

## ABSTRACT

Title of Document: INHIBITORY EFFECT OF SELECTED SPICE AND FRUIT SEED EXTRACTS ON LIPID OXIDATION IN FISH OIL AND THEIR RADICAL SCAVENGING AND ANTIMICROBIAL PROPERTIES

Marla West Luther, M.S. 2006

Directed By: Associate Professor, Dr. Liangli Yu, NFSC

Ethanol extracts of cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flours and cranberry seed meal were evaluated for their capacity to suppress lipid oxidation, preserve fatty acids, inhibit microbial growth, and scavenge DPPH and peroxy radicals (ORAC), and total phenolic content (TPC). All tested extracts suppressed lipid oxidation in fish oil. At a concentration range of 2.6-5.3 mg flour or meal equivalents/mL all extracts exhibited antibacterial activity against *Escherichia coli* and *Listeria monocytogenes*, except cumun-3 and green river parsley against *L. monocytogenes*. All tested seed flour and meal extracts exhibited DPPH radical quenching activity. Chardonnay exhibited the strongest ORAC of 663  $\mu\text{mol TE/g}$  and highest TPC of 99 mg GAE/g seed flour. The data from this study suggest the potential of developing natural food preservatives from these seed flours and meal for improving food stability, quality, safety and consumer acceptance.

INHIBITORY EFFECT OF SELECTED SPICE AND FRUIT SEED  
EXTRACTS ON LIPID OXIDATION IN FISH OIL AND THEIR  
ANTIMICROBIAL AND RADICAL SCAVENGING PROPERTIES

By

Marla West Luther

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Advisory Committee:  
Dr. Liangli Yu, Chair  
Dr. Thomas Castonguay  
Dr. Jianghong Meng

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## Table of Contents

Acknowledgements.....	ii
List of Abbreviations .....	v
List of Figures .....	vi
List of Tables .....	vii
Chapter 1: Literature Review.....	1
1.1.0 Significance, Rationale, and Hypothesis .....	1
1.2.0 Lipid Structure & Function.....	4
1.2.1 Lipids and Food Quality .....	4
1.2.2 Chemical Structure of Food Lipids.....	6
1.2.3 Degree of Saturation & Geometric Configuration of FA .....	7
1.2.4 Fatty Acid Chain Length.....	9
1.2.6 Sensory Properties .....	9
1.3.0 Health Benefits and Risks of Lipid Intake.....	10
1.3.1 Caloric Content & Recommendations .....	10
1.3.3 Lipids and Disease Risk.....	11
1.3.2 Polyunsaturated Fatty Acids .....	13
1.4.0 Lipid Oxidation.....	16
1.4.1 Chemical Mechanism of Lipid Oxidation.....	16
1.4.2 Isomerization of FA .....	18
1.4.3 Available Autoxidative Preservatives.....	19
1.5.0 Relevant Analytical Methods.....	21
1.5.1 Extraction Methods.....	21
1.5.2 Measuring Lipid Oxidation.....	22
1.5.3 Fatty Acid Analysis by Gas Chromatography .....	23
1.5.4 Radical Scavenging Capacity Assays and TPC of Foods.....	24
1.6.0 Introduction to Food Microorganisms .....	26
1.6.1 Microbial Related Food Quality Concerns .....	26
1.6.2 Microbial Related Food Safety Concerns .....	26
1.6.3 Classes of Microorganisms .....	27
1.7.0 Bacteria in Food Products.....	28
1.7.1 Fermented Food Products and Probiotics .....	28
1.6.4 Intrinsic Growth Factors .....	29
1.6.5 Extrinsic Growth Factors .....	31
1.8.0 Methods of Food Preservation.....	32
1.8.1 General Considerations.....	32
1.8.2 Physical Approach .....	33
1.8.3 Chemical Approach .....	34
1.8.4 Phytochemicals as Natural Antimicrobials.....	35
1.8.5 Objectives to Identify Natural Preservatives .....	38
1.9.0 References.....	39

Chapter 2: Inhibitory Effect of Selected Spice and Fruit Seed Extracts on Lipid Oxidation in Fish Oil and Their Radical Scavenging and Antimicrobial Properties. ....	46
2.1.0 Introduction.....	46
2.2.0 Materials and Methods.....	49
2.2.1 Materials & Preparation of Antioxidant Extracts .....	49
2.2.2 Oxidative Stability Index (OSI).....	50
2.2.3 Fatty Acid Composition.....	51
2.2.4 DPPH Radical Scavenging Activity .....	52
2.2.5 Oxygen Radical Absorbing Capacity (ORAC).....	53
2.2.6 Total Phenolic Content (TPC) .....	53
2.2.7 Antibacterial Activity.....	54
2.2.8 Color Measurement.....	55
2.2.9 Statistical Analysis.....	55
2.3.0 Results and Discussion .....	56
2.3.1 Lipid Stability and Maintenance of Essential Fatty Acids.....	57
2.3.2 Free Radical Scavenging Properties .....	62
2.3.4 Antimicrobial Activity.....	66
2.4.0 Conclusion .....	69
2.5.0 References.....	71

## **List of Abbreviations**

AA – Arachidonic Acid  
AI – Adequate Intake  
Aw – Available Water  
AOAC – Association of Analytical Chemists  
CDC – Center for Disease Control  
CHD – Coronary Heart Disease  
EDTA – Ethylenediamine tetracetic acid  
EPA – Eicosapentaenoic Acid  
DHA – Docohexanoic Acid (sp?)  
DPPH – 2,2-diphenyl-1-picrylhydrazyl radical  
DV – Daily Value  
ERH – Equilibrium Relative Humidity  
FA – Fatty Acid  
FDA – Food & Drug Administration  
GC – Gas Chromatography  
LA – Linoleic Acid  
LCFA – Long Chain Fatty Acid  
LCPUFA – Long Chain Polyunsaturated Fatty Acid  
MAP – Modified Atmospheric Packaging  
MUFA – Monounsaturated Fatty Acid  
ORAC- Oxygen Radical Absorbance Capacity  
OSI – Oxidative stability index  
PUFA – Polyunsaturated Fatty Acid  
TPC – Total Phenolic Content  
WHO – World Health Organization

## List of Figures

Figure 1: Free fatty acids & structural components of common lipids in foods.

