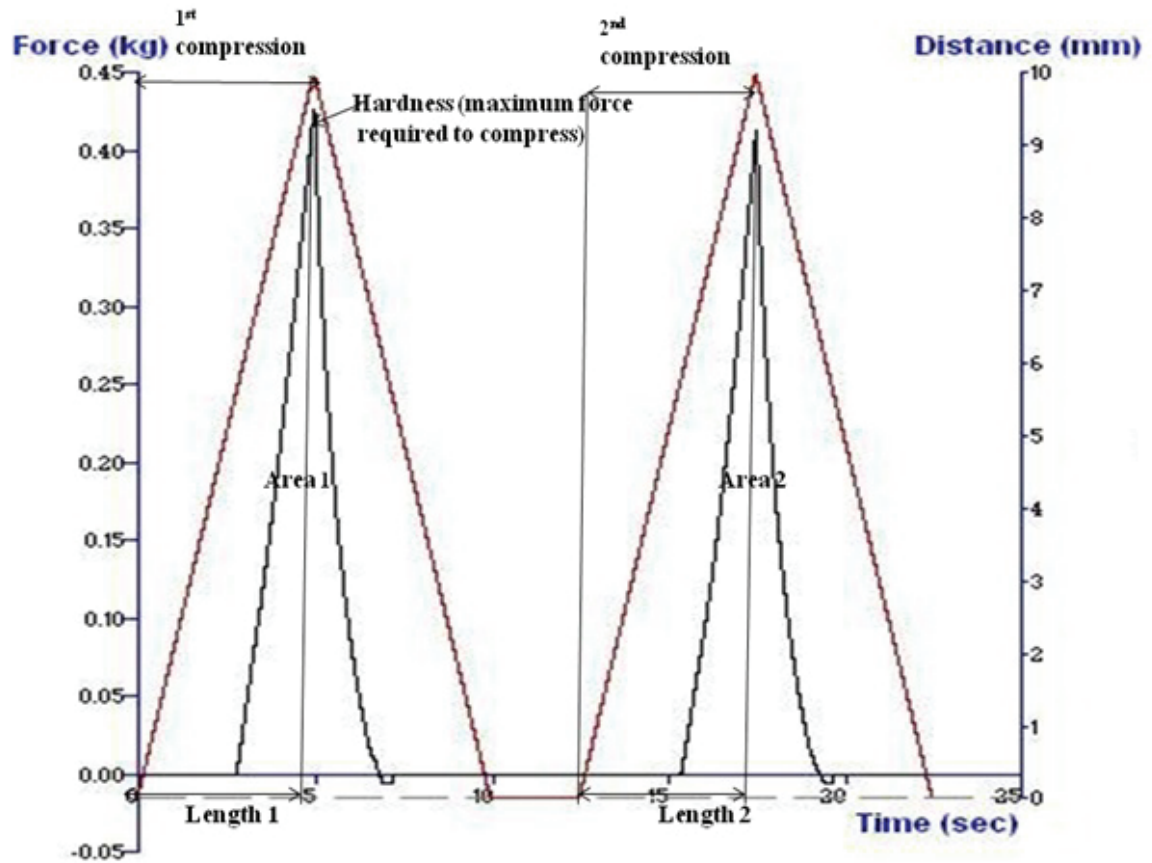
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APPENDIX I

