Blending palm oil with flaxseed oil or menhaden fish oil to produce enriched omega-3 oils for deep-fat frying

by

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Abstract

Two of the major dietary food sources of omega-3 fatty acids are flaxseed oil and fish oil; the former being a rich source of PUFA (e.g. α-linolenic acid (α –LA)), while the latter is a source of HUFA (e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)). In this study, palm oil, a commonly used vegetable oil that is widely available in Asian and African countries, was blended with flaxseed (omega 3-PUFA) and fish oil (menhaden oil) (omega-3 HUFA); respectively, to obtain blended oils that both contained a 1: 4 ratio of omega-3:omega 6 fatty acids. Rosemary extract (0.02% w/w) was added to the oil blends to stabilize the oil during use for deep-fat frying. Eight hours of heating at 180 °C was used to determine the stability of omega-3 fatty acids and uptake from the omega-3 enriched palm oil into fried potatoes. Lipid oxidation and thermal degradation of the palm oil blends, along with retention of α –LA, EPA, and DHA were measure of oil blends stability. Linoleic acid content in flax-palm oil blend did not change during frying when in the presence of different antioxidant treatments. The α –LA content of heated flax-palm oil blend was significantly reduced (P<0.05) after 8 hours of frying. Meanwhile, linoleic acid and EPA content in the fish-palm oil blend revealed significant (P<0.05) decreases in concentration after 8 hours of frying regardless of the presence of antioxidant. The DHA concentration was significantly lower when present in the absence of antioxidant (P<0.05). Totox significantly increased (P<0.05) in the blended oils after 8 hours of frying; albeit the extent of oxidation and thermal degradation was reduced when rosemary extract was added. A significant uptake of omega-3 fatty acids in both the
omega-3 PUFA (e.g. α-LA) and HUFA (e.g. EPA and DHA), respectively, occurred in potatoes fried in the respective blended oils. Although omega-3 fatty acid uptake was prevalent in potatoes fried in both blended oils, the effect of heating reduced the optimal 1:4 ratio of omega-3:omega-6 to a 1:6-1:7 ratio. This loss in omega-3, relative to omega-6, was attributed to thermal oxidation; a reaction not totally preventable by adding antioxidants to the frying oils. These functional omega-3 enhanced oils when used to process potatoes gave forth products that represented 1/10th suggested intake for EPA+DHA and 1/50th the daily requirement for α-LA.
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List of Abbreviations

ACM  Alkaline contaminant materials
α–LA  Alpha-linolenic acid
ANOVA  Analysis of variance
BHA  Butylated hydroxylanisole
BHT  Butylated hydroxytoluene
CV  Coefficient of variation
EPA  Eicosapentaenoic acid
FID  Flame ionization detector
DHA  Docosahexaenoic acid
GC  Gas chromatography
HPLC  High performance liquid chromatography
HUFA  Highly unsaturated fatty acid
PUFA  Polyunsaturated fatty acid
RBD  Refined, bleached, and deodorized.
TBHQ  Tertiary-butylhydroquinone
w-3/n-3  Omega 3 fatty acids
w-6/n-6  Omega 6 fatty acids
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General Introduction

Palm seed is a common agricultural commodity that is a source of many different edible fats and oils in Asian and African countries. Palm oil is mainly used as a cooking oil and is produced in South East Asia, Africa, and Central and Latin America (Hui, 1996). There are four major fatty acids present in refined, bleached, and deodorized (RBD) palm oil; these include palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids (Jaswir et. al, 2000).

Many studies have suggested that high intake of omega-3 fatty acids could exert a strong positive influence on human health (Kolanowski et. al, 1999); however, dietary intake of these fatty acids are usually low (Kolanowski et. al, 1999). Flaxseed is a major omega-3 fatty acid source containing α-linolenic acid, along with other non-lipid constituents such as certain sources of phytoestrogens, lignans, and soluble fibers (Bloedon, 2004; Kitts et. al, 1999). Another source of omega-3 fatty acid is fish oil, which is known to be the main dietary source of very long chain omega-3 polyunsaturated fatty acids (Gunstone, 1996; Hyunh et. al, 2007).

In general, an increase in omega-3 fatty intake in the diet has been associated with a decreased risk of cardiovascular disease, as well as additional benefits in brain development (Ruxton et. al, 2005). Scientific evidence has shown that the desirable ratio of omega-3 to omega-6 intake should be in the range of 1:5 to 1:10 (Kris-Etherton et. al, 2000). For example, Kang (2003), reported that a low balanced diet of omega-6/omega-3 fatty acids had a beneficial effect in different cell types, such as decreased susceptibility
of heart cells to arrhythmia, reduced response of endothelial cells to inflammatory agents, and increased apoptosis of cancer cells, as well as enhanced survival of neurons against ischemia. In general, there is reason to support the trend for increased omega-3 fatty acid intake in human diets. One strategy to accomplish this is to include frequent intake of food products enriched in omega-3 fatty acids in the diet (Kolanowski et. al, 1999).

Many physical and chemical changes occur during frying of palm oil. These include changes in colour formation, induced oxidation, polymerization, and hydrolysis (Augustin and Berry, 1983; Varela et. al, 1986; and Lawson, 1995). A primary concern is lipid oxidation, since in commercial deep-fat frying, the fat is continuously exposed to air and light for approximately 12 hours per day at 180 °C (Gwo et. al, 1985).

Synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxylanisole, and tertiary-butylhydroquinone are used in a variety of foods on the current market to prevent lipid oxidation (Chang et al., 1977). The maximum amount of synthetic antioxidants added is 0.02% (weight/weight), or 200 mg/kg of fat in food (Flick et al., 1992; Food Act and Regulations, 1996). With the increase in consumer concern over the use of synthetic antioxidants, employing natural antioxidant substances may be viable alternatives to replace the conventional stabilizers (Cuveiler et. al, 1994). Studies by Che Man and Tan (1999), showed that two plant-derived antioxidants; namely rosemary and sage, had the potential to replace the use of synthetic antioxidants in food processing. Moreover, Berner and Jacobson (1973), Chang et al. (1977), and Nakatani (1989), reported in detail, that rosemary and sage provided antioxidative characteristics in food.
The purpose of this thesis was to create omega-3 enriched palm oils by blending two different omega-3 sources (flaxseed oil and menhaden fish oil) along with the addition of antioxidants (e.g. BHA and rosemary extract) to palm oil, and perform deep-fat frying experiments with the formulated products at 180 °C for 8 hours. Furthermore, oleoresin rosemary at 0.02 % concentration was used to investigate the potential of stabilizing the omega-3 enriched palm oil during deep-fat frying through addition of this natural antioxidant.

The hypotheses of this research were:

1. Flaxseed oil and menhaden fish oil, can be useful sources for blending to palm oil, to provide specific oil blends that contain characteristics 1:4 ratio of omega-3:omega-6 PUFA and HUFA, respectively.

2. The addition of antioxidants, are effective at lowering total oxidation and degradation during frying of PUFA/HUFA-containing blended oils.

3. Omega-3 PUFA and HUFA content of flaxseed and fish oil blended palm oil, respectively, can be protected from thermal oxidation and degradation reactions with the addition of rosemary extract.
Research objectives:

1. Palm oil will be blended with flaxseed oil and fish oil, respectively to establish a reproducible ratio of 1:4 (omega-3:omega-6) using mass quatitation of fatty acid content.

2. Rosemary extract will be added to flaxseed or fish oil-blended palm oil to decrease the total oxidation and degradation during deep-fat frying.

3. Rosemary extract will be added to protect the optimal omega-3/omega-6 fatty acids content of blended oil during deep-fat frying.

4. Potato will be used as a model food system to demonstrate the uptake of omega-3 into potato products during deep-fat frying using blended palm oil
Chapter 1. Literature Review

1.1 Palm Oil

Palm oil is a vegetable oil that is widely used in Asian and African countries and is considered the second largest source of edible oil in terms of world production; comprising of about 50 % of world’s traded oil (Schroeder et al., 2006). Palm oil is mainly used as a cooking oil and is produced in South East Asia, Africa, and Central and Latin America (Hui, 1996). Although many countries produce palm oil, Malaysia and Indonesia are the two major countries that export palm oil (Hui, 1996).

Palm oil is obtained through an extraction process from the fleshy orange-red mesocarp of fruits derived from the palm tree (Elaeis guineensis). Palm fruit contains 45-55% oil (Edem, 2002) and crude palm oil is characterized as a semi-solid oil material at room temperature with a melting point of about 36 °C (Gunstone, 1987). Crude palm oil can be fractionated into a liquid oil, palm olein (65-70% with melting point 48-50 °C) and a solid fraction, stearin (30-35 % with melting point 48-50 °C) (Gunstone and Norris, 1983).

Crude oils and fats obtained from plant sources are further processed to remove a variety of substances such as fatty acids, phospholipids, carbohydrate, proteins, water pigments, and lipid oxidation products, which may impart undesirable flavour, colour, as well as the functional quality of the oil itself. Refined, bleached, and deodorized (RBD) palm oil is obtained from crude palm oil after it is subjected to several commercial refining processes, which include settling and degumming, neutralization, bleaching, and
deodorization (MPOPC, 1996). In the settling step, substances such as water, proteinaceous materials, phospholipids, and carbohydrates are removed through heating and separation of the aqueous phase (to be withdrawn) (MPOPC, 1996; Nawar, 1996). For example, some oils that are particularly rich in phospholipids (e.g. soybean oil), degumming is performed by adding 2-3% water to the oil; this is then followed by mixing and agitation at 50 °C until the hydrated phospholipids are separated by settling or centrifugation (Nawar, 1996). The neutralization step removes the free fatty acids that are present in the oil, since these fatty acids may contribute to lipid oxidation (MPOPC, 1996). In this step, caustic soda is mixed with heated oil, after which the mixture is allowed to stand until the aqueous phase settles. The aqueous solution is then removed and used for making soap (Nawar, 1996). The next process in edible oil processing is bleaching, or the removal of colour material, since it may lead to photooxidation and rancidity; this can be achieved by heating the oil to 85 °C and treating it with adsorbents (e.g. activated carbon) (Nawar, 1996). The final processing step is deodorization where the volatile compounds with undesirable flavours are removed by steam distillation under reduced pressure (MPOPC, 1996). In this stage, citric acid is often added to sequester traces of pro-oxidant metals.

There are four major fatty acids found in refined, bleached, and deodorized palm oil; these include palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids (Jaswir et al., 2000). Jasperson and Pritchard (1981), reported that the proportion of typical component fatty acids in palm oil consisted of 40% palmitic acid, 5% stearic acid, 43 % oleic acid, and 10% linoleic acid, with traces of other minor fatty acids such as myristic acid (2%) also present. Kochhar (2000), reported that the proportion of fatty
acids in palm oil consisted of 44% palmitic acid, 4.5% stearic acid, 39.2 % oleic acid, and 10.1 % linoleic acid, with trace amounts of other minor fatty acids also present. These reports suggest that each palm oil consisted of different fatty acid proportions depending on the source of material.

Palm oil is considered to be an oxidative stable oil due to a fatty acid composition that contains low polyunsaturation and relatively high antioxidant content. Natural antioxidants in palm oil are present in relative high concentrations and consist of three forms of tocotrienols (eg. α, γ, and δ), unlike many vegetable oils that contain only α-and γ-tocopherol. Previous reports suggested that the total tocopherol and tocotrienol content of palm oil is about 600-1000 ppm, with 72-82% of comes from homologue tocotrienols (Schroeder et al., 2006). Tocopherols have been reported to be excellent radical chain breaking antioxidants (La-Saquer et al., 2004). In addition, provitamins A, α and β-carotene (400-3500 ppm) are also present in palm oil, the relative amount depending on the refining process (Ooi, 1999). Moreover, the presence and amount of carotenoids has an important role in influencing the colour of palm oil (light yellow to orange-red) (Markom et al., 2001).

1.2 Flaxseed Oil

Flaxseed oil is a highly used edible oil in India, Asian countries, as well as the Western world (Bera et al., 2006). Flax (Linum usitatissimum) is one of the oil crops that is produced and plays an important economic role in Canada, which produces about 40 % of the world’s flaxseed (Oomah and Mazza, 1999). Currently, Canada is considered as the world’s largest exporter of flax seed, contributing about 75% of the world trade
Flaxseed is considered to be an excellent source of essential fatty acids, viscous fiber components and phytochemicals, such as lignans and protein (Bloedon, 2004; Carter, 1993; and Kitts et al., 1999). Flaxseed oil has many potential physiological benefits which can be attributed primarily to the high content of α-linolenic acid (Carter, 1993). These benefits include the inhibition of eicosanoids synthesis and alteration of prostanoid production, that results in blood pressure reduction in hypertensives as well as possible lowering effects for triglycerides and cholesterol (Berdanier, 2000). Moreover, dietary α-linolenic acid has also been shown to inhibit lymphocyte proliferation in animals and immune response in humans (Carter, 1993). A detailed study by Kelly (1995), showed that the intake of alpha-linolenic acid may be beneficial to individuals with autoimmune disorders. Other benefits of dietary α-linolenic acid include the retardation of tumor growth and also a role in preventing metastasis (Johnston, 1995). The presence of α-linolenic acid in the diet may also be essential for human’s neurological development, especially during fetal and early postnatal life (Cunnane, 1995).

The high dietary fiber content of flaxseed, such as gums and mucilage is also thought to have health benefits; which include roles in risk prevention of diabetes, coronary heart disease, colon cancer, as well as the incidence of obesity (Salmeron et al., 1997; Reddy, 1999; Katan, 1996; and Sparti et. al, 2000).

1.3 Fish Oil

Fish oils make a relatively minor contribution, about 2%, to the annual production of edible oils and fat; with main use in margarine production after hydrogenation
omega-3 fatty acids from fatty fish such as herring, capelin, menhaden, sardine, and anchovy (Kinsella, 1987). The composition of fish oil may vary depending on a variety of factors such as fish species, sex, and season (Turner et al., 2006). Fish oil is considered the best source of long chain polyunsaturated omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) (Turner et al., 2006; Kinsella, 1987; and Gunstone, 1996), in which many health benefits derived form the presence of these fatty acids.

1.4 Omega-3 Fatty Acids

Omega-3 fatty acids are considered to be essential fatty acids that are obtained only from the diet (Whitney and Rolfes, 2002; Berdanier, 2000). The primary member of omega-3 fatty acids in vegetable oils is α-linolenic acid (Ruxton et al., 2005; Whitney and Rolfes, 2002; and Berdanier, 2000). With the availability of dietary α-linolenic acid PUFA, further elongation and desaturation reactions in the human body can make the highly unsaturated 20 and 22 carbon member (e.g. eicosapentaenoic acid and docosahexaenoic acid) of the omega-3 series (Ruxton et al., 2005; Whitney and Rolfes, 2002; and Berdanier, 2000). Figure 1 shows the metabolic pathway of omega-3 fatty acids starting with alpha-linolenic acid and enzymatically transformed through elongation and desaturation to eicosapentaenoic acid (Gunstone, 1996). Further elongation and desaturation of eicosapentaenoic acid will yield tetrahexaenoic acid (Sprecher, 1999). Tetrahexaenoic acid is then translocated from the endoplasmic reticulum to peroxisomes.
where two carbons are removed by β-oxidation to yield DHA (Ruxton et al., 2005).

Omega-3 fatty acids play an important role in human health, through eicosanoids
produced from the metabolic pathway of omega-3 fatty acids (Ruxton et al., 2005). This
pathway regulates a number of cell response-processes, such as inflammation,
aggregation, vasodilation, and blood pressure (Berdanier, 2000).