Figure 2: Mechanism of lipid oxidation.

Figure 3: Lipid isomerization.

Figure 4: Formation of chelating complex illustrated through EDTA.

Figure 5: Redox mechanism illustrated through alpha-tocopherol.

Figure 6: OSI measurement of rancidity and induction point.

Figure 7: Gas chromatography – fish oil FA chromatograph.

Figure 8: Phenolic acids

Figure 9: Monoterpenes

Figure 10: Flavonoids

Figure 11: Oxidative stability index

Figure 12: DPPH radical scavenging capacity of selected seed flour and meal extracts

Figure 13: ORAC of selected seed flour and meal extracts

Figure 14: Total phenolic content of selected seed flour and meal extracts



## **List of Tables**

Table 1: Fatty acid composition of seed meal and flour extract preserved fish oil

Table 2: Antibacterial and bacteriocidal effect of selected antioxidants

Table 3: Effect of antioxidants on fish oil color

## Chapter 1: Literature Review

### 1.1.0 Significance, Rationale, and Hypothesis

In 1996 the World Health Organization (WHO) declared access to nutritionally adequate and safe food a right of every individual; yet, despite new knowledge and improvements in technology, food free from contamination, spoilage, and disease remains one of the biggest challenges in agriculture and the food industry (WHO 1996). In practicality, “illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (WHO, 1984).” In 2000 food safety was accepted as an essential public health function. (Kafenstein 2002) The identification and proper utilization of safe food preservatives is therefore a necessary priority in order to provide an adequate and safe food supply.

Food preservatives are important for reducing food spoilage and waste, improved distribution of goods, and superior convenience. The use of direct food additives to minimize deterioration and maintain nutritional value has thus risen substantially over the past 25 years in the United States; however, the benefits provided are not without potential risk (Tollefson 1988, Timbo and others 2004). The Food Additive Amendments of 1958 to the Federal Food, Drug and Cosmetic Act (FFD&CA) passed by Congress was enacted to ensure that there is reasonably certain proof that a food additive will not harm the consumer from its proposed use. It is important to note that proof beyond a reasonable doubt is not required

and therefore not all potential adverse effects are likely to be uncovered by the FDA (Tollefson 1988).

Unfortunately, synthetic preservatives are not completely safe, and have been linked to a number of reported incidences of food sensitivity such as sulfites. The FDA estimates that 1 in 100 people are sulfite-sensitive and may experience adverse reactions after consuming foods containing sulfites (Warner 2000). Although sensitivities are not true food allergies, severe reactions in sulfite-sensitive individuals such as severe chest pain, cardiac arrhythmias, gastrointestinal distress, neurological distress, and anaphylactic episodes do occur (Timbo and others 2004). Thus, the Center for Food Safety and Nutrition (CFSAN) developed Antimicrobial Resistance and Monitoring System (ARMS) to monitor food/illness/injury relationships and to monitor adverse reactions of people to food components. Subsequently, in 1986 the FDA banned the use of sulfating agents on fresh fruits and vegetables with the exception of potatoes and grapes (Tollefson 1988). A growing number of studies are also analyzing the link between pseudoallergic reactions to preservatives, specifically aromatic components and benzoates, and patients with chronic urticaria and angioedema (Colins-Wiliams 1983, Ortolani and others 1984, Zuberbier and others 2002, Nettis and others 2004, Arcella and others 2005). In addition, a number of mechanisms by which food preservatives such as nitrosamines may induce cancer have been proposed including: (1) impurities in the additive, (2) the additive may react with a food constituent forming a new carcinogenic compound (3) metabolic or fermentation products may convert the additive (Fairweather 1981).

Recently a number of studies have been conducted on herb and fruit seeds and their components seed oils and meals, and concluded that various herb and fruit seed extract have antimicrobial and/or antioxidant activity (Rauha and others 2000, Gill and others 2002, Ahn and others 2003, Chorianopoulos and others 2004, Dadalioglu and Evrendilek 2004, Ozkan and others 2004, Proetos and others 2006). Fruit seeds, a byproduct of the fruit processing industry, are readily available with 180,000 kg of caneberry seeds alone in 2003 (Bushman and others 2004). While generally discarded as waste, these seeds may be ground into seed meal or cold-pressed to separate the seed oils and flours. Utilizing seeds, seed meals, flours, and oils may benefit small fruit producers and processors by enhancing the value of otherwise waste products and enlarge their marketing capacity.