Texture profile analysis curve obtained from Instrumental Texture analyzer

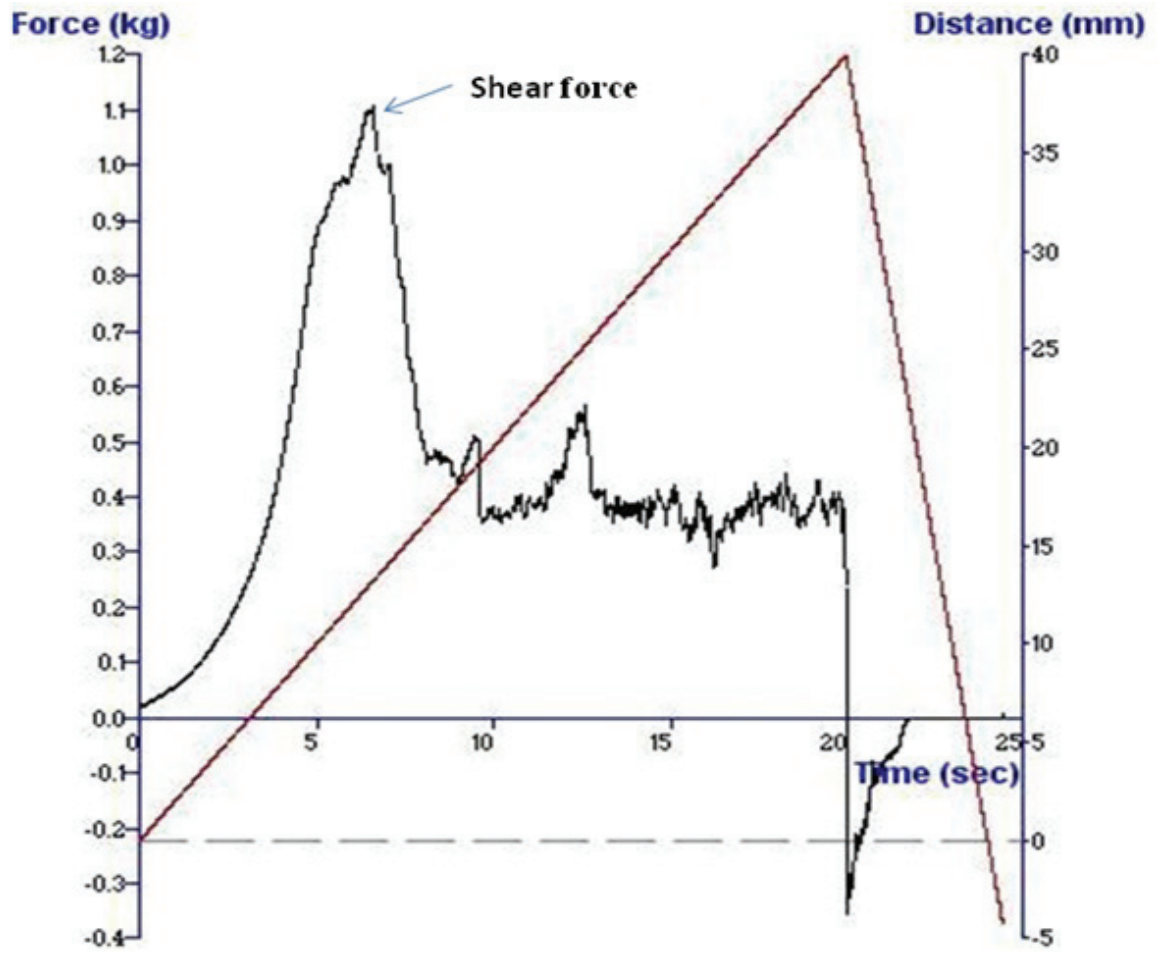


$$\text{Springiness} = \text{Length 2} / \text{Length 1}$$

$$\text{Gumminess} = \text{Area 2} / \text{Area 1} * \text{Hardness}$$

$$\text{Chewiness} = \text{Gumminess} * \text{Length 2} / \text{Length 1}$$

Warner-Bratzler shear force curve generated from Texture analyzer



APPENDIX II



**Nova Scotia
Agricultural
College**

Research Ethics Board

P.O. Box 550
Truro, Nova Scotia
Canada B2N 5E3
Ph: 902-893-4413
Fx: 902-893-3430
nsac.ca/reb

June 9, 2010

Dear Sadish Srinivassane:

I refer to your research ethics application entitled "Sensory evaluation of omega-3 enriched chicken frankfurters". On behalf of the REB, I am pleased to approve your research ethics application as revised. I will take this opportunity to remind you of the following REB administrative and reporting requirements:

1. You are required to submit an annual report by the NSAC REB anniversary year of your research project, and every subsequent year until the research project is completed. For the avoidance of doubt, your first annual report should be submitted no later than June 9, 2011. Research ethics annual and final report forms are available on the NSAC Research Ethics Board website: (<http://nsac.ca/research/researchers/ethics.asp>).
2. If the research methods or instruments change, then please send the changes and/or revisions to the Chair of the REB as soon as possible. The Chair will determine if the changes need to be presented to the entire REB.
3. If an adverse event occurs, such as violation of privacy or complaint by a human participant in your study, please inform the REB within a week of the occurrence.
4. Keep a complete record of all materials related to this research project in a secure location accessible for review by the REB or TriCouncil auditors, including a copy of your applications to the REB, all correspondence with and from the REB (such as those related to adverse events and amendments), and any original signed consent forms and data forms.

Best wishes with your research.

Sincerely,

Emmanuel Yiridoe, Ph.D.
Chair, Research Ethics Board

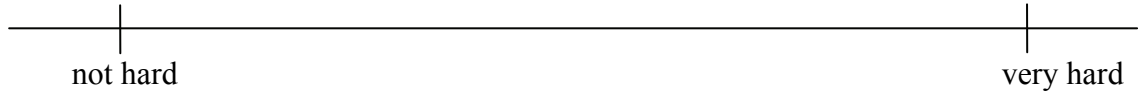
Cc: Carolyn Terry, REB Secretary
Bruce Rathgeber, Supervisor

NSAC. Embrace Your World.

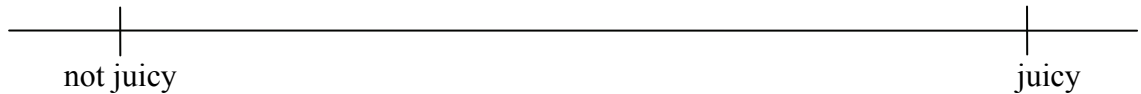
Score Sheet for Sensory Analysis Test

Please evaluate and score the products for texture (hardness, juiciness) and fishy flavor. Code numbers are mentioned on the containers.

1. Please rate the following for: **Hardness**.....



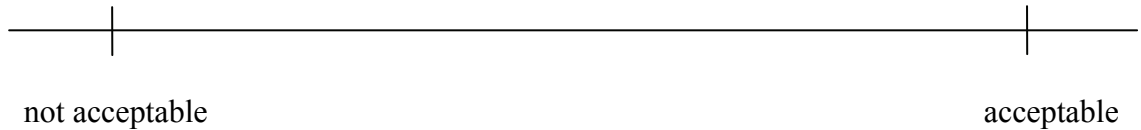
2. Please rate the following for: **Juiciness**.....



3. Please rate the following for: **Fishy flavor**.....



4. Please rate the following for: **overall acceptability**.....



Comments:.....
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.....
.....
.....

APPENDIX III

Table AIII.1 Microbiological count of Omega-3 enriched chicken frankfurters used in sensory evaluation

Chicken frankfurters formulations	Aerobic colony count (ACC)
Flax oil 1.2%	45.2 x 10 ¹
Flax oil 2.4%	129.5x 10 ¹
Microencapsulated fish oil 1.2%	84 x 10 ¹
Microencapsulated fish oil 2.4%	70 x 10 ¹
Microencapsulated fish oil 3.6%	85.2 x 10 ¹
Control	64 x 10 ¹

No detectable *Staphylococcus aureus* and *E.coli*