Many studies have suggested that a high dietary intake of omega-3 fatty acids can
exert a strong positive influence on human health (Kolanowski et al., 1999). However, it
is common that the intake of these fatty acids is low (Kolanowski et al., 1999). Two
major dietary sources of omega-3 fatty acids are flaxseed oil and fish oil. Flaxseed is the
richest known source of α-linolenic acid (Bloedon, 2004), where fish oil is a well known
source of very long chain, highly unsaturated omega-3 polyunsaturated fatty acids
(Turner et al., 2006).

Recent research has suggested that modern industrial processing of vegetable oil
has played an important role in reducing omega-3 fatty acid content in human diets.
Simopoulos (2003), described the efficiency of economically large-scale production
practices used to process crude oils that results in a reduction of omega-3 content in some
vegetable oils. In detail, many oil processes such as partial selective hydrogenation will
reduce the α-linolenic acid content of soy-bean oil while leaving a high concentration of
linoleic acid.
Figure 1. Metabolic pathways of the omega-6 fatty acids and omega-3 fatty acids (Ruxton et al., 2005 and Gunstone, 1996).
Simopoulos (1999), emphasized that the current ratio of omega-3:omega-6 fatty acids in human diet is currently 1:20-30, which is far below an optimal health range of 1:1-2 to receive health benefits. It is commonly believed that the desirable ratio of omega-3 to omega-6 intake should be somewhere in the range of 1:1 to 1:10 (Health and Welfare of Canada, 1989; Kris-Etherton et al., 2000). Scientific evidence is now available to show increased health benefits for the 1:4 to 1:10 n-3:n-6 ratios. For example, Goodstine et al. (2003) and Bagga et al. (2002), both reported that increases in omega-3 fatty acid intake were effective at reducing the risk of breast cancer. In detail, Chajes and Bougnoux (2003), suggested that the n-3:n-6 ratio of approximately 1:1-2 had the most protective effect against the development and growth of mammary and colon cancers. Kang (2003), also found that a low balance of omega-6/omega-3 fatty acids had a beneficial effect in different cell types, such as decreased susceptibility of heart cells to arrhythmia, reduced inflammatory response of endothelial cells, and increased apoptosis of cancer cells and enhanced survival of neurons against ischemia. In separate research, Yehuda (2003), described that a n-3/ n-6 ratio of 1:4 was the best ratio for improving learning performance, elevating pain threshold, improving sleep, and improving thermoregulation. Increased intake of alpha-linolenic acid can also decrease the risk of coronary artery disease, as reported by Zampelas et al. (2003) and Pella et al. (2003).

In general, there are indications for a trend to increase omega-3 fatty acid intake in the human diet (Kolanowski et al., 1999). Many researchers have suggested that the ratio of omega-3 to omega-6 in the diet should be at least 1:4-5 (Salem et al., 1998; Simopoulos et al., 1999; and Sanders, 2000). Kolanowski et al. (1999), suggested that a
good way to raise the omega-3 intake without making major changes in eating habits, is
to enrich frequent and common consumed food products with omega-3 fatty acids.

1.5 Omega-6 Fatty Acids

The primary member of the omega-6 fatty acids in vegetable oils is the essential fatty acid, linoleic acid (Ruxton et al., 2005; Whitney and Rolfes, 2002; and Berdanier, 2000). Similar to α-linolenic acid, the human body can metabolize and produce the 20 carbon polyunsaturated fatty acid (arachidonic acid) through further elongation and desaturation reaction from linoleic acid (Ruxton et al., 2005; Whitney and Rolfes, 2002; and Berdanier, 2000). The omega-6 fatty acid metabolic pathway shown in Figure 1 illustrates the conversion of linoleic acid to gamma-linolenic acid, which can be further elongated and desaturated to yield arachidonic acid using the same series of enzymes involved in the metabolism of omega-3 polyunsaturated fatty acid (Ruxton et al., 2005). Unlike the case of omega-3 fatty acids, the normal human diet containing vegetable oils and meats provides enough omega-6 fatty acids to meet the body’s need (Whitney and Rolfes, 2002).

Similar to omega-3 fatty acids, various possible health promoting effects through eicosanoid production derived from the omega-6 metabolic pathway have been associated with the presence of omega-6 fatty acids. Moreover, many reports have shown that omega-6 and omega-3 fatty acids are precursors of potent lipid mediators in tissues that have important roles in regulation of inflammation, blood viscosity, vasoconstriction/vasodilation, and blood pressure (Hudert et al., 2006; Calder, 2006; and Berdanier, 2000).
1.6 Eicosanoids

Eicosanoids are produced in the human body using essential fatty acids as the substrate (Whitney and Rolfes, 2002; Berdanier, 2000). Eicosanoids are described as a diverse group of “hormone like” compounds, but they are different from hormones in important ways (Whitney and Rolfes, 2002). For example, hormones are secreted in one location and travel to specific targets, whereas eicosanoids only affect the cells in which they are produced (Whitney and Rolfes, 2002).

Eicosanoids have important participating roles in the immune response, inflammation, pain, blood pressure, blood clot formation, etc. (Whitney and Rolfes, 2002; Berdanier, 2000). Eicosanoids produced from the elongation and desaturation of omega-3 and omega-6 fatty acids exhibit antagonistic activities in immune response, inflammation, pain, blood pressure, as well as blood clot formation. As an example, generally omega-6 polyunsaturated fatty acids promote inflammation, whereas, the omega-3 polyunsaturated fatty acids show anti-inflammatory properties (Hudert et al., 2006). Inflammation is a normal reaction as the host response to infection and injury; however, excessive inflammation may contribute to various chronic human diseases (Calder, 2006). Inflammation can be attributed to the production of inflammatory cytokines, arachidonic acid-derived eicosanoids (prostaglandins, thromboxane, leukotrienes, and other oxidized derivative), inflammatory agents, and adhesion molecules (Calder, 2006). In particular, long chain omega-3 fatty acids have been shown to have anti-inflammatory properties by inhibiting the formation of omega-6
polyunsaturated-derived proinflammatory products, such as eicosanoids that are involved in generation of reactive oxygen species, release of cytokines, and the expression of adhesion molecules (Hudert et al., 2006; Calder, 2006).

1.7 Lipid Oxidation

Lipid oxidation occurs in food products and edible oil products that contain high amount of lipids, especially a high proportion of polyunsaturated fatty acids. Chan (1987), reported that polyunsaturated fats quickly autoxidize even at ambient or subambient temperatures. Autoxidation (Figure 2) is one of the major causes of food spoilage and is of great economic concern to the food industry because it leads to the rancidity of edible oils and fat-containing foods (Nawar, 1996), which reduces shelf-life. The mechanisms of autoxidation are described as follows (Nawar, 1996; Shahidi and Wanasundara, 1992):

Initiation: \( \text{(Initiator} \rightarrow \text{free radicals)} \)

\[
\begin{align*}
\text{LH} & \rightarrow L' \\
L' + O_2 & \rightarrow LOO' 
\end{align*}
\]

Propagation: \( \text{LOO'} + L'H \rightarrow \text{LOOH} + L' \)

Termination: \( \text{LOO'} + L'' \rightarrow \text{LOOL'} \\
\text{LOO'} + L'OO' \rightarrow \text{LOOL'} + O_2 \\
L' + L' \rightarrow LL'
\]

Where:

LH and L’H : fatty acid
L' and L’” : free radical
In the initiation step, the presence of initiators with an unsaturated fatty acid forms a free radical, which then reacts with oxygen to form a peroxy radical. Initiators such as transition metals have an important role in catalyzing the removal of a hydrogen ion from an unsaturated fatty acid (Turner et al., 2006). The initiation of lipid oxidation is also influenced by hydroperoxide decomposition, or by presence of metal catalysts which act as initiators. The reaction of free radical with oxygen (oxygenation) is very rapid; therefore, the peroxyl radical is considered to be a primary product of autooxidation. The propagation step can be best described as the continuation and acceleration of the chain reaction following initiation. During the propagation step, peroxy radicals produce a free radical by abstracting a hydrogen atom from an adjacent fatty acid. The new free radical will then oxygenate and form a peroxy radical which can further accelerate the oxidation step. In the final termination step, free radicals generated will react with each other and form neutralized non-radical species. Alcohols, aldehydes, acids, ketones, and other less reactive substances are also produced as secondary products as a result of hydroperoxide decomposition.

The reaction of substrate fatty acid with oxygen that produces free radicals is thermodynamically unfavorable (activation energy is about 35 kcal, or 146 kJ/mol),
therefore catalysts are required for the production of the first few radical initiated to start
the propagation reaction (Nawar, 1996).

Photo-oxidation is another oxidation reaction that occurs in oils; where light
exposure (UV radiation) and a photo-sensitizer (chemicals that can be excited by UV
radiation; e.g. chlorophyl) convert triplet oxygen to singlet oxygen. This in turn reacts
with fatty acid double bonds to form lipid hydroperoxide (Turner et al., 2006). Photo-
oxidation products can also initiate further autooxidation reactions (Frankel, 1991).
The mechanism of photo-oxidation is as follow (Cuppert et al., 1996; Turner et al., 2006):

Initiator (e.g. UV light)

\[ ^3O_2 \rightarrow ^1O_2 \]
\[ ^1O_2 + LH \rightarrow LOOH \]

Where:

\[ ^3O_2 \] : triplet oxygen
\[ ^1O_2 \] : singlet oxygen
LOOH : hydroperoxide
LH : unsaturated fatty acid
Figure 2. Generalized scheme for autooxidation of lipids (constructed from Nawar, 1996)
1.8 Frying

Deep-fat frying is one of the oldest cooking methods, and a very popular method for food preparation at both home and commercial industry scale uses (Rimac-Brncic et al., 2004). Frying is a complex process which involves several chemical and physical changes to food, many not related to the oil itself such as starch gelatinization, protein denaturation, water vaporization, and crust formation (Rimac-Brncic et al., 2004). There are two types of frying; deep-fat frying and shallow frying. Deep fat frying is widely used in a larger scale industry, as an example, the multimillion-dollar snack food industry (Singh, 1995). Deep-fat-frying is a common way of cooking food in chips and the french fry industry (Guillaumin, 1988). This deep-fat frying process usually uses a large amount of oil and fat, in which the food is completely immersed in the hot oil or fat at temperatures greater than 160 °C (Guillaumin, 1988). In contrast, shallow frying is similar to household frying and uses a smaller amount of oil or fat; thus, this household frying may increase the degradation of lipids due to peroxide formation and decomposition during reheating, cooking and cooling cycle (Guillaumin, 1988).

Fried foods are popular since they have enhanced organoleptic characteristics that yield unique flavour and texture (Varela, 1998). However, the frying process can lead to oxidative and hydrolytic degradation and polymerization of the frying oil (Rimac-Brncic et al., 2004). Frying is a complex process involving numerous factors which depend on the actual process, the source of frying fat, and the food itself (Figure 3) (Singh, 1995; Varela, 1988). Time, temperature, and method used to fry foods have an important role in the actual process of frying. Meanwhile the chemical composition, physical and
physicochemical constants, and additives in the fat are the main key variables in frying fat. The food source should also not be overlooked, since each food must be fried in a specific way and the weight/frying fat volume as well as surface area/volume ratios influence the uptake of oil into the food.

Frying is basically a dehydration process; where water and materials within the food are heated and release into the hot oil (Varela et al., 1986; Nawar, 1996). During frying, the interactions between the food and oil may lead to the development of volatiles in the food (e.g. sulfur compounds and pyrazine derivatives in potato). Endogenous lipids from the food itself can be released into the surrounding medium which may affect the oxidative stability of the new mixture compared to the original frying fat (Nawar, 1996). Many physical and chemical changes occur during the frying of palm oil (Figure 4). These changes include colour formation, oxidation, polymerization, and hydrolysis (Augustin and Berry, 1983; Varela et al., 1986; and Lawson, 1995). When food is introduced to the hot oil, oxidation starts as the oxygen is also introduced into the oil (Lillard, 1983). Hydroperoxides, aldehydes, ketones, acids, hydrocarbons, and many polymeric compounds are produced as a result of oxidation (Varela, 1988; Lillard, 1983). As the food starts absorbing the frying fat, the food lipids and the colour pigments are solubilized and released into the frying fat; at the same time, moisture is also released from the frying food (Varela, 1988; Lillard, 1983). This moisture leads to lipolysis (Lillard, 1983). Lipolysis is described as hydrolysis of ester bonds in lipids which can occur by enzymatic action, or by exposure to heat and moisture, and thus resulting in the liberation of free fatty acids (Nawar, 1996).
Figure 3. Factors that may affect the frying process of food (constructed from Varela, 1988).
Lipolysis occurs during frying due to the presence of water in the food system when exposed to heat (Nawar, 1996). The quantity of free fatty acids during frying is associated with decreases in smoke point and surface tension of the oil; thus, the net result being that at the end of lipolysis, fatty acids that have been liberated are now more susceptible to oxidation (Nawar, 1996).
Although, many physical and chemical changes occur during frying, an additional concern of thermal oxidation of lipid in commercial deep-fat frying is the extent that the food product is also exposed to air and light (e.g. approximately 12 hours per day at 180 °C) (Gwo et al., 1985). Thermal oxidation is a major deterioration reaction occurring as a result of frying oils and leads to losses in oil quality (Alexander, 1978), as well as the reduced safety of fried foods (Pearson et al., 1983; Wu and Nawar, 1986; and Kitts, 1996). For example, excessive heating of oils and fats forms antinutritional compounds, such as enzyme inhibitors (Ruiz-Gutierrez and Murina, 1982) and accelerate loss of antioxidant vitamins such as vitamin E (Sheehy et al., 1994; Liu and Lee, 1998). These nutrient losses have been attributed to growth depression and adverse histologic changes of gastrointestinal tissues (Alexander, 1983; Clark and Serbia, 1991). It also has been reported that oxidized lipid will enhance peroxidation of membrane macromolecules (Hayam et al., 1997), genotoxicity (Kitts, 1996), and angiotoxicity (Addis, 1986; Nawar 1996).
1.9 Antioxidants

In order to reduce lipid oxidation, many synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary-butylhydroquinone (Figure 5) are added to a variety of foods (Chang et al., 1977). Antioxidants are described as substances that can delay onset, and slow-down the rate of oxidation of the autooxidizable materials in foods (Nawar, 1996). Antioxidants may act at different levels in the oxidative sequence by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals, binding to metal-ion catalysts, decomposing primary products to non-radical compounds, and chain-breaking to prevent continued hydrogen abstraction from substrates (Shahidi, 1996). Currently, lipid soluble monohydric or polyhydric phenols, with various ring substitutions are widely used in the food industry (e.g. BHA, BHT, and TBHQ) (Nawar, 1996). These phenolic antioxidants
have been shown to inhibit lipid oxidation by trapping peroxy radicals (Cuppert et. al, 1996). Moreover, phenolic structures can act as a free radical terminator which can interrupt or inhibit the formation of peroxyl radicals (Basaga et al., 1997). A study by Agustin and Berry (1983), showed that BHA was a more effective antioxidant than BHT in palm olein during static heating; however, both BHA and BHT were relatively ineffective at retarding the deterioration of the oil during intermittent frying of potato chips. Detail stability tests conducted by Hamama and Nawar (1991), using thin-layer chromatography and gas chromatography, showed that BHT had the highest stability followed by BHA and TBHQ. Primary antioxidants are often used in combination with other phenolic antioxidants, or with various metal sequestering agents for maximum efficiency (Nawar, 1996).