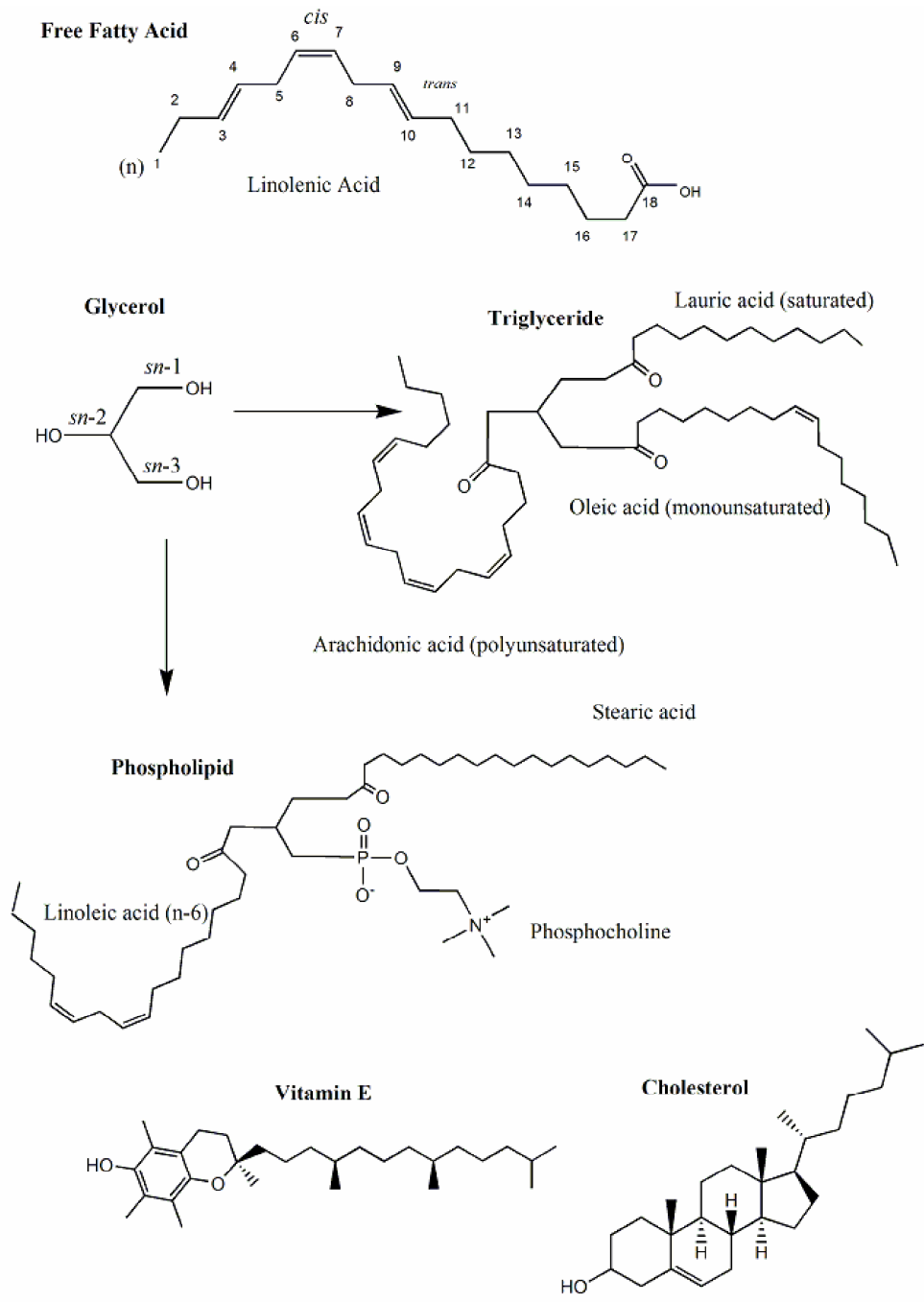
Interest in natural preservatives continues to increase as they are presumably safe as they naturally occur in foods that have been used for centuries. In addition, they may have anticarcinogenic effects and inhibit biologically harmful reactions *in vivo* (Frankel 1996). These naturally occurring antimicrobials and antioxidants are small hydrophobic organic biomolecules commonly found in spices and herbs. Spices and herbs are primarily used to enhance the palatability of foods, however some researchers state that the ultimate reason for using spices is that they help cleanse foods of unwanted microorganisms and thereby contribute to health. Most natural antimicrobial biomolecules contain aromatic structures similar to those in chemical preservatives; and complete mechanisms of antibiotic resistance remains elusive.

The potential use of these natural preservatives may assist in the development of novel mild preservative regimes tailored to the organoleptic quality needs of specific natural food products. Consumers are requiring higher quality products that are appetizing, minimally processed, and preservative free, with an extended shelf-life. (Brul & Coote 1999)

### 1.2.0 Lipid Structure & Function

#### 1.2.1 Lipids and Food Quality

Food quality may be measured by its nutritive value and consumer acceptance. Lipids play a pivotal role in nutrition as a general source of energy, and carriers of lipophilic, biologically active compounds, as well as providing essential fatty acids not produced *in vivo* by humans. Lipids are important factors in consumer acceptance in most products. They have the ability to affect organoleptic properties, emulsify lipophilic agents such as carotenoids to enhance the color of foods as well as agents that contribute to the aroma of a product (Brown 2000). Lipids that have been exposed to light, oxygen, pressure, and temperature may oxidize, altering sensory properties, producing off-odors and flavors, discolorations, and poor texture (Sikorski and Kolakowska 2003).



**Figure 1: Selected Fatty Acids & Structural Components of Common Lipids in Food**

### 1.2.2 Chemical Structure of Food Lipids

Lipids are generally hydrophobic molecules primarily composed of carbon, hydrogen, and oxygen. Physically they are insoluble in water and soluble in organic solvents (Fahy and others 2005). Fatty acyls, also known as free fatty acids, have a terminal methyl group and terminal carboxyl group and are best described by their hydrocarbon chain length, number and position of double carbon to carbon bonds, and location within a glyceride molecule. In practice, many are known by their trivial names, however the International Union of Pure and Applied Chemistry (IUPAC-IUB 1977) notations or formula descriptions, describing the chain length and number of double bonds separated by a colon, are also commonly used. Specifically, the IUPAC nomenclature describes the first carbon a double bond exists at in unsaturated FA by the addition of n- the carbon number counting from the methyl end. (Nichols and Sanderson 2003 & Scrimgeour 2005)

Triglycerides, a glycerol backbone with three acylated fatty acids, comprise 95% of all edible lipids (Roche 1999). As illustrated in Figure 1, phospholipids differ from triglycerides with the addition of a phosphorous-containing compound between one of the fatty acids and the glycerol backbone while other structural lipids have large ring-based structures, such as sterols. Sterols are important constituents of hormones, cholesterol, bile, natural antioxidants such as tocopherols and tocotrienols, and pigments such as carotenes and chlorophyll (O'Brien 2004).