The use of antioxidants is well regulated in the food industry; the maximum amount of synthetic antioxidants added is at 0.02% (weight/weight) or 200 mg/kg of fat in food (Flick et al., 1992; Food Act and Regulations, 1996). With an increase in consumer's concern over the use of synthetic antioxidants in foods, natural antioxidant substances may be a viable alternative to replace the conventional synthetic stabilizers (Cuveiler et al., 1994). A study by Che Man and Tan (1999), showed that two plant-derived antioxidants, namely rosemary and sage, had a potential to replace the use of synthetic antioxidants in food processing. Berner and Jacobson (1973), Chang et al. (1977) and Nakatani (1989), reported in detail, that rosemary and sage provided antioxidant characteristics in food. Chang et al. (1977), found that rosemary extracts were effective antioxidants in steamed lard, chicken fat, sunflower oil or corn oil, where the
addition of 0.02 % rosemary extract into the fats and oil reduced peroxide values by 50 
%

1.10 Natural Antioxidants

The use of natural antioxidants as potential alternatives to synthetic antioxidants in formulated food products has become a popular area for research development designed for consumers that are concerned about the safety of chemical stabilizers widely used in food. From the safety point of view, herbs and spices are targets to search for natural antioxidants (Yalnishlieva et al., 2006). Some ground herbs and spices have been reported to have protective antioxidant activity; these include ground clove, ginger, oregano, sage, thyme, and rosemary (Shahidi et al., 1995; Wong and Kitts, 2006).

Pokorny (1991), described factors that should be considered for natural antioxidants having advantages over synthetic ones which include:

1. Are readily acceptable by consumer.
2. Are considered to be safe.
3. Legislation does not require safety test for natural antioxidants.
4. History of use: many natural antioxidants have been used in food, or have been mixed with food by people over hundreds of years.
5. May contribute to the nutraceutical value of edible oil functionality, not only as a stabilizer.
1.11 Rosemary

Currently, one of the most effective antioxidant spices widely used in food processing is rosemary (Yalnishlieva et al., 2006). The antioxidant activity of extracts derived from rosemary (*Rosmarinus officinalis L*) comes from the diphenolic diterpenes, carnosol and carnosic acid, which account for over 90% of the antioxidant activity of rosemary (Frankel et al., 1996; Aruoma et al., 1992). Phenolic structures have an affinity to interrupt, or inhibit peroxy racidals by acting as a free radical acceptor (Basaga et al., 1997). Moreover, Basaga et al. (1997), also found that rosemary extract had additional protective activities as an oxygen scavenger in a concentration dependent manner. Studies by Hadolin et al. (2004) and Arouma et al. (1992), reported that carnosic acid (Figure 6) was a major phenolic diterpene present in fresh rosemary and chiefly responsible for the antioxidant activity; however, carnosic acid is relatively unstable. Hadolin et al. (2004), further explained that carnosic acid could be converted to carnosol upon heating and carnosol could degrade further to produce other compounds, such as rosmanol and 7-oxyderivatives of rosmanol. Che Man et al. (2003), Frankel et al. (1996), and Chrysam (1994), working with a standardized oleoresin rosemary extract, reported many different phenolic components which were believed to act in synergy to provide antioxidant activity. Reports by Economou et al. (1991), Fukumoto and Mazza (2000), Kahkonen et al. (1999), and Zheng and Wang (2001), have demonstrated and described the success of using rosemary to prevent oxidation of fats, which further show the feasible use of rosemary in food products as natural antioxidant. Moreover, D’Evoli et al. (2006), described in detail the positive result of rosemary to counteract the oxidation of plant sterols in extra virgin olive oil during heating, thus preventing formation of
potentially harmful compounds to human health. Some researchers have discussed the suitability of rosemary as an antioxidant for deep-fat frying, especially in the presence of ascorbyl palmitate (Gordon and Kourimska, 1995). Frying experiments using potatoes in rapeseed oil by Reblova et al. (1999), showed that the rosemary extracts inhibited the formation of polar substances and polymers as well as the decomposition of polyunsaturated triacylglycerols. With these findings, the antioxidant properties of rosemary look promising to be a useful natural stabilizer in processed food products.

1.12 Sage

Along with rosemary, sage has also been found to be an effective antioxidant compared to other herbs and spices (Chipault et al., 1952; Chipault et al., 1955). Sage (Salvia officinalis) is a widely used and cultivated herbal plant in Turkey (Baskan et al., 2007) and elsewhere. The herb is used for its medicinal properties and also for culinary processes, as well as for food flavouring and seasoning (Baskan et al., 2007; Yanishlieva et al. 2006). Sage is also known for its suitability to treat sore throats and is a popular beverage in Turkish coffee houses (Baskan et al., 2007). Sage contains a high amount of di-triterpenoids, phenolic acids, and flavonoids (Lu and Foo, 2002; Ulubelen and Topçu, 1998). The antioxidative activity of sage is correlated with the presence of carnosic acid and rosmarinic acid, which is similar to rosemary, since both plants belong to the Labiatae family (Figure 6) (Cuvelier et al., 1996; Deans and Simpson, 2000). Baskan et al. (2007), reported that genetic factors and environmental conditions play an important role in the level of carnosic acid and rosmarinic acid in the dry sage leaves.
Figure 6. Structure of antioxidant compounds from rosemary (Cuppert et al., 1996)
1.13 Methods Used to Evaluate Fatty Acid Composition and Measure the Stability of Oils during Frying.

Lipid analysis is ideally performed using gas chromatography (Pike, 1998) to measure individual, specific fatty acid composition, distribution and position of fatty acids in lipid. Studies concerned with researching factors influencing fat stability and oxidation, detection of adulterants, as well as the level and effectiveness of antioxidants have employed this methodology (Pomeranz and Meloan, 1994). Gas chromatography, following methyl ester derivatization is widely used to measure the fatty acid composition of food products (Ackman, 1992). Moreover, gas chromatography is now the most commonly employed technique for separation of methyl esters for quantitative analytical purposes (Gunstone, 1996). Ackman (1972), described that the main reason for using short chain fatty acid esters with gas chromatography analysis was to prevent tailing or ghosting peaks, since short chain fatty acids are associated with the vapor phase and adsorbed to almost any convenient surface. Moreover, Pike (1998), described that sample preparation, which include saponification and esterification of fatty acids to produce fatty acid methyl esters was usually performed to increase volatility prior to gas chromatography analysis.

Measurements of heat abused oil are mainly based on the formation of volatile and non-volatile components formed during frying. Most of the volatile decomposition products are distilled and are removed during frying. The non-volatile decomposition products can remain in the frying fat and are absorbed by the fried food (Stevenson et al., 1984). These non-volatile decomposition products can lead to further degradation of both
the oils as well as other heat labile nutrients (e.g. proteins, vitamins, etc) (Stevenson et al., 1984).

Many different methods have been reported for use in measuring the stability of oil during frying. These include peroxide value, p-anisidine value, iodine value, viscosity, free fatty acid content, colour, and alkaline contaminant materials (Pike, 1998; Jaswir et al., 2000; and Nawar, 1996).

The peroxide value measures the amount of peroxides, or hydroperoxides that are formed during oxidation of fats and oil (Berger, 1984). This measures the miliequivalents of peroxides per kilogram of fat that is obtained through a titrimetric determination of the amount of peroxide or hydroperoxide groups. Thus, peroxide value is a widely known measurement of initial products (primary products) of lipid oxidation (Pike, 1998).

The p-anisidine value is also widely used as a quality indicator measuring oxidative deterioration of oils and fats, with amount of aldehydes, secondary products of lipid oxidation, (mainly 2-alkenals and 2,4-dienals) being measured (IUPAC, 1987; Pike, 1998). The principle of the p-anisidine measurement, is that aldehydes will react with p-anisidine reagent to form a chromogen that can be detected spectrophotometrically (Pike, 1998). Combining the peroxide value measure with the p-anisidine value can be used to assess total oxidation; often referred to as “totox”. This value reflects the total lipid oxidation of the sample using the following equation: totox = p-anisidine value + (2 x peroxide value) (Pike, 1998).

Iodine value, or iodine number, can also be used as a measure for total number of unsaturated double bonds present in oil (Pike, 1998). It is defined as the grams of iodine
absorbed per 100 g sample (Pike, 1998). Generally, the higher the iodine value, the greater the degree of lipid unsaturation.

Viscosity parameters can be used as an indirect measure of lipid oxidation (AOCS, 1983). Viscosity can be described as the measurement of internal friction between molecules in a fluid. Thus, viscosity of oils decreases slightly with an increase in the degree of unsaturation. Lawson (1995), reported that the viscosity of highly polymerized oils is much greater than that of a fresh oil; thus, viscosity was used to determine the condition of fats following deep frying. A viscometer is normally used to determine the viscosity of fats and oils (AOCS, 1983).

The presence of free fatty acid in oils, or fats, reflects the concentration of an acid which is non-esterified due partly from hydrolysis and partly from further oxidation of secondary oxidation products, which are formed during frying (Kun, 1990). Free fatty acid content can be defined as the percentage by weight of a specified fatty acid; which, may be derived from hydrolysis of triacylglycerols (Pike, 1998). In detail, Pike (1998) described that free fatty acid and acid value can be converted from one to the other using a conversion factor. Acid value is described as the mg of KOH to neutralize the free acids present in 1 g of fat or oil (Pike, 1998).

Changes in colour formation also occur in frying of palm oil (Lawson, 1995); thus colour of the oils can be used as an indicator of oil quality. The Lovibond method and the spectrophotometric method are widely used in measuring the colour of fats and oils (Pike, 1998). The Lovibond method uses red and yellow colour standards, where, the colour of oil in a standard sized tube is visually compared with the standard (Pike, 1998). In the spectrophotometric method, samples are heated to 25-35 °C and placed in a cuvette for
absorbance reading at 460, 550, 620, and 670 nm wavelengths (Pike, 1998). Thus, the photometric color index is calculated using this equation:

Photometric color index = 1.29(A460) +69.7(A550) +41.2(A620) -56.4(A670)
(Pike, 1998).

Gill (1998) reported that alkaline contaminant materials (ACM) were potent surfactant-like materials that could lower interfacial tension of frying oil; thus leading to the retention of the oil in the food product. ACM, such as soaps and other highly surfactant-like materials are formed during frying with polar components (Blumenthal and Stockler, 1986). ACM surfactants that are formed naturally in oil during oil degradation reactions, through the interaction between fatty acids with metal ions, can be used to estimate thermal abuse of cooking oil. Gill (1998) and Jaswis et al. (2000), reported that ACM can be determined spectrophotometrically.
Chapter 2. Quantitative Measurement of Fatty Acid Content in Native and Blended Oils

2.1 Introduction

In general, fat and oil processing involves various chemical procedures which include settling and degumming, neutralization, bleaching, and deodorization (MPOPC, 1996). The ideal fat or oil should have excellent oxidative stability, contain enough solid fat (e.g. for use as margarines or shortenings), be low in saturated fatty acids, and high in polyunsaturated fatty acids (List, 2004). In reality, it is difficult to obtain such an oil or fat that can achieve all of these requirements. Therefore, additional processes such as hydrogenation, interesterification, and blending are often performed to gain desirable characteristics of fats and oils (Rossell, 1985). Hydrogenation of fats involves the addition of hydrogen to double bonds in the fatty acid chain which converts liquid oils to semi-solid or plastic fats, and also leads to improvement of the oxidative stability of the edible oil (Nawar, 1996). Hydrogenation is achieved through the reaction of hydrogen gas in the presence of a metal catalyst such as nickel (Ackman, 1998). Although hydrogenation improves the stability of the edible oil, one negative effect correlated with hydrogenation is the production of trans fats (List, 2004), which has been associated with risks of heart disease in humans (Ascherio, 2002; Hu et al., 2001).

Another way of producing customized fats is by using an interesterification method. Interesterification can be described as the hydrolysis of the ester bond between the fatty acid and glycerol, followed by the subsequent reformation of the ester bond
among the mixed free fatty acids and glycerol (Gunstone, 1996; Nawar, 1996; and Tarrago-Trani et al., 2006). One way of performing interesterification is through chemical interesterification, which is a random process; however, the resulting interesterified triglyceride can be controlled by the relative amounts of each type of fat (Tarrago-Trani et al., 2006). Enzymatic interesterification involves the use of microbial lipases (e.g. lipase Sn 1,3 which attack at position 1 and 3 of the triacylglycerol), which selectively interact with specific triacylglyceride ester bonds (Tarrago-Trani et al., 2006). When a specific triacylglyceride is desired, enzymatic interesterification is usually preferred (Kellens, 2000). Although interesterification seems to be desirable in edible oils and fats processing, one negative effect is the high cost associated with this procedure.

With the negative effects associated with hydrogenation and costs associated with interesterification, oil blending can be considered another alternative to gain desirable characteristics of certain fats and oils. Blending of oil has been reported successful in various edible oils sources, such as groundnut oil, soybean oil, palm kernel oil, coconut oil, and karanja oil (Semwal, 2001; De et al., 1999; and Rossell, 1985)

In this experiment, two omega-3 fatty acids sources (e.g. flaxseed oil and menhaden fish oil) were blended into palm oil, a common vegetable oil used in Asia and Africa, to derive two specific omega-3 enriched palm oil blends that contained different omega-3 fatty acids and which could be used as common cooking oils. The purpose of this experiment was to utilize a quantitative approach for establishing the 1:4 omega-3:omega-6 ratio that would enable reproducible formulations.
2.2 Materials and Methods

2.2.1 Materials

Refined, bleached, and deodorized palm oil was donated by P.T. Intiboga Sejahtera (Jakarta, Indonesia). Flaxseed oil was provided by Polar Foods, Inc. (Fischer Branch, MB). Commercially stabilized menhaden fish oil was kindly provided by Omega Pure (Houston, TX). Oleoresin Rosemary (Herbalox Brand, Type O) was purchased from The Ingredient Company (Brampton, ON). All reagents were analytical grade. Butylated hydroxyanisole (BHA) was purchased from Fischer Scientific (Toronto, ON). Heptadecanoic acid methyl ester (C17:0) and sodium thiosulphate were obtained from Sigma Aldrich (St. Louis, MO). A Supelco 37 Component FAME mix of C4-C24 (Bellefonte, USA) was used as a fatty acid standard.

Upon receiving palm oil, flaxseed oil, and fish oil sources, all oils were stored at subzero temperature of -35 °C. All oil samples were covered with aluminum foil and stored in boxes to minimized light exposure (from freezer bulb) during frozen storage.

2.2.2 Experimental Design

Standard fatty acid calibration curves were constructed to quantify the linoleic acid, linolenic acid, EPA, and DHA content of oil samples for blending calculations. Palm oil was blended with two omega-3 fatty acids sources, namely flaxseed oil and fish oil, as well as two antioxidant treatments (e.g. 0.02% rosemary and 0.02 % BHA). Omega-3 sources were blended with palm oil, omega-6 fatty acids source, based on weight calculations derived from preliminary GC data on all experimental oils. GC analysis of the new oil blends was performed to ensure that the ratio of omega-3:omega-6 ratio was within a 1:4 ratio.
All data are presented as mean values ± standard deviation. Linear regression analysis (Microsoft Excel, Microsoft Corporation) was performed for calibration curves. One way ANOVA (Microsoft Excel, Microsoft Corporation) and least significant difference test at \( \alpha = 0.05 \) were used to analyze the data obtained from lipid oxidation analysis.