### 1.2.3 Degree of Saturation & Geometric Configuration of FA

Degree of saturation refers to the number of double bonds in a fatty acyl and may be present in either the cis or trans configuration. Double bonds are also described by their location on the fatty acid chain. Polyunsaturated fatty acids (PUFA) are often grouped into n-3, n-6, and n-9 classes according to the carbon at which the first double bond is located from the methyl end. These classes are important as although the number of double bonds in these classes may differ. Lipids sharing the same location of the first double bond often have similar health related properties. For example, n-3 LCPUFA in part regulate eicosanoid production and are therefore clinically important in the immune system for regulation of autoimmune and inflammatory diseases in humans (Harbige 2003, Rees and others 2006).

Varying degrees of saturation (illustrated in Figure 1) such as the structures of lauric acid, oleic acid, and arachidonic acid illustrate saturated fatty acids (contain no double bonds), monounsaturated fatty acids (MUFA) (one double bond), and polyunsaturated fatty acids (PUFA) (more than one double bond) respectively. Fats of animal origin contain larger quantities of saturated fatty acids. These pack together tightly and maintain a solid state at ambient temperatures. Oils from plant sources typically have a greater number of double bonds and therefore they are more aqueous at ambient temperatures (with a few exceptions such as avocado (Brown 2000)). Typically, lipids from any source will consist of a large number of different fatty acids which may vary significantly depending on the species, growing conditions, and environmental factors for plants, as well as diet, health,



maturity, and fat sample location in animals (O'Brien 2004). For example, Menhaden oil (*Brevoortia tyrannus*) from fish is composed of at least 44 different fatty acids (Stansby 1990).

Configuration about the double bond, identified as *cis* or *trans*, also alters the physical properties of lipids. As illustrated by conjugated linoleic acid (CLA) in Figure 1, a single fatty acid may have both types of double bond. *Cis* conformation, with alkyl group on the same side of the double bond induces a more pronounced bend in the chain, loosening fatty acid arrangement. Fatty acids in the *cis* conformation are not as thermodynamically stable as *trans* and subsequently have a lower melting point. The *cis* configuration is also more readily produced naturally; however, *trans* fats are also found in nature. Vaccenic acid and rumenic acid which have been found in dairy fats are considered natural although they may be the result of bacteria modification (Scrimgeour 2005). *Trans* fatty acids are unsaturated fatty acids with the alkyl groups on opposite sides of the double bond inducing a small change in shape and therefore the melting-point of such fatty acids is similar to its saturated counterpart (Nawar 1996). *Trans* fatty acids are generally geometrical isomers that originate from hydrogenation, the process of reacting hydrogen gas with the *cis*-double bonds in the carbon chain, or a thermally (temperatures above 200°C) induced radical mechanism (Wolff 1993). This process of isomerization is discussed in further detail in Section 1.4.0.

### 1.2.4 Fatty Acid Chain Length

Most edible fatty acids are straight chain aliphatic carboxylic acids which vary in length from 4-22 carbons, with 18 carbons being the most prevalent in edible fats and oils (Scrimgeour 2005). Short chain fatty acids from hydrolytic rancidity may contribute to undesirable rancid flavor and off-odors, as well as desirable flavors in some aged cheese products and fermented yogurt and breads (Nawar 1996). Long chain fatty acids (LCFA) are essential for many cellular functions including structural and physiological integrity of cell membranes, and eicosanoid production.

Fatty acid chain length and degree of saturation also influence their arrangement in a triglyceride. LCPUFAs such as linoleic acid, are preferentially located at the *sn*-2 position of a triglyceride. Saturated fatty acids occur primarily in the *sn*-1,3 positions with the exception of some animal fats, particularly fat from swine (Nawar 1996). Overall melting behavior of triglycerides is dependent on fatty acid composition.

### 1.2.6 Sensory Properties

Lipids function in foods in a variety of ways to influence aroma, texture, appearance, and mouth-feel. Lipid degradation products during preparation and storage impart distinctive taste to foods, such as bacon, olive oil, fish and cheeses while in fish, the fresh fish smell is lost as PUFA's enzymatically and microbially are degraded into short chain FA's and alcohols signifying spoilage (Brown 2000). In contrast, the desirable flavor of some cheeses is developed by lipid

oxidation of short chain FA's into ketones and aldehydes (Kolakowska and Sikorski 2003). Physically, the type and quantity of lipid included in food products alters texture. Lipids are commonly described as fats or oils. Typically, they are distinguished from one another by their physical state at ambient temperature with fats being solid at ambient temperatures and oils being liquids. In processed foods they may be incorporated into flour mixtures where they act as a tenderizer, add volume, structure, flakiness, and play a critical role in staling (Brown 2000). Lipids also improve the appearance of foods by providing color by carrying lipid soluble color compounds such as carotenes (yellow-red), chlorophylls (green), and gossypols (yellow) and can be added to the top of baked goods to prevent moisture loss and add sheen (Brown 2000, Nichols and Sanderson 2003). Lastly, unique melting points, plasticity, and solubility alter the overall mouth-feel of products such as ice-cream, making effective fat substitutions difficult.

### 1.3.0 Health Benefits and Risks of Lipid Intake

#### 1.3.1 Caloric Content & Recommendations

Nutritionally, lipids offer a dense, easily stored source of energy providing 9 kcal/g while proteins and carbohydrates provide only  $\cong 4$  kcal/g. Fat in the diet also aids in the transport of fat soluble vitamins and other less polar components such as cholesterol. In the United States however, increased caloric consumption is contributing to obesity and other chronic diseases. Therefore, the *Dietary*

*Guidelines for Americans 2005*, recommends that Americans consider the following regarding lipid intake:

- *“Keep total fat intake between 20 to 35 percent of calories, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts, and vegetable oils.”*
- *“Consume less than 10% of calories from saturated fatty acids and less than 300 mg/day cholesterol.”*
- *“Look for foods low in saturated fats, trans fats, and cholesterol to help reduce the risk of heart disease (5% DV or less is low, 20% DV or more is high).”*
- *“Most of the fats you eat should be polyunsaturated and monounsaturated fats.”*
- *“Keep trans fatty acids consumption as low as possible.”*

### 1.3.3 Lipids and Disease Risk

Lipid quantity and type are important factors in determining the health beneficial or health deleterious effects. Data from the US National Health and Nutrition Examination Surveys (NHANES II & III) indicate that excessive energy intake is a major contributor to the increase in average body weight over the last 20 years. 2004 Center for Disease Control and Prevention (CDC) data observing obesity trends in the United States indicates 7 states with an obesity prevalence of 15-19%, 33 states with 20-24% and 9 states with more than 25% based on body mass index (BMI) data. (Harnack and others 2000) Obese and overweight persons

have substantially raised risks of morbidity and mortality from hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and respiratory problems, and cancers of the endometrial, breast, prostate, and colon (NIH 1998).

Certain lipids have been associated with disease, while others are essential for optimal health. Excessive intake of energy rich saturated fats, *trans* fats, and cholesterol increase the risk of high lipid levels in the blood contributing to cardiovascular vascular disease (CVD) (Caterina and others 2006). Saturated FA's increase total plasma cholesterol and decrease low density lipoprotein (LDL) receptor-mediated catabolism, thereby increasing LDL cholesterol. In contrast, shorter chains FAs also slightly increase high density lipoproteins (HDL) (Woodside and Kromhout 2005). Trans-fats have also been shown to elevate LDL and lower HDL cholesterol. Trans-FA's in adipose tissue is also associated with myocardial infarction (Clifton, Keogh, and Noakes 2004). In addition, trans-fats increase intestinal permeability, which may improve intestinal absorption. This could lead to adverse effects such as the promotion of cancer cell invasion and metastasis. Improved intestinal permeability is possibly the mechanism by which incidence of allergenic rhinitis, atopic diseases, and asthma are positively associated with trans-FA intake (Roche HM et. al. 2001, Stender and Dyerberg 2003).

In contrast, some FAs have health beneficial effect whereby a 2004 FDA bulletin now allows a qualified health claim for olive oil stating:

*“Limited and not conclusive evidence suggests that eating about 2 tablespoons (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day.”*

PUFA, specifically essential fatty acids are required to prevent essential fatty acid deficiency, characterized by retarded growth, dermatitis, kidney lesions, and premature death (Gropper, Smith, & Groff, 2004).

### 1.3.2 Polyunsaturated Fatty Acids

Essential polyunsaturated fatty acids (PUFA) are FAs that can not be synthesized in the human body and are therefore required from dietary sources. The principle essential fatty acids are linoleic acid (n-6) and alpha-linolenic acid (n-3). LCPUFA such as arachidonic acid (AA), eicosapentanoic acid (EPA) and docohexanoic acid (DHA) may be considered essential under specific conditions of inadequacy or infancy (Newton 2001, Yehuda and others 2005). PUFA's are important to health as they have been reported in research to prevent and treat coronary heart disease, hypertension, diabetes, arthritis and inflammation, autoimmune disorders, and some cancers, and are required for normal growth and development (Stansby 1990, Raatz and others 2001, Wijendran and Hayes 2004, Zamaria 2004). Consumption of adequate amounts of PUFA is especially important in maternal and infant nutrition as DHA and AA are essential for proper

and full phospholipid membrane development in the retina and brain, especially during the first two years of life (Newton 2001). Increasing fish intake is the most obvious way to increase n-3 PUFA intake. Fish oil contains a plentiful supply of n-3 fatty acids such as EPA and n-6 fatty acids such as DHA, both highly unsaturated and essential fatty acids (Roche 1999).

Optimal EFA intake is also dependent upon n-3/n-6 fatty acid intake ratio. This ratio is important to balance AA and EPA which determine eicosanoid type and efficacy which regulate thrombosis as well as immune and inflammatory response (Roche 1999, Harbige 2003). The n-6/n-3 FA ratio was close to 1:1 for those living in the Paleolithic period 40,000 years ago. Through the agricultural revolution and diet changes rising consumption of n-6 FA through margarine, salad dressing, mayonnaise, nuts, and cooking oils have altered the ratio of n-6 to n-3 FA's to approximately 10:1 in the United States today. (Simopoulos 2004, Kris-Etherton et.al. 2003, Miljanovic 2005). The approach to rebalancing this ratio lies in decreasing n-6 intake while increasing total n-3 FA intake as well as EPA and DHA.

Several ratios of n-6/n-3 have been proposed for optimal health. Wijendran and Hayes (2004) suggested that an adequate intake (AI) of LA is 6%, LNA 0.75%, EPA 0.25% and DHA 0.5% of the caloric diet for healthy adults. This corresponds to a 6:1 ratio of n-6/n-3 fatty acids. The International Society for the Study of Fatty Acids and Lipids suggested a smaller ratio of 2:1 n-6/n-3 with a total caloric consumption of LA of only 2-3% and LNA 1% (Wijendran and Hayes 2004). Similarly, a n-6/n-3 ratio of 1:2.3 was suggested by Kris-

Etherton with an additional 0.7g EPA and DHA combined per day (2000). The adequate intake for females age 19 to 30 in the U.S. 2005 Dietary Guidelines for Americans list the USDA food guide for LA at 18g/day and 1.7g/day of ALA, slightly higher than the Institute of Medicine recommendations of 12 and 1.1g/day respectively (2005).

The current AI recommendation from the U.S. Dietary Reference Intakes for Energy and Macronutrients is 4.4 – 17 g/d n-6 FAs and 0.5 – 1.6 g/day n-3 FAs depending on life stage group (Institute of Medicine 2005). This intake may be accomplished by the American Health Association Dietary Guideline of two fish meals per week and use of ALA containing vegetable oils (Kris-Etherton 2000). The actual U.S. intake of EPA and DHA combined is ~ 10 times less than the recommended AI, however, it is important to note that deficiency symptoms for both n-3 FAs and n-6 FAs are nonexistent in the U.S. although current intake does not meet the AI (National Institute of Medicine 2005).