2.2.3 Fatty Acid Analysis

The fatty acid compositions of different starter edible oil materials were determined using gas chromatography as reported by Yuan and Kitts (2002). Fatty acid methyl esters in each sample were measured using a Shimadzu Model GC-17 A flame-ionization gas chromatograph (Mandel Scientific Co. Ltd. Guelph, Canada), containing a fused-silica capillary column (J&W Scientific DB-23) of 30 meter length, 0.25 mm internal diameter, and 0.25 um film thickness. The initial column temperature was set at 120 °C with an increase temperature applied at a rate of 3 °C/min until 220 °C was reached. The injector and detector had a working temperature of 250 °C. A fatty acid standard obtained from Supelco (Supelco 37 Component FAME mix C4-C24) was used to identify different fatty acids in each sample. Quantitation of fatty acids was made using an internal standard (heptadecanoic acid methyl ester, C17:0) in comparison with standard curves derived for each of the major fatty acids under investigation.

Samples were prepared by adding the internal standard (0.2575 g of heptadecanoic acid methyl ester in 250 ml of hexane) to the oil sample (0.1 g). Then, 5 ml of 0.5 N potassium hydroxide/ methanol was added to dissolve lipids and the test tubes were left overnight at room temperature. The next day, 2.5 ml (one full pasteur pipette) of petroleum ether was added to each sample and a phase separation was
accomplished in 20 minutes. After phase separation, the upper layer (contains ether and nonsaponifiable matter) was discarded. The next step was adding 2 or 3 boiling chips and 5 ml of Boron Trifluoride. Samples were heated in a beaker containing 100 ml boiling water. After 15 to 20 minutes, cold water was poured into the beaker to terminate the reaction. Samples were allowed to cool to room temperature for 60 minutes. Deionized water (2 drops) and 5 ml of hexane was added into each tube, FAMEs were dissolved in the top hexane layer and the hexane layer was transferred to a tube containing a drying agent (Na₂SO₄: NaHCO₃, 4:1) before being dispensed into a GC vial for analysis.

2.2.4 Fatty Acid Calibration Curves

Gas chromatography was performed to derive fatty acid area measurement (linoleic acids, α-linolenic acids, EPA, and DHA) using an internal standard (heptadecanoic acid). Calibration curves were obtained by comparing the area ratio of FAMEs (linoleic acid, α-linolenic acid, EPA, and DHA) with the internal standard on X-axis and weight ratio of FAMEs (linoleic acid, α-linolenic acid, EPA, and DHA) with the internal standard on the Y-axis. A linear regression was used to derive the equation for mass to mass ratio and area to area ratio for the internal standard relative to the specific fatty acid.

2.2.5 Blending Procedure

A quantitative measure of the amount of omega-3 fatty acids derived from flaxseed oil or fish oil (menhaden oil), respectively, when added to palm oil was determined by absolute calculation based on quantitative measurement of fatty acid composition of palm oil, flaxseed oil, and fish oil. Upon blending, a palm oil sample was
removed from frozen storage and placed at room temperature overnight. Flaxseed, fish oil, and rosemary were added to the palm oil sample, and mixed together in Rubbermaid containers using a regular spoon stirrer for 5 minutes. Blending was performed in a cold room set at 4 °C to minimize the heat friction from the stirrer.

2.2.6 Peroxide Value

The AOAC official method (AOAC 965.33, 2003), with minor modification, was used to determine sample oil peroxide values. Samples weighing 5.00 ± 0.05 g were transferred to a 250 ml Erlenmeyer flask, to which 30 ml of an acetic acid-chloroform mixture was added. Saturated potassium iodide (1ml) was added to the flask and the sample was mixed by swirling the flask for 1 minute. Samples were stored in the dark for 5 minutes, after which, 30 ml of deionized water was added and followed by the addition of 1% starch solution (0.5 ml). The mixture was then titrated with 0.01 N sodium thiosulphate until the blue colour disappeared. The peroxide value (P.V.) was calculated using the following equation:

\[
P.V. \text{ (meq O}_2/\text{kg fat)} = [(\text{Volume (ml) of sodium thiosulphate}) \times (\text{N of sodium thiosulphate used})] \times 1000
\]

2.2.7 Determination of p-anisidine Value

The IUPAC (1987) method was used to quantify aldehydes (mainly 2-alkenals) present in oil using the p-anisidine values. Oil samples were weighed to approximately 2 g (recorded to 3 decimal places) and placed in a 25 ml volumetric flask, then filled to volume with iso-octane. Absorbance readings at 350 nm were taken in a 1.0 cm cell using a Shimadzu UV-160 spectrophotometer (Canada), employing iso-octane as the blank. The next step was to measure 5 ml of oil solution into a glass test tube and add 1.0 ml of
a 0.25% p-anisidine solution (p-anisidine in acetic acid). Samples were shaken to mix and then stored in darkness for 10 minutes. Absorbance was taken at 350 nm against a sample blank (5 ml of iso-octane plus 1 ml p-anisidine reagent). The p-anisidine value (p-A.V) was calculated according to the following equation:

\[ p-A.V. = \frac{15(1.2A_s-A_b)}{m} \]

where, \( A_s = \) the absorbance of the fat solution with the addition of p-anisidine reagent; \( A_b = \) the absorbance of the fat solution; and \( m = \) mass (g) of oil used.

### 2.2.8 Total Oxidation Number (Totox)

Totox is a measure of the total oxidation (Che Man and Hussin, 1998; Rossell, 1994), and is calculated according to the following equation:

\[ \text{totox} = (2\times P.V.) + p-A.V. \]

### 2.3 Results

#### 2.3.1 Calibration Curves and Blending Experiment

The primary fatty acids present in palm oil, flaxseed oil, and fish oil (menhaden oil) are presented in Table 1. Palm oil contained high proportions of palmitic acid, stearic acid, oleic acid, and linoleic acid. The primary fatty acids present in flaxseed oil were identified to be \( \alpha \)-linolenic acid, linoleic acid, and palmitic acid. Eicosapentaenoic acid, docosahexaenoic acid, and palmitic acid are the principle fatty acids present in the fish oil source. Table 2 presents the calibration curves for linoleic acid, \( \alpha \)-linolenic acid, EPA, and DHA, that were constructed from fatty acids mass and area ratios analysis (Appendix III.). Using calibration curves described in Table 2, weight of omega-6 (e.g. linoleic acid) and omega-3 (\( \alpha \)-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) fatty
acids in all three oil sources were calculated and are presented in Table 3. Table 4 presents the different fatty acids in flaxseed-palm oil and fish-palm oil blends that are expressed as relative percent and absolute mass ratio. Since the objective of this study was to add omega-3 fatty acids to palm oil, it is important that mass ratio analysis be used since it is a better approach to report the fatty acid composition of the newly developed oil blends. Based on the fatty acids concentration data, an accurate determination of 1:4 (omega-3: omega-6) ratio for the flax-palm oil blend was obtained, which involved adding 6.10 grams of flaxseed oil per 100 grams of palm oil. In the case of the fish-palm oil blend, 4.46 grams of fish oil (menhaden oil) per 100 grams of palm oil was required to achieve a 1:4 (omega-3: omega-6) ratio. From data given in Tables 4, 5, and 6, results of blending flaxseed oil and fish oil, respectively to palm oil, produced final ratios of 1:3.7 and 1:3.8 (omega-3: omega-6) respectively (Appendix I and II).

2.3.2 Peroxide Value, p-anisidine Measurement, and Total Oxidation Number

Lipid oxidation products in starting experimental oils (Figure 7) showed that fish oil (menhaden oil) had the highest (P<0.05) peroxide value and p-anisidine measurement followed, by flaxseed oil and palm oil, respectively. The highest peroxide value and p-anisidine measurement of all samples was found with the fish oil (menhaden oil) which ranged between 3.91-4.37 and 2.03-2.95, respectively. Total oxidation measurement (totox), a combination of peroxide value and p-anisidine measurement, is presented in Table 7 and shows that the fish oil sample had the highest degree of lipid oxidation followed by flaxseed oil and palm oil, respectively. ANOVA analysis of data presented revealed that there was a significant difference (P<0.05) in totox between palm oil, flaxseed oil, and fish oil.
Table 1. Fatty acid profile of percent major fatty acid present in palm oil, flaxseed oil, and fish oil.¹

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Palm Oil (%/100 g)</th>
<th>Flaxseed Oil (%/100 g)</th>
<th>Fish Oil (%/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric Acid</td>
<td>0.40 ± 0.07</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>1.32 ± 0.24</td>
<td>N/D</td>
<td>5.48 ± 0.98</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>36.15 ± 1.45</td>
<td>9.00 ± 2.28</td>
<td>20.45 ± 0.64</td>
</tr>
<tr>
<td>Palmitoleic Acid</td>
<td>N/D</td>
<td>N/D</td>
<td>7.0 ± 0.59</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>5.36 ± 0.46</td>
<td>4.77 ± 0.82</td>
<td>4.32 ± 0.12</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>47.21 ± 2.38</td>
<td>19.42 ± 1.61</td>
<td>14.92 ± 0.87</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>9.80 ± 1.34</td>
<td>16.36 ± 1.66</td>
<td>N/D</td>
</tr>
<tr>
<td>α-Linolenic Acid</td>
<td>N/D</td>
<td>51.52 ± 2.55</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td>EPA</td>
<td>N/D</td>
<td>N/D</td>
<td>22.23 ± 0.62</td>
</tr>
<tr>
<td>DHA</td>
<td>N/D</td>
<td>N/D</td>
<td>24.60 ± 0.80</td>
</tr>
</tbody>
</table>

¹ Results shown as mean ± standard deviation of three replicates measured twice in a month, and values in parenthesis represent CV = coefficient of variation. N/D = under 0.01 %.

Table 2. Calibration curves for linoleic acid, α-linolenic acid, EPA, and DHA.¹

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Calibration Curves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic Acid</td>
<td>Y = 0.9492 X - 0.0403, R² = 0.996</td>
</tr>
<tr>
<td>α-Linolenic Acid</td>
<td>Y = 1.1486 X + 0.0198, R² = 0.999</td>
</tr>
<tr>
<td>EPA</td>
<td>Y = 0.8386 X - 0.016, R² = 0.995</td>
</tr>
<tr>
<td>DHA</td>
<td>Y = 1.0571 X - 0.0158, R² = 0.999</td>
</tr>
</tbody>
</table>

¹ Y = fatty acid weight ratio and X = chromatogram area ratio.
Table 3. Fatty acid composition of linoleic acid, α-linolenic acid, EPA, and DHA content of palm oil, flaxseed oil, and fish oil.  

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Oil Source</th>
<th>Palm Oil (g/100 g)</th>
<th>Flaxseed Oil (g/100 g)</th>
<th>Fish Oil (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic Acid</td>
<td>9.03 ± 0.29</td>
<td>10.7 ± 0.93</td>
<td>N/D</td>
<td></td>
</tr>
<tr>
<td>α-Linolenic Acid</td>
<td>(0.03)</td>
<td>(0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>N/D</td>
<td>N/D</td>
<td>20.44 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>N/D</td>
<td>N/D</td>
<td>29.1 ± 0.27</td>
<td></td>
</tr>
</tbody>
</table>

Results shown as mean ± standard deviation of three replicates measured twice in a month, and values in parenthesis represent CV = coefficient of variation. N/D = under 0.01 g/100g

Table 4. Results of blending flaxseed oil and fish oil with palm oil to achieve a 1:4 (omega-3:omega-6) ratio.

<table>
<thead>
<tr>
<th>Oil Blend</th>
<th>Method for Determining Blending Ratio</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Percent Fatty Acid (g)</td>
<td>Mass Ratio (g)</td>
</tr>
<tr>
<td>Flaxseed Blended Palm Oil</td>
<td>5.12</td>
<td>6.10</td>
</tr>
<tr>
<td>Fish Oil Blended Palm Oil</td>
<td>5.14</td>
<td>4.46</td>
</tr>
</tbody>
</table>

Values represent g of respective oils added to 100 g of palm oil to achieve a 1:4 (omega-3:omega-6) ratio. Denotes procedures for qualitative (e.g. relative percent fatty acid, %/100g oil) versus quantitative (e.g. mass ratio, g/100g oil) to obtain a 1:4 omega-3:omega-6 ratio. Denotes the difference between qualitative and quantitative procedures to calculate oil blends with a 1:4 omega-3:omega-6 ratio.
Table 5. Fatty acid profile of major fatty acids present in the flaxseed-palm oil blend.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Flaxseed Oil Blended Palm Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Percent Fatty Acid (%/100 g)</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>0.21 ± 0.20 (0.95)</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>1.0 ± 0.02 (0.02)</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>40.95 ± 0.59 (0.014)</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>4.70 ± 0.12 (0.03)</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>40.05 ± 0.08 (0.002)</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>10.78 ± 0.16 (0.015)</td>
</tr>
<tr>
<td>α-Linolenic Acid</td>
<td>2.32 ± 0.41 (0.18)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Results shown as mean ± standard deviation, and values in parenthesis represent CV = coefficient of variation.
Table 6. Fatty acid profile of major fatty acids present in the fish-palm oil blend.¹

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Fish Oil Blended Palm Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Percent Fatty Acid (%/100 g)</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>0.18 ± 0.18 (1)</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>0.88 ± 0.50 (0.57)</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>44.72 ± 6.26 (0.14)</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>5.09 ± 0.92 (0.18)</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>34.90 ± 7.81 (0.22)</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>11.38 ± 1.20 (0.11)</td>
</tr>
<tr>
<td>EPA</td>
<td>0.72 ± 0.02 (0.03)</td>
</tr>
<tr>
<td>DHA</td>
<td>2.14 ± 0.27 (0.13)</td>
</tr>
</tbody>
</table>

¹Results shown as mean ± standard deviation, and values in parenthesis represent CV = coefficient of variation.
a. Peroxide value.

![Graph showing peroxide values for Palm, Flax, and Fish oils.]

b. P-anisidine measurement.

![Graph showing P-anisidine values for Palm, Flax, and Fish oils.]

Figure 7. Lipid oxidation products in experimental oils. Results shown are the average of three sample measurements with standard deviation. a. = Peroxide value and b = P-anisidine measurement.

*Refers to significant difference (P<0.05) from palm oil.

**Refers to significant difference (P<0.05) from palm oil and flaxseed oil.
Table 7. Total oxidation (totox) of experimental oils.\(^1\)

<table>
<thead>
<tr>
<th>Oils</th>
<th>P.V. (meq O(_2/\text{kg fat}))</th>
<th>p-A.V.</th>
<th>Average Totox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil</td>
<td>0.08 ± 0(^a)</td>
<td>1.44 ± 0.46(^a)</td>
<td>1.60 ± 0.4(^a)</td>
</tr>
<tr>
<td>Flaxseed Oil</td>
<td>0.47(^b) ± 0.12(^b)</td>
<td>2.04 ± 0.05(^b)</td>
<td>2.97 ± 0.2(^b)</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>4.14 ± 0.23(^c)</td>
<td>2.49 ± 0.46(^b)</td>
<td>10.77 ± 0.48(^c)</td>
</tr>
</tbody>
</table>

\(^1\)Values represent mean ± standard deviation of three measurements.
\(^a, b, c\) Represent statistical difference at P<0.05 within a column.
P.V. = peroxide value. p-A.V. = p-anisidine measurement. Totox = total oxidation measurement.