Supplements are available to help consumers increase their EFA and PUFA intake. The fish oil capsules in the US market today may provide only 180 mg EPA and 120 mg DHA per capsule (Kris-Etherton and others 2000). Without additional n-3 FA in the diet, three capsules would therefore be required to meet the 1g n-3/d suggested intake for Coronary Heart Disease (CHD) patients while 1.5-3g n-3/d have shown beneficial (Kris-Etherton 2003). A balanced intake has been shown to improve health by; reducing the risk of dry eye syndrome in women, reduced lung cancer cell invasion, helping to maintain bone mineral



density, preventing atherosclerosis, and regulation of inflammation. (Weiss et al. 2005, Xia et. al. 2005, Yamashita et. al. 2005)

### 1.4.0 Lipid Oxidation

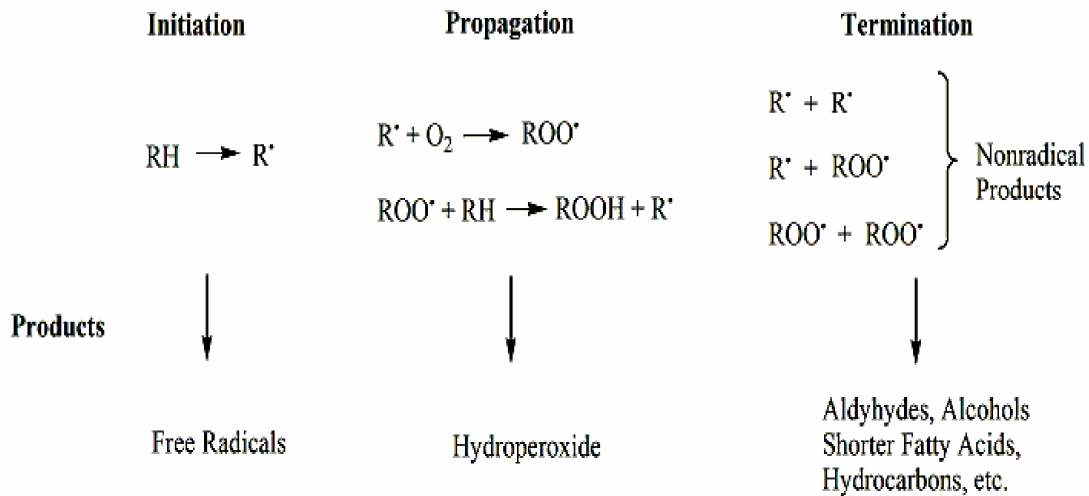
#### 1.4.1 Chemical Mechanism of Lipid Oxidation

When lipids oxidize they alter sensory properties of a product, even if they constitute only a small percentage of the product (Kolakowska and Sikorski 2003). Unsaturated FAs are particularly subject to free radical oxidation and their secondary volatile aldehyde byproducts may cause off-flavors at concentrations lower than 1 ppm (E. N. Frankel 1982). Rancid products are thus less acceptable to consumers and have decreased nutritional value. Lipids may be oxidized both enzymatically and nonenzymatically, however, the primary mechanism involved in lipid oxidation is “autoxidation” which is the reaction of a lipid with molecular oxygen (Nawar 1996).

Rate of oxidation is mediated by the number, position, and geometrical configuration of double bonds, and FA attachment to a glycerol (Nawar 1996). Oxidation of lipids occurs at double bonds and adjacent allylic carbons (Scrimgeour 2005). Bond configuration influences reactivity as *trans* is more stable than *cis*, and unconjugated double bonds are more stable than conjugated. Free fatty acids oxidize more rapidly than those esterified to a glycerol. External factor that affect the rate of autoxidation through a free radical mechanism are: (1) chemicals known to decrease the rate of other well-established free radical reactions, (2) the catalytic effect of light and free-radical producing substances,

(3) increased quantities of hydroperoxide, and (4) longer induction periods correlated with pure substrates. (Nawar 1996)

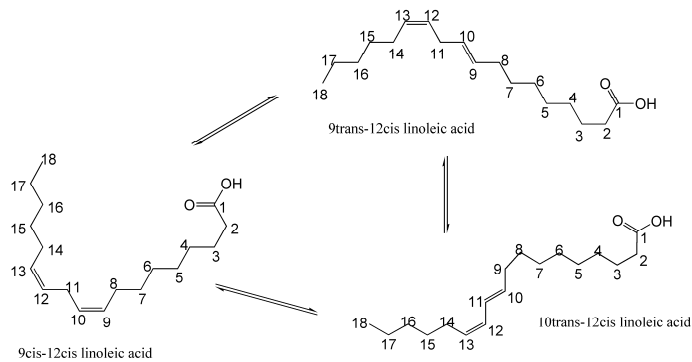
Free radical oxidation is defined as a three stage process of initiation, propagation, and termination of radical reactions (Fig.2). The initiation step includes the initial reaction of a lipid with a free radical that is present in the food matrix or in vivo such as transition metals, light, reactive oxygen species (ROS), reactive nitrogen species (RNS) or irradiation. A lipid free radical then begins the propagation phase. During propagation unstable hydroperoxides, the primary products of lipid oxidation, are formed with the addition of oxygen. Thus, new lipid radicals are continually generated. This process of propagation continues until two peroxide radicals react or when a peroxide reacts with an antioxidant terminating the sequence of new lipid radical formation and forming nonradical secondary products. (Kolakowska 2003, Huang and others 2005)



**Figure 2: Mechanism of Lipid Autoxidation with primary and secondary products.**

## 1.4.2 Isomerization of FA

Isomerization is a change in double bond position or geometry while retaining the same chemical formula. Double bond position changes influence the number carbon(s) at



**Figure 3: Isomerization of Linoleic Acid**

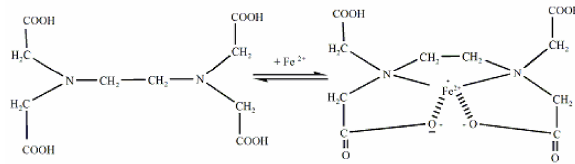
which the double bonds take place however the overall number of double bonds in the fatty acid remains constant. Figure 3 demonstrates positional changes as the double bond moves from between carbons nine and ten to between carbons ten and eleven. This Figure also demonstrates geometric configuration changes as the 9*cis*-12*cis* linoleic acid under certain conditions may be changed to 9*trans*-12*cis* linoleic acid.

Geometrical isomerization begins with lipid oxidation. Lipid oxidation may be triggered by light, transition metals, hydroperoxide breakdown or pigments (Wolff RL 1993). In the absence of the double bond a single oxygen is formed on the unsaturated carbon(s), creating a free radical and a partial double bond which stretches across the five carbon atoms where a triplet oxygen atom may then attack at either outside carbon. The intermediate may then re-form into its original *cis* configuration, or it's more stable *trans* isomer once again leaving a

free radical on the bridge carbon to which a hydrogen may bond (Wolff et al, 1996). This process is beneficial for producing stable frying oils and lipid products to provide texture in foods.

### 1.4.3 Available Autoxidative Preservatives

The most common additives used to prevent lipid oxidation are free radical scavengers which function by

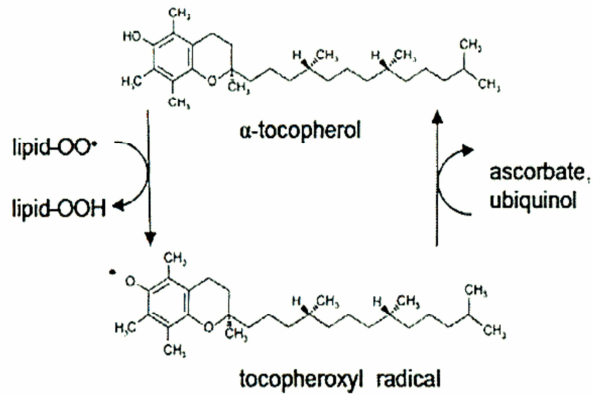


**Figure 4: Chelating capacity of EDTA**

quenching reactive radicals produced in the initiation and propagation steps of oxidation (Scrimgeour 2005). Two classes of preservatives are chelating agents and antioxidants. Chelating agents, also known as sequestrants, work by forming complexes with metal ions that

would otherwise aid in reactions that produce discoloration, oxidative rancidity, turbidity, and flavor changes in food products (Lindsay 1996).

Ethylenediamine tetracetic acid (EDTA) salts, citric acid, and phosphates are the most commonly used chelating agents in food products



**Figure 5: The oxidation and reduction of alpha-tocopherol** (Tucker and Townsend 2005).

(Lindsay 1996). As illustrated by iron (Fe) in figure 4 transition metals are bound to the chelating agent which may make important micronutrients unavailable in the diet if used extensively.

One common group of antioxidants is vitamin E which includes eight fat soluble isomers which are comprised of functional methyl group(s) on a chromane ring with a phytyl tail. Of the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  isomers of vitamin E, alpha-tocopherol is considered the most effective lipid-soluble antioxidant and has demonstrated greater antioxidant properties than other tocopherols (Singh and others 2005, Tucker and Townsend 2005). Figure 5 illustrates alpha-tocopherols ability to quench lipid oxidation by forming the tocopheroxyl radical, which may then be converted back into alpha-tocopherol by reducing agents such as ascorbic acid (Tucker and Townsend 2005). It is important to note that continual increase does not increase activity as the capacity of alpha-tocopherol to inhibit lipid oxidation was studied at concentrations from 25 to 1500  $\mu\text{mol}$  alpha-tocopherol/kg rapeseed oil triglyceride and concluded and the antioxidant capacity increased to a concentration of 100 $\mu\text{mol}/\text{kg}$ , however subsequent increases may decrease antioxidant efficacy (Ohm and others 2005). In addition, some studies suggest that  $\gamma$ -tocopherol is a more effective free radical scavenger and a mixture of tocopherols is more effective at inhibiting lipid oxidation in human erythrocytes than  $\alpha$ -tocopherol alone, so antioxidant effect may vary in food products versus *in vitro* (Wolff 1996 and Liu 2002).