2.4 Discussion

With respective calibration curves for linoleic, α-linolenic, EPA, and DHA, fatty acid data obtained from the GC chromatograms could be reported in terms of mass concentration (e.g. g PUFA/100 g blended oil). Unlike using the percent fatty acids based on peak area, which can be affected by the presence of other fatty acids in the samples, as well as possible less than 100% oil derivatization efficiency, expression of fatty acids concentrations is more accurate since the former method includes the correction of the internal standard (heptadecanoic acid). The use of internal standards in fatty acid composition has been emphasized by Varela (1988), who explained that following the use of percentage in fatty acid composition was valid only if the other fatty acids remained unchanged. In the case of frying oils, it is likely that polyunsaturated fatty acid content in particular will change and derivatization efficiency may vary with boiling point. Varela (1988), further discussed that in some experiments, the proportion of saturates could also be misleading, such as to overestimated increases when there in fact was a small or no change at all in the saturated and monosaturated fatty acid content when results were expressed as a percentage. Varela (1988), concluded that using the
percentage of three main fatty acids as the main analysis of the data, leads to inaccuracies that comprise a false increase in saturated or monosaturated fatty acids during frying.

Rounds and Nielsen (1998), also emphasized that the use of internal standard in fatty acid chromatography measurements to be very important, since this method could minimize errors that originate from sample preparation, the analytical apparatus, as well as the derivatization and extraction methods proficiency of the operator. Moreover, with the use of internal standard, injection volumes and detector response are not required to be accurately measured and to be constant since changes that do occur will not affect the internal standard and analyte ratios (Rounds and Nielsen, 1998). One main disadvantages of this method is finding a suitable standard that does not interfere with the analyte measurement.

Numerous publications have reported variable results in fatty acids analysis which occur as a result of different extraction and derivatization methods. For example, Bondioli and Della (2002), described that their specific method, tested on refined sunflower, olive, soybean, and degummed sunflower oils, did not permit satisfactory conversion of free fatty acids to methyl ester due to the present of hydroperoxides, phospholipids, and various impurities. Carvalho and Malata (2005), discussed that current analytical methods published mainly focus on the amount of fatty acids extracted; however, the limitations of procedural methods that involve derivatization steps likely do not produce the same results if there are different sample conditions. Thus, Carvalho and Malata (2005), placed further emphasis on ranking the relative importance of specific analysis steps, such as the derivatization process, to improve the measure of fatty acids in biological materials with high accuracy, high efficiency, and minimum costs. Jochm et al.
(2001), also described that different supplemented food samples required different methods of preparing samples for semi-quantitative omega-3 analysis, due to the unique properties of omega-3 fatty acids.

Looking at the quality of the starting oil samples in this study, our highest peroxide value ranged between 3.91-4.37 (Figure 7), which is considered acceptable and compares to the upper limit that is set by the International Olive Oil Council (1995) at P.V. = 20 meq/kg. From these quality control data, it can be concluded that our initial starting oil materials used to create the different oil blends, were of acceptable quality. Notwithstanding this, however, absolute quantitation of fatty acid content in the oils used for blending was performed.

2.5 Conclusion

Based on the mass ratio weight data for individual fatty acids, a final ratio of 1:4 (omega-3: omega-6), was achieved by adding 6.10 grams of flaxseed oil per 100 grams of palm oil for α-linolenic acid/linoleic acid. For the fish oil-palm oil blend, 4.46 grams of fish oil was needed to be added to 100 grams of palm oil to achieve a 1:4 ratio for EPA-DHA:linoleic acid. Calculations of fatty acid content, based on percent weight and absolute weight for the amount of flaxseed oil and fish oil added to palm oil; respectively, gave different results. The data herein agrees with previous studies by Varela (1988), who described that the use of an internal standard to calculate mass content of fatty acids was more accurate then using a simpler relatively percent of fatty acid peak area calculation. Considering the variances in fatty acid content that might be caused from the three major fatty acids in all samples, determining mass weight was proven to be an
important first step in generating a reproducible omega-3:omega-6 ratio. The experimental results show that the ratio of omega-3: omega-6 for the flaxseed-palm oil blend (1:3.7) and fish-palm oil blend (1:3.8), respectively were very close to the aimed ratio of 1:4. Finally, the quality of study materials used in these blends, based on presence of lipid oxidation products was found to be acceptable to make the different oil blends for future experiments.
Chapter 3. Quality Changes of Heated Flaxseed Oil-Palm Oil and Fish Oil-Palm Oil Blends Containing Natural Antioxidant Stabilizers

3.1 Introduction

Flaxseed contains a high concentration of α-linolenic acid (C18:3), which has been associated with many health benefits such as the prevention of blood platelet aggregation (Holub, 1990,) and also anti-hypercholesterolemic effects (Carter, 1993). On the other hand, fish oil is widely known as a good source of highly unsaturated omega-3 fatty acid (HUFA), which include eicosapentaenoic acid and docosahexaenoic acid (Park et. al, 2004). Similar to flaxseed oil, many researchers have suggested that fish oils offer many important health benefits. Health benefits associated with highly unsaturated omega-3 fatty acids include, prevention of cardiovascular disease, anti-inflammatory effects, antiarrhythmic effects, and anti-hypertensive effects, prevention of immune disorder, as well as reduced diabetes symptoms (Carrero et. al, 2004; Simopoulos, 1990; and Kinsella, 1986).

Unfortunately, the omega-3 fatty acids intake in the Western style diet is currently below the recommended minimum of two servings of fish a week, a major reason for this being the low consumer acceptance for oily fish (Kolanowski and Laufenberg, 2006). Omega-3 fatty acid intake can be increased by consumption of dietary health supplements, which include omega-3 fatty acid mixtures. Another effective approach towards achieving increased intake of omega-3 fatty acids is the enrichment of frequently
consumed foods that are enriched with omega-3 fatty acids sources (Kolanowski and Laufenberg, 2006).

The potential health benefits derived from the consumption of flaxseed and flaxseed oil, are an incentive to find ways to incorporate flaxseed, or its components into regular food products. In the baked goods industry, flaxseed enrichment has been widely incorporated and used. Carter (1993), reported that ground or whole flaxseed can be added to almost any baked product and will contribute to a nutty flavor to bread, waffles, pancakes, and breakfast cereals. Flaxseed flour is currently being used by bakeries in the United States (Burckhardt, 1989). Cold-pressed flaxseed oils have also been used to make salad dressings (Carter, 1993). A study by Bernal-Gomez and Mancini-Filho (2003), reported feeding flaxseed oil to hens, for the purpose of producing novel omega-3 enriched eggs, rich in alpha-linolenic and docosahexaenoic acids. Manthey et al. (2002), also reported the success of fortifying spaghetti using ground flaxseed, where the presence of alpha-linolenic acid was stable during processing and cooking.

One main limitation of fish oil enrichment in formulated food products is the associated off-flavours due to fishy flavour (Kolanowski et al., 1999), which can be solved with the use of microencapsulation and prevented in part by the addition of antioxidants (Bimbo, 1998; Neil and Younger, 1998). Microencapsulation is described as the transformation of liquid oil into a stable powder where the wall materials of the microcapsule protect the core substance against influences of environment origin (Kolanowski et al., 2004). Materials such as combinations of gelatin, maltodextrine or other sugars and starch, plant gums, milk powder, and milk or whey protein have been reported to be successful in the microencapsulation process (Neil and Younger, 1998;
Heinzelmann et al., 2000; Keogh et al., 2001; and Lamprecht et al., 2001). One relevant example, is the Roche Vitamins’ (Parsippany, N.J.) omega-3 powder (encapsulated fish oil), which is used to fortify dry beverage mixes, baked goods, breakfast foods, and hot cereals. The use of microencapsulation also plays an important role in protecting the oil from oxidation (Labell, 2000). Another example is the success of using microencapsulated tuna oil in a bread product (Yep et al., 2002), as well as the success of Ocean Nutrition (Dartmouth, NS), which markets an omega-3 enriched bread using omega-3 powder. Although there are many studies which show the potential use of microencapsulation in food products, the cost of this technology to the food processor is a main hurdle to overcome.

Park et al. (2004), reported another way of enriching food products with omega-3 fatty acids, described as an oil-in-water emulsion, as well as the bulk oil of algal oil, incorporated into surimi. Unlike the addition of bulk oil, which decreases the gel strength of surimi, oil-in-water emulsions showed a greater potential for use in enriching surimi with omega-3 fatty acids from algal oil, since the emulsion had no effect on the gel strength (Park et al., 2004).

These specific examples indicate a potential mechanism to raise the omega-3 intake of the consumer without making major changes in dietary habits (Kolanowski et al., 1999).

In this chapter, frying experiments using the omega-3 enriched flaxseed oil-palm oil and fish oil-palm oil blends created in Chapter 2 were conducted to investigate if these blended oils would be used for deep-fat frying. Moreover, the feasibility of using
rosemary as a natural antioxidant was also accessed in the newly developed oil blends during frying experiment.

3.2 Materials and Methods

3.2.1 Materials

Refined, bleached, and deodorized palm oil was donated by P.T. Intiboga Sejahtera (Jakarta, Indonesia). Flaxseed oil was provided by Polar Foods, Inc. (Fischer Branch, MB). Commercially stabilized menhaden fish oil was kindly provided by Omega Pure (Houston, TX). Oleoresin Rosemary (Herbalox Brand, Type O) was purchased from The Ingredient Company (Brampton, ON). All reagents used were analytical grade. Butylated hydroxyanisole (BHA) was purchased from Fischer Scientific (Toronto, ON). Heptadecanoic acid methyl ester (C17:0), bromophenol blue, sodium thiosulphate, and α-tocopherol standard were obtained from Sigma Aldrich (St. Louis, MO). A Supelco 37 component FAME mix of C4-C24 standards, β-tocopherol, γ-tocopherol, and δ-tocopherol were obtained from Supelco (Bellefonte, USA).

All palm oil, flaxseed oil, and fish oil samples were stored at subzero temperature of -35 °C and covered with aluminum foil before held in sealed boxes to minimized light exposure during storage.

3.2.2 Experimental Design

Palm oil was blended with the flaxseed oil and menhaden fish oil as omega-3 sources as described in Chapter 2. Omega-3 sources were blended to palm oil (omega-6 source) based on an absolute weight calculation derived from palm oil, flaxseed oil, and fish oil fatty acid profile data. GC analysis of the new oil blends were performed to
confirm that the ratio of omega-3:omega-6 ratio was within a 1:4 range. Three different antioxidant treatments (e.g. 0.02% rosemary, 0.02 % BHA, and a control (without antioxidant)) were also added to the omega-3 enriched palm oil for the frying experiment. The frying experiment was performed at 180 °C for 8 hours to evaluate the usefulness of these newly blended oils as cooking oils.

Two way ANOVA (Microsoft Excel, Microsoft Corporation) and a Least Significant Difference (LSD) test, set at $\alpha = 0.05$, were used to analyze data obtained from the frying experiment. Linear regression analysis was performed to obtain analyte standard curves. All fatty acid data and total oxidation measurements are presented as mean ± standard deviation. Thermal degradation data and tocopherol concentration in oils are expressed as an average of duplicate measurements.

3.2.3 Fatty Acid Calibration Curves

Please see Chapter 2 for details.

3.2.4 Blending Procedure

The weight of omega-3 sources, flaxseed oil and fish oil added to palm oil was determined by a calculation that was based on absolute weights of omega-6 and omega-3 contents of flaxseed oil, fish oil, and palm oil, as detailed in Chapter 2.

3.2.5 Frying Experiment

Two deep-fat fryers in this experiment (Beatty DF 142F and Garland E22-28FT, Canada) with similar capacity were used. Blended oil (3 kg) was used for each frying experiment. Oil was heated in the fryers to reach a temperature of 180 °C ± 10 °C, in 10 minutes, and the temperature was kept within this range. Time measurements started when the temperature reached 180 °C ± 10 °C. Through-out the experiment, and prior to
sample collection taken at 30, 120, and 480 minutes, an infrared temperature gun was used to monitor temperatures within an expected range of 180 °C ± 10 °C. All samples were collected, cooled at room temperature, and stored at 4 °C in brown transparent tubes until analytical measurements could be performed.

3.2.6 Fatty Acid Analysis

The fatty acid composition of the oil samples were determined by gas chromatography as reported by Yuan and Kitts (2003). Details are given in Chapter 2.

3.2.7 Peroxide Value (AOAC method)

The AOAC official method (AOAC 965.33, 2003), with minor modification was used to determine sample oil peroxide values. Details are given in Chapter 2.

3.2.8 Determination of p-anisidine Values

The IUPAC (1987) method was used to determine the p-anisidine values. Details are given in Chapter 2.

3.2.9 Total Oxidation Number (Totox)

Totox was calculated from P.V. and p-anisidine values as shown in Chapter 2.

3.2.10 Alkaline Contaminant Materials – Thermal Degradation Experiment

Analysis of alkaline contaminant materials (ACM) was adapted from a procedure used by Jaswir et al. (2000). The test reagent solution contained American chemical society grade bromophenol blue (0.04 g) which was dissolved in 750 ml of methanol, thus resulting in a pale yellow solution. This was further diluted by adding distilled water (75 ml) which produced a gold colored solution. A final step of in the reagent preparation involved adding five drops of sodium hydroxide (0.01 N), which gave a greenish tinge,
followed by the addition of hydrochloric acid (0.01 N), which restored a pure yellow color.

Absorbance values for 0, 0.005, and 0.01 g/g of sodium palmitate in flax-palm oil blend and fish-palm oil blend were measured at wavelengths of 520 nm, 540 nm, and 560 nm, respectively using a spectrophotometer (Shimadzu UV-160). Of these three wavelengths, linear regression plots of ACM absorbance taken at 560 nm maximum absorption were the best to generate two standard curves for both flax-palm oil blend and fish-palm oil blend for quantification of the concentration of ACM in thermally processed oils.

ACM was extracted from the heat processed oil by adding 2 ml of the reagent solution to 2 ml of processed oil. The oil and reagent were quickly vortex and allowed to stand at room temperature for 1 minute to allow separation. The test solution (upper layer) was subsequently collected and filtered into a disposable polystyrene cuvet (1x1 cm) through a filter disk (Millipore, Type HA 0.45 um pore size) to obtain a transparent solution. The color of the upper layer correlated directly to the amount of ACM extracted from the oil. The absorbance of the upper layer was measured at a wavelength of 560 nm, and the concentration of ACM could be determined from the standard curves.

3.2.11 Tocopherol Analysis

The tocopherol content in experimental oils was determined by high performance liquid chromatography (HPLC), using an Agilent 1100 series (Wilmington, USA) HPLC, equipped with an Agilent LiChrospher® Si 60 column (4.6x250 mm) (5 um). The AOCS Ce 8-89 oil method (AOCS, 1997), without saponification (e.g. 99.5% hexane and 0.5 % isopropanol as the mobile phase), was chosen for tocopherol separation. UV detection
was set at 292 nm. Calibration curves for α, β, γ, and δ-tocopherol standards were constructed to quantify the tocopherol content in experimental oils as part of the method set-up procedure. A range of concentrations of α, β, γ, and δ-tocopherol were used and individual tocopherol calibration curves were constructed to convert absorbance readings to absolute concentrations (ug/ml) of tocopherol in each oil sample. Preparation of each sample, involved dissolving 2 grams of oil in 10 ml of hexane, followed by 30 seconds of vortexing. Samples were allowed to stand at room temperature for 30 minutes and 20 ul of sample was injected onto the HPLC-column. The tocopherol content in oil samples (ug/g) was then calculated based on the weight of sample and volume of hexane.

3.3 Results

3.3.1 Oil Blending Experiment (Chapter 2)

Using the mass ratio weight data for individual fatty acid content of standard oils (Table 3), obtained from calibration curves (Table 2), it was confirmed that 6.10 grams of flaxseed oil was needed to be blended into 100 grams of palm oil (Table 4) to achieve a flax-palm oil blend that contained a ratio of 1:4 (omega-3: omega-6). Meanwhile, a ratio of 1:4 (omega-3: omega-6) for the fish-palm oil blend was obtained by adding 4.46 grams of menhaden fish oil to 100 grams of palm oil (Table 4). Table 5 reports the new fatty acid composition of the blended flaxseed-palm oil with the absolute weight of linoleic acid and α-linolenic acid possessing a final 1:4 ratio (omega-3: omega-6). Moreover, the weight of fatty acids derived from GC analysis of the fish-palm oil blend (Table 6) also confirms that the omega-3:omega-6 ratio did not exceed 1:4. The weight of fatty acids for both oil blends were also used as the reference sample or zero-time controls for similar
oil blends that were used in the frying experiments. This was done out of necessity due to
the limited amount of oil available for frying experiments.

3.3.2 Flax-Palm Oil Blend

3.3.2.1 8 Hours Frying Experiment at 180 °C of Flax Blended Palm Oil

During frying, the linoleic acid content in the flax-palm oil blend was not
statistically different among antioxidant treatments and frying times (Table 8). By
comparing with the reference for linoleic content, all samples showed no significant
decrease in linoleic acid content after 8 hours of frying.

In contrast, the α-linolenic acid content of heated oil was significantly reduced
(P< 0.05) after 8 hours of frying, when compared to the reference (Table 9). The decrease
in α-linolenic acid was first observed to start at 30 minutes of frying (compared to
reference). ANOVA further indicated that the α-linolenic acid content of flax-palm oil
blend during frying was not significantly different between 30 minutes and 2 hours of
frying. When antioxidants were added to the oil, no further significant losses were
observed. This was not the case with blended oil samples that did not contain added
antioxidant. In this case, α-linolenic acid content did not change significantly, compared
to oil samples containing BHA and rosemary antioxidants. This result suggests that the
rosemary and BHA provided some protection against α-linolenic acid thermal oxidation
during deep-fat frying. Alternatively, small changes in both linoleic acid and α-linolenic
acid content of these oils following thermal treatment suggests also that stabilization of
PUFAs could be due to the presence of natural antioxidant present in both palm oil and
flaxseed oil.
3.3.2.2 Total Oxidation

Figure 8. shows the significant increase in totox, or total oxidation number (P<0.05) of the flax-palm oil blend that occurred during 8 hours of heating. A significant difference (P< 0.05) in totox was also observed between different antioxidant treatments. Oil blended samples without added antioxidant had the relatively highest totox, compared to samples containing BHA and rosemary, respectively. Blended oil samples treated with BHA had the lowest totox at 30 minutes and 2 hours of heating, followed by blended oil sample stabilized with rosemary (p< 0.05). After 8 hours of frying oil, samples stabilized with rosemary had the lowest totox, followed by the sample containing BHA. In general, a trend for lower total oxidation in blended oil samples indicated that BHA was the most effective stabilizing agent, followed by the rosemary extract.

3.3.2.3 Alkaline Contaminant Materials (ACM) - Thermal Degradation Product

Figure 9 shows that the ACM content present in the flax-palm oil blend can be determined spectrophotometrically at an optimal wavelength of 560 nm due to the highest absorbance measurements observed in the range of 510 -570 nm. A calibration curve constructed at 560 nm (Figure 10) was used to determine the alkaline contaminant concentration in flaxseed oil blended palm oil (Table 10).

After 30 minutes of deep-fat frying of the flax-palm oil blend, alkaline contaminant materials were not detected, which suggests that thermal degradation was not always observed (Table 10). After 2 hours of frying, detectable concentrations of ACM occurred in the flax-palm oil blend which did not contain antioxidant and the rosemary stabilized blend. However, Lower concentrations of ACM were detected in the rosemary treated flax-palm oil. After 8 hours of frying, the control (e.g. unstabilized)
flax-palm oil blend had a higher sodium palmitate content than the blended sample containing rosemary extract. No sodium palmitate could be detected in flax-palm oil samples stabilized with BHA. These results further emphasize that the antioxidant effectiveness of rosemary extract produced lower ACM content when used in deep frying (Jaswir et al., 2000). Notwithstanding, these results, rosemary extract was not as effective as BHA in protecting against thermal degradation of blended oils.

3.3.2.4 Tocopherol Content

Standard calibration curves for α, β, γ, and δ tocopherol, obtained by HPLC enabled quantitation of tocopherol isomers in oils used in this experiment (Table 11).

The palm oil sample contained mainly α-tocopherol; whereas, γ-tocopherol was the major isomer found in the starting flaxseed oil (Table 12). Blending flaxseed oil to palm oil resulted in the detection of α-tocopherol as the major tocopherol isomer, followed by γ-tocopherol. The effects of blending oils did not necessarily enhance the antioxidant content of the oils, but rather a significant loss (P<0.05) in α-tocopherol was observed in all samples after 8-hours of deep-fat frying. The blended flax-palm oil stabilized with BHA retained a small amount of δ-tocopherol after 8 hours of deep-fat frying. The presence of δ-tocopherol in the BHA stabilized oil blend; after 8 hours of deep-fat frying, and the loss of all other isomers, suggests that there might be a specific mechanism which preserved or altered only the δ-tocopherol isomer during frying. Further research is needed to help explain the specific mechanism for this possible structural change or preservation of different tocopherol isomers in oils that are heated at high temperatures. Moreover, the presence of δ-tocopherol in the BHA treated sample after 8 hours of deep-fat frying further supports the trend obtained with reduced totox
value and alkaline contaminant materials also detected in these samples. Thus, lower lipid oxidation and lipid degradation products due to the presence of BHA helped stabilized the flax-palm oil blend.

3.3.3 Fish-Palm Oil Blend

3.3.3.1 8 Hours Frying Experiment at 180 °C of Fish Blended Palm Oil

Statistical analysis of the linoleic acid content in the fish-palm oil blend during frying (Table 13) revealed significant decreases in concentration after 8 hours of frying, and no significant differences among the different antioxidant treatments. Although there was no significant different among treatments, this trend suggests that after 8 hours of exposure to frying temperature, linoleic acid content of the fish-palm blended oil was not influenced by the presence of antioxidant. From 30 minutes of frying on, however, significant decreases (P<0.05) in linoleic acid content occurred in the blended oil compared to the non-heated control oil.

Significant decreases were observed after exposure to 8 hours frying temperature for EPA concentration in the blended fish-palm oil; however, no significant differences were observed with antioxidant treatments (Table 14). Although ANOVA did not show any significant changes in EPA concentration during 30, 120, and 480 minutes of frying, blended oil samples not containing antioxidant treatment had a relatively lower EPA content after 480 minutes of exposure to frying temperature. Compared to the non-heated control sample of the blended fish-palm oil, significant decrease (P<0.05) in EPA content occurred as early as 30 minutes of frying.

Blended fish-palm oil stabilized with antioxidants, BHA and rosemary, retained greater (P<0.05) concentrations of DHA than the unstabilized blends (Table 15). There
was however, no significant difference in DHA content in the blended oils that contained BHA and rosemary treatments. After frying blended oils for 8 hours, a significant decrease (P<0.05) in DHA retention was observed. This result was the same that DHA concentration was significantly decreased (P<0.05) after only 30 minutes and at 2 hours, respectively, of frying. Compared to the non-heated control sample of the blended fish-palm oil, the decrease in DHA content in the oil blend also occurred as early as 30 minutes of frying. Similarly, linoleic acid, EPA, and DHA concentrations were lower in all heated oils compared to non-thermally treated oil.

Similar with the flax-palm oil blends, an insignificant effect of added antioxidant treatments on retained linoleic and EPA content could reflect the presence of natural antioxidants (eg. tocopherol and added TBHQ) presence in palm or fish oils prior to blending. Throughout the blending and heating of the fish-palm blended oil experiment, the presence of α-linolenic acid could not be detected.

### 3.3.3.2 Total Oxidation

Blended fish-palm oil exhibited a significant increase in total oxidation (P<0.05) during 8 hours of heating when compared to the non heated oil (Figure 11). A significant difference (P<0.05) was also observed among the antioxidant treatments. Samples of blended oils not containing added antioxidant had the highest totox, whereas BHA and rosemary treated samples gave relatively lower totox, respectively. At 30 minutes and 8 hours of heating oils, those samples oils treated with BHA had the lowest totox followed by oils stabilized with rosemary. After 2 hours, blended fish-palm oil samples containing rosemary had the lowest totox values followed by samples containing BHA. In general, these results obtained with fish-palm oil blends were similar to that obtained with the
flax-palm oil blend, in so much that the trend of total oxidation indicated that BHA treatment provided the greater protection than rosemary against total oxidation. Both BHA and rosemary stabilized oils produced lower totox values (P<0.05) than control, unstabilized oils. The insignificant effect of adding antioxidants suggests that the total oxidation number was not mainly affected by linoleic acid and EPA content during 8 hours of frying.

3.3.3.3 Alkaline Contaminant Materials (ACM) - Thermal Degradation Product

Similar with the flax-palm oil blend, the best wavelength range for determining the ACM content in the fish-palm oil blend was 510-570 nm. Figure 12 shows that the ACM content present in the fish-palm oil blend can be determined spectrophotometrically at an optimal wavelength of 560 nm due to the highest absorbance values observed at this wavelength. A calibration curve used to determine the sodium palmitate concentration at 560 nm in the fish-palm oil blend is presented in Figure 13.

After 30 minutes and 2 hours, respectively, of frying oils, the alkaline contaminant materials were not detected in all blended fish-palm oil samples. This finding suggests that thermal degradation had not occurred to a level that could be detected in this assay procedure (Table 16). After 8 hours of frying blended oils, sodium palmitate concentrations in fish-palm oil samples containing BHA were the lowest, followed by blended oil samples containing rosemary. Similar with the flax-palm oil blend, these results further emphasize the fact that rosemary has antioxidant activity which effectively lowered ACM content in thermally heated oils, but it was not as effective as BHA in doing so.
3.3.3.4 Tocopherol Content

Both the palm oil and fish oil samples, as well as the blended fish-palm oil contained mainly α-tocopherol (Table 17). Similar with the flax-palm oil blend, all the fish-palm oil blends showed a loss in α-tocopherol after 8 hours of deep-fat frying. Only oil blend samples treated with BHA contained detectable amount of δ-tocopherol. The presence of δ-tocopherol in BHA treated blended oils after 8 hours of deep-fat frying, suggests again that there might be a specific mechanism which preserved or altered tocopherol structure during frying. Moreover, the presence of δ-tocopherol in fish-palm oil blends stabilized with BHA after 8 hours of deep-fat frying, further supports the trend observed with lower totox and lower alkaline contaminant results.

Table 8. Effect of BHA and rosemary on linoleic acid content of heated flax-palm oil blends.¹

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Treatment</th>
<th>Treatment</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>9.40 ± 0.64</td>
<td>9.55 ± 2.17</td>
<td>8.90 ± 0.53</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>9.29 ± 0.22</td>
<td>9.59 ± 0.72</td>
<td>8.08 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>480</td>
<td>8.11 ± 1.1</td>
<td>8.03 ± 0.59</td>
<td>8.76 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are expressed as g linoleic acid/100 g. Values represent mean ± standard deviation of three measurements.
Zero time linoleic acid concentration: 9.09 ± 0.77 g/100g.
Table 9. Effect of BHA and rosemary on α-linolenic acid content of heated flax-palm oil blends.  

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>120</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td>1.52 ± 0.18</td>
<td>1.56 ± 0.21</td>
<td>1.71 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>1.47 ± 0.03</td>
<td>1.50 ± 0.15</td>
<td>1.61 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>1.24 ± 0.07</td>
<td>1.42 ± 0.14</td>
<td>1.37 ± 0.02</td>
</tr>
</tbody>
</table>

Values are expressed as g α-linolenic acid/100 g. Values represent mean ± standard deviation of three measurements.

Zero time α-linolenic acid concentration: 2.44 ± 0.59 g/100g.  

a, b, c Represent statistical difference at P<0.05 in frying times.

Figure 8. Total oxidation (totox) of heated flax-palm oil blends.  

Time 0 is the reference blended oil; □ (- antioxidant), ■ (+ BHA), □ (+ rosemary).  

Results shown are mean ± standard deviation of three measurements.  

a, b, c, d Represent statistical difference at P<0.05 in frying times.  

x, y, z Represent statistical difference at P<0.05 among treatments.
Figure 9. Absorbance for sodium palmitate in flax-palm oil blend; • (0 g/g), ▲ (0.005 g/g), ▲ (0.01 g/g), taken at wavelengths (510-570 nm). Results shown are the mean of duplicate analysis.
Figure 10. Calibration curve constructed for sodium palmitate (0, 0.005, 0.01 g/g) concentrations in flax-palm oil blend. $Y = 6.25X + 0.1946$, $R^2 = 0.999$, $X =$ concentration of sodium palmitate (g/g), $Y =$ absorbance at 560 nm. Results shown are the mean of duplicate analysis.

Table 10. Concentration of alkaline contaminant materials in heated flax-palm oil blends measured at 560 nm. ¹

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Treatment</th>
<th>BHA</th>
<th>Rosemary</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>N/D</td>
<td></td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>120</td>
<td>1.6</td>
<td>N/D</td>
<td>N/D</td>
<td>0.01</td>
</tr>
<tr>
<td>480</td>
<td>2.1</td>
<td>N/D</td>
<td>N/D</td>
<td>0.69</td>
</tr>
</tbody>
</table>

¹Values are expressed as g ACM/100 g. Values represent the average of duplicate analysis. 

N/D = not detected or <0.001 g/100g. 

Reference zero time control = N/D.
Table 11. HPLC calibration curves for standard tocopherol.¹

<table>
<thead>
<tr>
<th>Tocopherol Isomer</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Y = 8.88 X - 2.13, R² = 0.999</td>
</tr>
<tr>
<td>β</td>
<td>Y = 7.66 X - 1.73, R² = 0.999</td>
</tr>
<tr>
<td>γ</td>
<td>Y = 10.02 X - 1.93, R² = 0.999</td>
</tr>
<tr>
<td>δ</td>
<td>Y= 8.17 X + 7.77, R² = 0.996</td>
</tr>
</tbody>
</table>

¹X = tocopherol amount (ug/ml) and Y = area measurement.

Table 12. Tocopherol contents in native and blended oils before and after frying.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tocopherol Isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α (ug/g)</td>
</tr>
<tr>
<td>No Frying</td>
<td></td>
</tr>
<tr>
<td>Palm Oil</td>
<td>154</td>
</tr>
<tr>
<td>Flaxseed Oil</td>
<td>N/D</td>
</tr>
<tr>
<td>Flax-palm Blend</td>
<td>127.5</td>
</tr>
<tr>
<td>Frying (8 hrs @180 °C)</td>
<td></td>
</tr>
<tr>
<td>Flax-palm Blend</td>
<td>N/D</td>
</tr>
<tr>
<td>Flax-palm Blend +BHA</td>
<td>N/D</td>
</tr>
<tr>
<td>Flax-palm Blend +Rosemary</td>
<td>N/D</td>
</tr>
</tbody>
</table>

¹N/D is below the detection limit 10 ug/g.
Table 13. Effect of BHA and rosemary on linoleic acid content of heated fish-palm oil blends.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>BHA</th>
<th>Rosemary</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>8.36 ± 0.93b</td>
<td>9.54 ± 1.94b</td>
<td>8.23 ± 0.51b</td>
</tr>
<tr>
<td>120</td>
<td>8.85 ± 1.87b</td>
<td>8.94 ± 1.08b</td>
<td>8.78 ± 1.33b</td>
</tr>
<tr>
<td>480</td>
<td>6.87 ± 0.87c</td>
<td>7.97 ± 1.55c</td>
<td>8.27 ± 1.05c</td>
</tr>
</tbody>
</table>

Values are expressed as g linoleic acid/100g. Values represent mean ± standard deviation of three measurements.
Zero time linoleic acid concentration: 10.02 ± 0.82 g/100g. a
a, b, c Represent statistical difference at P<0.05 in frying times.

Table 14. Effect of BHA and rosemary on EPA content of heated fish-palm oil blends.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>BHA</th>
<th>Rosemary</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.44 ± 0.12b</td>
<td>0.44 ± 0.05b</td>
<td>0.45 ± 0.06b</td>
</tr>
<tr>
<td>120</td>
<td>0.43 ± 0.03b</td>
<td>0.43 ± 0.08b</td>
<td>0.43 ± 0.01b</td>
</tr>
<tr>
<td>480</td>
<td>0.41 ± 0.08b</td>
<td>0.43 ± 0.04b</td>
<td>0.42 ± 0.03b</td>
</tr>
</tbody>
</table>

Values are expressed as g EPA/100 g. Values represent mean ± standard deviation of three measurements.
Zero time EPA concentration: 0.56 ± 0.11 g/100g. a
a, b Represent statistical difference at P<0.05 in frying times.

Table 15. Effect of BHA and rosemary on DHA content of heated fish-palm oil blends.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>BHA</th>
<th>Rosemary</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.1 ± 0.33bx</td>
<td>1.25 ± 0.18by</td>
<td>1.13 ± 0.1 by</td>
</tr>
<tr>
<td>120</td>
<td>0.85 ± 0.14cx</td>
<td>1.12 ± 0.07cy</td>
<td>1.02 ± 0.08 cy</td>
</tr>
<tr>
<td>480</td>
<td>0.82 ± 0.12dx</td>
<td>1.01 ± 0.11dy</td>
<td>0.99 ± 0.1 dy</td>
</tr>
</tbody>
</table>

Values are expressed as g DHA/100g. Values represent mean ± standard deviation of three measurements.
Zero time DHA concentration: 2.09 ± 0.15 g/100g. a, b, c, d
a, b, c, d Represent statistical difference at P<0.05 in frying times.
x, y Represent statistical difference at P<0.05 among treatments.
Figure 11. Total oxidation (totox) of heated fish-palm oil blends. Time 0 is the reference blended oil; (- antioxidant), (+ BHA), (+ rosemary). Results shown are mean ± standard deviation of three measurements. a, b, c, d Represent statistical difference at P<0.05 in frying times. x, y, z Represent statistical difference at P<0.05 among treatments.
Figure 12. Absorbance values for sodium palmitate in fish-palm oil blend; ◆ (0 g/g), □ (0.005 g/g), ▲ (0.01 g/g), taken at wavelengths (510-570 nm). Results shown are the mean of duplicate analysis.
Figure 13. Calibration curve constructed for sodium palmitate (0, 0.005, 0.01 g/g) concentrations in fish-palm oil blend. \( Y = 22.95 X + 0.2021 \), \( R^2 = 0.904 \), \( X = \) concentration of sodium palmitate (g/g), \( Y = \) absorbance at 560 nm. Results shown are the mean of duplicate analysis.

Table 16. Concentration of alkaline contaminant materials in heated fish-palm oil blends measured at 560 nm.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N/D</td>
<td>BHA</td>
</tr>
<tr>
<td>30</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>120</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>480</td>
<td>2.1</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as g ACM/100 g. Values represent the average of duplicate analysis.

N/D = not detected or <0.001 g/100 g.

Reference zero time control = N/D.
Table 17. Tocopherol contents in native and blended oils before and after frying. ¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tocopherol Isomer</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α (ug/g)</td>
<td>β (ug/g)</td>
<td>γ (ug/g)</td>
<td>δ (ug/g)</td>
</tr>
<tr>
<td>No Frying</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm Oil</td>
<td>154</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>Fish Oil</td>
<td>697.5</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>Fish-palm Blend</td>
<td>118</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>Frying (8 hrs @ 180 °C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish-palm Blend</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>Fish-palm Blend</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>496.5</td>
</tr>
<tr>
<td>+ BHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish-palm Blend</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
<tr>
<td>+ Rosemary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ N/D is below the detection limit 10 ug/g.

3.4 Discussion

Using only general visual observations (data not shown), the colour of the frying oil became darker from the original light yellow colour and was transformed to a brown colour after 8 hours of deep-fat frying. Tan et. al (1985), described this typical colour change in heated oils to represent the combined effects of oxidation, polymerization, and other chemical reactions which occur in the oil. In addition to changes in lipid content associated with individual fatty acid components, colour changes of heat processed oil may also represent chemical modification of phenolic compounds, such as chlorophyll pigments present in the darker palm oil (Tan et al., 1985). Moreover, this darkening oil phenomena noticed with heat treatment was very useful and practical since it identified the fact that the oil had undergone thermal oxidation, and in fact had deteriorated. This
change in oil colour enabled a visual marker to be used to detect oil quality changes for making decisions to cease the use of further frying (Tan et al., 1985).

In general, 8 hours of frying of blended flax-palm oil produced a final average decrease in linoleic acid (11.66 %) which corresponded to a greater decrease in α-linolenic acid (45.08 %). Similarly, average losses of linoleic acid (18.46 %), EPA (25 %), and DHA (55.02 %) were observed after 8 hours heating of fish-palm oil blends. The results produced a change in the intended ratio of 1:4, and produced a 1:6-1:7 final ratio for omega-3:omega-6 fatty acids. The extent of decreased retention of polyunsaturated fatty acids (e.g. linoleic acid, α-linolenic acid, EPA, and DHA) content, following frying, compared to non-thermal heated oils, agrees with reports by Che Man et. al (2003) and Tyagi and Vasishtha (1996). Che Man et al. (2003), reported that during 5 days of repeated frying of banana chips in RBD palm oil at 180 °C, resulted in an average loss of 19% for omega-6 fatty acid (C18:2). In separate research, Tyagi and Vasishtha (1996), also reported final average loses of 45.72 % for omega-6 fatty acid (C18:2) and 60.78 % for omega-3 fatty acid (C18:3) after 70 hours of frying of soybean oil at 180 °C.

Analysis of lipid oxidation products in oil blends in this experiment showed that the totox increased during frying, and furthermore that the addition of antioxidants to the oils effectively reduced the totox. Similar findings, with heated palm oil have been reported by Che Man et al. (2003) and Che Man et al. (1999). Totox reflects both primary and secondary products of lipid oxidation; therefore, the increase in total oxidation number during heating or frying of the oil is attributed to the formation of hydroperoxides and conjugated dienes (Che Man et al., 2003; Che Man and Hussin, 1998; and Rossell, 1994).
Alkaline contaminant materials are potent surfactants, which lower the interfacial tension of frying oil and also the retention of the oil on food products (Gill, 1998). Jaswir et al. (2000), reported that ACM surfactants could be used as a measure of thermal abuse of cooking oil, since they are formed naturally during oil degradation reactions through the combination of fatty acids with metal ions. The experimental results shown in this study confirmed the effectiveness of using both BHA and rosemary extract to lower ACM content in deep-fat frying oil (Jaswir et al., 2000). However, rosemary extract was not found to be as effective as BHA in preventing thermal degradation of the flax-palm oil and fish-palm oil blends, respectively.

The effectiveness of different antioxidant treatments for both flax-palm oil and fish-palm oil blends could be evaluated by monitoring both the totox and alkaline contaminant measures. BHA consistently produced a protective effect to the heated oils which is contradictory to a previous report by Coppen (1998), who suggested that the addition of BHA failed to significantly increase the stability of oils containing tocopherol. BHA is considered an effective antioxidant, especially when it is used in combination with other primary antioxidants (Nawar, 1996). Detail research on thermal treatment of sunflower oil by Allam and Mohamed (2002), suggested that thermal stability of the antioxidant ascorbyl palmitate could be enhanced by the addition of BHA. BHA is one of the most commonly used antioxidants which is known for its chemical stability, low cost, and availability (Yang et al., 2002).

The tocopherol analysis of blended oils following heat treatment indicated that the tocopherol mixture from flaxseed oil, menhaden fish oil, and palm oil, carried over to the blended product; however, heating of these oils at frying temperature destroyed
tocopherol content. The only possible exception to this was with the δ-tocopherol. Barrera-Arellano et al. (2002), reported that tocopherol, especially α-tocopherol was quickly lost at frying temperatures. In general, vegetable oil sources with a high vitamin E activity are not effective antioxidants under thermal conditions, as reflected by the descending antioxidant activity of tocopherol observed for δ > γ > β > α when exposed to heat and oxygen (Nawar 1996).

3.5 Conclusion

The results of these experiments show that it is feasible to enriched palm oil with omega-3 fatty acids from either flaxseed oil or fish oil to produce an effective 1:4 (omega-3:omega-6) ratio. The retention of omega-3 (α-linolenic acid, EPA, and DHA) in samples collected throughout the thermal treatments, common to that used with deep-fat frying; however, indicated that the blended oils have indeed, some potential use for deep-fat frying. For example, the retention, albeit reduced absolute amount of omega-3 fatty acids in the thermally treated cooking oil blend, could be considered an additional source of omega-3 PUFA and HUFA for contributing to dietary requirements. Health Canada (2006) and Nielsen (2005) recommended that α-linolenic acid intake for males/females adults ranges from 1.0-1.6 g/day. Although there is no recommended daily intake of EPA and DHA, it was suggested that intake over 250 mg per day is sufficient enough to provide significant health benefits in humans (Nielsen 2005). Looking at the final ratio of omega-3:omega-6 in the blended oils and the changes in relative concentrations during thermal processing, it can be concluded that heat processing will result in a final ratio of 1:6 or 1:7 (omega-3:omega-6) from the non-heated 1:4 oil blend.
During these experiments, the antioxidant activity of rosemary reported by Berner and Jacobson (1973), Chang et al. (1977), Nakatani (1989), and Jaswir et al. (2000) in bulk oil sample, was also assessed herein from total oxidation measurement (totox) and alkaline contaminant determinations. Rosemary, a natural antioxidant, was not as effective as BHA in protecting the omega-3 fatty acids in both oil blends exposed to high temperature frying as shown also by lipid oxidation and degradation methods of analysis. Lipid oxidation and degradation of the thermally heated oil blends, as well as the loss of tocopherols were less in the blended oils that were stabilized with BHA, compared to rosemary.
4.1 Introduction

Deep-fat frying is commonly used in home food preparation and is one of the oldest and widest used cooking methods. This form of cooking is also important to the food industry and institutional food preparation where enhancing taste, appearance and texture of the foods is essential for consumer acceptance (Rimac-Brncic et al., 2004). One example of deep-fat frying popularity is the snack industry which is based on fried potato products such as chips and French fries (Saguy and Dana, 2003), processed in vegetable oils such as canola and palm.

Potatoes (*Solanum tuberosum*) belong to the family Solanaceae, and are native to the Andean regions of South America (O’Connor et al., 2001). Potatoes first originated in Europe in the 16th century and have been widely distributed worldwide (O’Connor et al., 2001). They are now a popular cultivated food staple with many new varieties easily developed to suit the local agricultural growing and consumer preference (Hawkes, 1978).

Potatoes are also widely used in deep-fat frying processing, since they are easily chopped and contain minimal fat. Studying the complex process in frying and bidirectional uptake of oil with frying is relatively simple using potatoes (Varela, 1988).

In this experiment, two newly developed blended oils characterized in Chapter 2 and 3 (flaxseed enriched palm oil and fish enriched palm oil with rosemary) were use in
deep fat-frying experiments to determine the extent of omega-3 fatty acids uptake in fried potatoes. In addition, the relative uptake of \( \alpha \)-linolenic acid compared to the longer chain omega-3 PUFAs (EPA and DHA) was examined.

4.2 Materials and Methods

4.2.1 Materials

Russet Baker potatoes were purchased at a local grocery store in Vancouver, BC. Please see Chapter 3 for details on other materials used.

4.2.2 Experimental Design

Two oil blends, stabilized with rosemary (0.02%); namely flaxseed oil enriched palm oil and fish oil enriched palm oil, were used as frying oils to process potatoes in this experiment. The uptake of omega-3 fatty acids, \( \alpha \)-linolenic acid, EPA, and DHA were recovered in fried potatoes from oils that has been exposed to heat durations of 30 minutes, 2 hours and 8 hours at 180 °C.

4.2.3 Fatty Acid Calibration Curves

Please see Chapter 2.

4.2.4 Blending Procedure

The weight of omega-3 oil sources added to the palm oil to create the different blends was determined by first quantitating fatty acid composition of flaxseed oil, menhaden fish oil and palm oil from the preliminary weight data. Details have been given in Chapter 2.
4.2.5 Frying Experiment

Two deep-fat fryers (Beatty DF 142F and Garland E22-28FT, Canada) with similar capacity were used in this experiment. For each frying experiment, 5 kg of rosemary-stabilized blended oils made from flax-palm oil and fish-palm oil, respectively, were used. Fryers were heated to 180 °C ± 10 °C in 10 minutes and the temperature was kept within this range. Time measurements started when the temperature reached 180 °C ± 10 °C. Through out the experiment, an infrared temperature gun was used to ensure that the temperatures were within this range of temperature. A day before the frying experiment, potatoes were cut into 2 X 2 cm slices and soaked in hot water; before being stored in plastic bags at -18 °C. Potatoes were fried for 8 minutes in the blended oils that had been previously exposed to high temperature for 30, 120, and 480 minutes. All potato samples were collected, cooled at room temperature, and stored in an aluminum foil cover at 4 °C until analytical experiments were performed.

4.2.6 Crude Fat Analysis

The Bligh-Dyer method for fat extraction, with minor modification, was performed on fried potato samples to recover the oil from potatoes. Potato samples weighing 4 grams were added to 10 ml chloroform and 20 ml methanol, and then blended using a spark proof blender (covered with ice to prevent heat friction) for 120 seconds. The next step was adding an extra 10 ml chloroform to create a biphasic solution, which was then mixed again for 30 seconds. Following the addition of 8 ml of water, samples were mixed again for 30 seconds. Mixtures were filtered using Whatman No.1 paper under vacuum to remove all solid residues. The filtrate was then transferred to 50 ml graduated cylinder and allowed to separate into two phases and the methanol phase was
suctioned off. An aliquot of the chloroform layer (10 ml) was transferred into a preweighted aluminum weigh boat. The aluminum weighing boat containing the sample, was placed in a boiling water bath to allow chloroform to evaporate, and then transferred to a drying oven set at 105 °C and dried for 15 minutes to remove residual chloroform. The final step was to allow samples to cool in a dessicator for 15 minutes.

Percent lipid in the potato sample was calculated using the following formula:

\[
\text{l lipid weight (g)} \times \frac{\text{ml of chloroform}}{\text{ml of aliquot weight of original sample (g)}}
\]

Note: lipid weight is the weight of aluminum weigh boat after fat extraction minus preweighted aluminum weigh boat.

4.2.7 Fatty Acid Analysis

The fatty acid composition of samples was determined by gas chromatography as reported by Yuan and Kitts (2003). Details have been given in Chapter 2.

4.3 Results

Table 18 summarizes the specifications of the Russet Baker potato used in this experiment, which were cut in 2 x 2 cm dimensions that ranged from 7-11 g wet weight. Potato contains very little fat as the USDA data shows (e.g. 0.08 g of fat per 100 g of potatoes). Moreover, omega-3 fatty acids were not detectable in potatoes prior to frying as shown in Tables 20 and 21. Table 19 shows the percent lipid in the fried potato samples calculated from a 10 ml aliquot collected from 4 g of fried potatoes. An average of 6.35 % of lipid on wet weight basis was found in fried potatoes. This represented a massive (e.g. 7837.5 %) increase in the final lipid content due to deep-fat frying.
Oil extracted from potatoes after frying with both fish-palm oil and flax-palm oil blends treated with rosemary (Table 20 and 21) contained detectable amounts of omega-3 fatty acids. The amount of omega-3 fatty acids extracted from fried potatoes ranged from 0.35-0.38, 0.07-0.09, 0.36-0.38 g per 100 g of oil for α-linolenic acid, EPA, and DHA, respectively. This represented an average percent transfer of 14.75, 13.69, and 17.86 of omega-3, α-linolenic acid, EPA, and DHA, respectively, from oil to potatoes. These results show that the omega-3 enriched palm oil blends could be used with deep-fat frying to increase the dietary intake of omega-3 fatty acids, present in fried food products.

Table 18. Range of specification parameters of Russet Baker potatoes.

<table>
<thead>
<tr>
<th>Size (cm x cm)</th>
<th>Weight</th>
<th>Total Lipid (fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet Weight (g)</td>
<td>Dry Weight (g)</td>
</tr>
<tr>
<td>2 x 2</td>
<td>7-11</td>
<td>5.80-9.12</td>
</tr>
</tbody>
</table>

1 Potato sample size (width x height).
2 Dry weight is obtained through air oven at 80 °C for 1 hour.
3 Total lipid per 100g of potatoes (wet weight), obtained from USDA.

Table 19. Percent lipid content of fried potatoes processed in oils exposed to different times of frying. 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oil blends 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flax-Palm Oil (%)</td>
<td>Fish-Palm Oil (%)</td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>6.36 ± 0.40</td>
<td>6.1 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>120 minutes</td>
<td>6.38 ± 0.38</td>
<td>6.25 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>480 minutes</td>
<td>6.38 ± 0.38</td>
<td>6.64 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

1 Results shown are the average of triplicate analysis, mean ± standard deviation.
2 Time refers to thermal treated at 180 °C for different for different durations before used to fry potatoes.
3 Oil blends contained rosemary extract.
Table 20. α-Linolenic acid content of fried Russet Baker potatoes with rosemary stabilized flax-palm oil blend.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α-Linolenic Acid (g/100 g)</th>
<th>Transfer to Potatoes (%) ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N/D</td>
<td>-</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0.35 ± 0.06</td>
<td>14.34</td>
</tr>
<tr>
<td>120 minutes</td>
<td>0.38 ± 0.1</td>
<td>15.57</td>
</tr>
<tr>
<td>480 minutes</td>
<td>0.35 ± 0.03</td>
<td>14.34</td>
</tr>
</tbody>
</table>

¹ Values of α-linolenic acid represent mean ± standard deviation of three measurements.
² Time refers to thermal treated at 180 °C for different durations before used to fry potatoes.
³ Values represent the percent omega-3 fatty acid in the potato sample compared to the reference original oil.
N/D: not detected.

Table 21. EPA and DHA content of fried Russet Baker potatoes with rosemary stabilized fish-palm oil blend.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EPA (g/100 g)</th>
<th>Transfer to Potatoes (%) ³</th>
<th>DHA (g/100 g)</th>
<th>Transfer to Potatoes (%) ³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N/D</td>
<td>-</td>
<td>N/D</td>
<td>-</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0.07 ± 0.01</td>
<td>12.5</td>
<td>0.38 ± 0.02</td>
<td>18.18</td>
</tr>
<tr>
<td>120 minutes</td>
<td>0.08 ± 0.01</td>
<td>14.29</td>
<td>0.36 ± 0.03</td>
<td>17.22</td>
</tr>
<tr>
<td>480 minutes</td>
<td>0.09 ± 0.01</td>
<td>16.07</td>
<td>0.36 ± 0.03</td>
<td>17.22</td>
</tr>
</tbody>
</table>

¹ Values of EPA and DHA represent mean ± standard deviation of three measurements.
² Time refers to thermal treated at 180 °C for different durations before used to fry potatoes.
³ Values represent the percent omega-3 fatty acid in the potato sample compared to the reference original oil.
N/D: not detected.

4.4 Discussion

The fried potatoes used in this experiment were found to absorb 6.27 % lipid from the oil blends during frying. Varela (1988), reported an uptake of oil in fried products to vary from as low as 5-6 % to higher levels of 40 %. This variation in oil uptake into potato was influenced heavily by different factors, such as thermal process conditions,
pretreatment of food, characteristics of the food itself, the starting oil content, and the chemical composition of the oil (Rimac-Brncic et al., 2004). Uptake of oil into food products has an important role in sensory properties of fried products. For example, the findings of Garayo and Moreira (2002) and Mellema (2003), both reported that ranges in oil content from 35-45 percent in thin slice potato chips were the main reason for both desirable texture and flavor attributes of fried potato products.

Fatty acid analysis of potatoes fried in the different oil blends showed that the omega-3 fatty acids derived from α-linolenic acid from the flaxseed-palm oil blend and DHA from the fish-palm oil blend, were assimilated into the fried potatoes during frying at similar amounts (e.g. 23mg/100g) and which was not characteristic of the different omega-3 oil sources in the blends. On the other hand, the relatively lower uptake of EPA into the fried potatoes could be explained by the markedly lower concentration of this fatty acid in the fish palm oil blend. On the basis of the average total amount of oil taken up into potato on frying, and the average concentration of omega-3 fatty acids content which contributed to the lipid uptake in the potato, it can be calculated that 23 mg of α-linolenic acid and 23 mg of DHA were transferred to 100 grams (wet weight) of fried potatoes from the flax-palm oil and fish-palm oil blends, respectively. The EPA content in the potato samples fried with fish-palm oil blend was considerably lower (e.g. 5 mg/100 g). Health Canada (2006) and Nielsen (2005), have recommended that an effective intake level of α-linolenic acid for males/females adults ranges from 1.0-1.6 g/day in order to receive expected health benefits. Although, there are no recommended daily intakes for DHA and EPA, it has also been suggested that 250 mg per day is a sufficient intake to provide some health benefits to humans (Nielsen, 2005). Considering
that a typical medium size serving of French fried potatoes ranges from 100-120 g, the
uptake of EPA+DHA combined in the fried potatoes studied herein represents
approximately 1/10\textsuperscript{th} of the suggested daily intake. Alternatively the uptake of \(\alpha\)-linolenic acid into the potato constitutes only 1/50\textsuperscript{th} the recommended allowance. Although this amount represents a considerable lower contribution for dietary \(\alpha\)-linolenic acid, it can be argued that it is much easier to find additional food sources that contain \(\alpha\)-linolenic acid which are available for consumer consumption. This study demonstrated the use of newly developed omega-3 enriched oil blends, developed herein, could fortify a frequently used and consumed food product to a level that would contribute to the required uptake of omega-3 fatty acids. Other omega-3 rich oil sources derived from vegetable (e.g. flax, or soybean, or canola) and fish oils (fish supplements or fatty fish) could also contribute to the omega-3 intake. A different example of food products that have been fortified with omega-3 fatty acids are the omega-3 enriched eggs and omega-3 enriched salad dressing.

A clinical study on healthy females conducted by Cunnane et al. (1993), showed that the consumption of raw, ground flaxseed rich in \(\alpha\)-linolenic acid was associated with a lowering of total serum cholesterol and especially a reduction in low density lipoprotein cholesterol. Other studies conducted in hamsters, further emphasized the health benefits associated with \(\alpha\)-linolenic acid intake and lower liver cholesterol content (Morise et al., 2005). With the fish-palm oil blend, the EPA/DHA enriched fried potatoes could feasibly contribute to health benefits. Bakker et al. (1989), reported that fish oil consumption, rich in EPA and DHA, had an important role in decreasing serum triglycerides in nephrotic syndrome patients. No changes were observed, however in total cholesterol or high density lipoprotein cholesterol levels. A study on fish oil supplementation in healthy
volunteers by Bell et al. (1996), further emphasized the effective role of EPA/DHA in reducing plasma triglyceride concentration and reducing very low density lipoprotein particles. Thus, these results indicate that omega-3 enriched palm oil blends can be used as cooking oils under the conditions employed herein and produce potential value-added benefits by increasing the omega-3 fatty acids intake in human diets.

4.5 Conclusion

Chapter 4 showed that it is feasible to enriched palm oil with omega-3 fatty acids from both flaxseed oil, or menhaden fish oil, to produce α-linolenic acid and HUFA enriched blends, respectively for use in frying potatoes. The presence of α-linolenic acid versus EPA/DHA omega-3 fatty acids in thermally processed potato samples from respective oil sources collected throughout the heating experiment shows that these blended oils can be used for deep-fat frying potatoes, especially when stabilized with antioxidant to reduce thermal degradation of omega-3 fatty acids.

The fried potato experiment conducted in this study showed that omega-3 fatty acids are taken up by the potatoes during frying in a relatively stable form. Upon extraction of the oil from the fried potatoes, uptake of α-linolenic acid (e.g. 0.36 g/100 g), EPA (e.g. 0.08 g/100 g), and DHA (e.g. 0.37 g/100 g) had occurred. The respective uptakes α-linolenic acid and EPA+DHA combined into fried potatoes represents 1/50th and 1/10th of the recommended and suggested daily intake of these fatty acids, respectively. Thus, this thesis has shown that omega-3 enriched palm oils can be used as a cooking oil to improve the dietary intake of omega-3 fatty acids.
Chapter 5. General Conclusion

In Chapter 2, it was shown that the use of expressing fatty acid determination quantitatively (e.g. g/100 g) enabled an accurate and reproducible formulation of a specific omega-3:omega-6 ratio in different oils blended with palm oil. The use of a mass ratio of omega-3 fatty acid concentration gave an accurate result for obtaining a 1:4 ratio (n-3:n-6) of omega-3 enriched palm oil blends for future quality control.

The third and fourth chapters demonstrated that the presence of omega 3 sources; derived from flaxseed oil and menhaden fish oil into palm oil, respectively, had relatively different character when exposed to a frying temperature for prolonged processing conditions. The presence of omega-3 fatty acids after 8 hours of deep-fat frying at 180 °C and the fact that they could be successfully assimilated into fried potatoes, suggest that it is feasible to enriched palm oil with these particular omega-3 fatty acids derived from both flaxseed oil and fish oil. Both rosemary and BHA treatments exhibited antioxidant effects with the two new omega-3 enriched palm oil blends during deep-fat frying, especially the positive effects associated with reduced the lipid oxidation and degradation. However, the protective action of the antioxidants shown herein with respective oil blends was not uniformly consistent.

Future quality control research that looks at the variables associated with different varieties of palm oils (e.g. plantation locations and climatic factors), the variety of omega-3 sources (e.g. species and climatic factors), and source of antioxidants are required to define and produce a specific set of specifications for blending procedures if
these products are to be mass produced and marketed to the public. Moreover, further studies at finding alternative stabilizers, other than BHA and rosemary, which may provide better protective effects for these newly developed omega-3 enriched palm oil blends would add important information considering storage and distribution concerns for products containing these omega-3 sources. A better antioxidant can be either a natural one, or even a combination of natural and synthetic antioxidants for use to stabilize these oil blends. Moreover, the effects of synergism should also be considered with the use of combination of antioxidants. Synergism is described as a condition when a mixture of antioxidants produces greater activity than the sum of the activities of the antioxidants when tested individually (Nawar, 1996). From a manufacturer stand point, knowledge of the shelf-life of such food products is very important to ensure the highest quality product, considering the required distribution time from processor to consumer (Fu and Labuza, 1997). Furthermore, this knowledge is also important to maximize the sales potential of food products (Man, 2002). In addition to product specification and a shelf-life study, a cost analysis study is needed to enhance product development, and to calculate the production costs as well as total retail cost. These calculations are needed in order for establishing consumer power for purchasing these oil blends, since these products may only be sold in a niche market. The RBD palm oil price (FOB Malaysia/Indonesia) is US$ 415 per 1000 kilograms or US$ 0.042 per 100 grams, and market prices of flaxseed oil supplement and fish oil supplement are approximately US$ 0.0216 per grams and US$ 0.159 per grams, respectively (personal communications, S. Darmoredjo, P.T. Indofood). The blending experiment in this thesis determined that 6.10 grams of flaxseed oil and 4.46 grams of fish oil were needed to be added to 100 grams of
palm oil in order to reach the targeted ratio of 1:4 (omega-3:omega-6). To reach this target, the amounts of flaxseed oil and fish oil to be added to palm oil will result in additional costs of US$ 0.13 and US$ 0.71, respectively, per 100 grams of palm oil, which has a current price of US$ 0.042 per 100 g of palm oil. Looking at this brief cost analysis calculation, the price of the omega-3 enriched palm oil could be expensive compared to the original price of palm oil, which may potentially limit its use as functional food frying oils under normal circumstances. Moreover, with an effective marketing strategy to highlight the value-added health benefits of the presence of omega-3 fatty acids in frying oil blends and foods subsequently fried in these oils, educating the consumer on the potential health benefits of the improved 1:4 ratio (omega-3:omega-6) could feasibly result in a willingness to pay the additional costs.

In conclusion, with the addition of different omega-3 fatty acid sources into a regular and popular cooking oil, such as palm oil from Southeast Asia, two new oil blends with unique 1:4 ratio (omega-3:omega-6) derived from α-linolenic acid and EPA/DHA, respectively, were achieved using flaxseed oil and menhaden fish oil sources. The ratio of omega-3 to omega-6 however was not retained following the deep-fat frying, a consequence of changes in the concentration of omega-3 and omega-6 fatty acids resulting from thermal degradation and oxidation. Adding a natural or synthetic stabilizer to the frying oil had limited success in maintaining the optimal 1:4 ratio (omega-3:omega-6).
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USDA. Nutrient data laboratory: russet baker potato.


Appendix figure I. Typical GC chromatogram of flaxseed-palm oil blend.

Note:

1. Hexane (solvent)
2. Palmitic acid (methyl ester)
3. Heptadecanoic acid (methyl ester), internal standard
4. Stearic acid (methyl ester)
5. Oleic acid (methyl ester)
6. Linoleic acid (methyl ester)
7. Linolenic acid (methyl ester)
Appendix figure II. Typical GC chromatogram of fish-palm oil blend.

Note:

1. Hexane (solvent)
2. Palmitic acid (methyl ester)
3. Heptadecanoic acid (methyl ester), internal standard
4. Stearic acid (methyl ester)
5. Oleic acid (methyl ester)
6. Linoleic acid (methyl ester)
8. Eicosapentaenoic acid (methyl ester)
9. Docosahexaenoic acid (methyl ester)
Appendix figure III. Calibration curves for linoleic acid, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid.
Appendix figure IV. Calibration curves for α, β, γ, and δ-tocopherol standards – HPLC/method set up.
Appendix figure V. Typical HPLC chromatogram of tocopherol analysis of flaxseed-palm oil blend.
Appendix figure VI. Typical HPLC chromatogram of tocopherol analysis fish-palm oil blend.