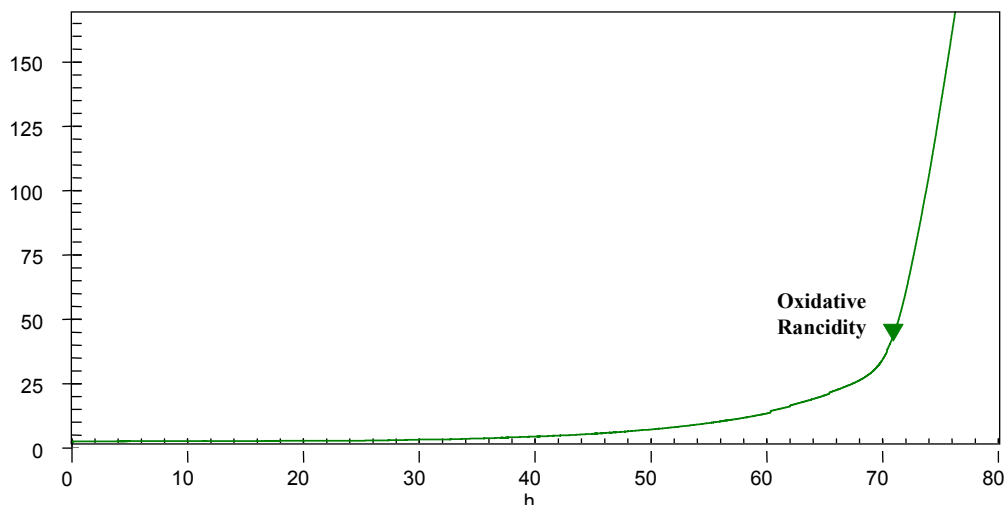
### 1.5.0 Relevant Analytical Methods

#### 1.5.1 Extraction Methods

Three types of extraction methods: continuous, semi-continuous and discontinuous are used to extract fat from foods. The Goldfish method is a continuous method and is preferable for rapid, efficient extraction. It is conducted with solvent in a boiling flask continuously flowing over food material held in a ceramic thimble. Fat content is calculated as the mass lost from the dried sample, or by the weight of the fat extracted in the solvent. The Soxhlet semicontinuous extraction method utilizes a siphon arm and condenser over solvent in a boiling flask so that the solvent builds up, surrounding the sample packed thimble for 5-10 minutes before siphoning back down into the flask for evaporation. This soaking effect avoids the channeling found in continuous methods, however it is time consuming. Samples used for these analyses must be pre-dried to allow for adequate solvent penetration, must be reduced in particle size to allow lipids to move out of the food matrix, and must be acid hydrolyzed to release lipids from proteins and carbohydrates. Lastly, the Mojonnier discontinuous milk fat method consists of a series of treatments and solvent being added to the sample in a Mojonnier fat extraction flask. The extracted fat is then dried to a constant weight and expressed as percent fat by weight. Soxhlet and Mojonnier methods are the most commonly used organic solvent methods using hexane, ethyl ether, and petroleum ether for dried and moist samples, respectively. Other instrumental methods such as infrared, ultrasonic, colorimetric, and density methods are also used for specific foods. (Min and Boff 2003)

### 1.5.2 Measuring Lipid Oxidation

Several methods of analysis have been developed to measure the changes in lipids as they undergo lipid oxidation and lipolysis (the hydrolysis of fatty acids from a glyceride molecule). Among the available analysis are: peroxide value, *p*-aniside value, volatile organic compounds (VOC), thiobarbituric acid reactive substances (TBARS), and oil stability index (OSI) (Pike 2003). These analyses measure either the primary lipid oxidation products, hydroperoxides, or the secondary products including aldehydes, ketones, organic acids, and hydrocarbons. Peroxide value is a measurement of primary lipid oxidation products. *P*-aniside, VOC, and TBARS measure the secondary products aldehydes, headspace volatile lipid oxidation products, and malonaldehydes respectively. These tests are conducted under normal utilization and storage of products. In contrast, OSI is an accelerated test which increases the rate of lipid oxidation by the addition of catalysts oxygen and heat, and possibly light, transition metals, and/or enzymes. OSI measures the length of time before a lipid sample is detectibly rancid (induction time) by measuring acidic volatiles (Figure 6). A Rancimat will then record the time it takes for the sample to become rancid by measuring the conductivity of the volatiles in distilled water. This is an effective method for analyzing antioxidant capacity for the prevention of lipid oxidation; however it should not be assumed that the reactions occurring under these conditions are exactly the same as those that would occur under actual storage conditions. (Pike 2003)



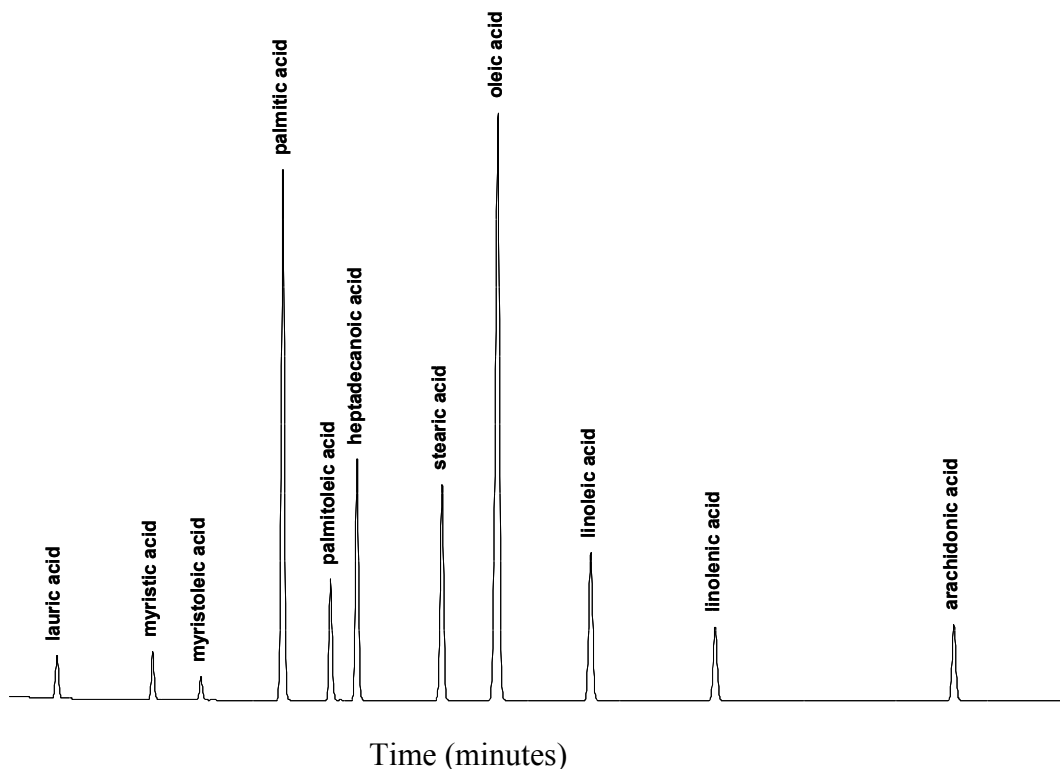
**Figure 6: Oxidative Stability Index Curve**

### 1.5.3 Fatty Acid Analysis by Gas Chromatography

The unique properties of individual fatty acids, their role in the food matrix, and impact on human health require the quantification of individual fatty acids. Gas chromatography (GC) is the preferred method to analyze fatty acid composition. Fatty acid composition is quantified by extracting lipids from a food matrix and converting them to fatty acid methyl esters (FAMES) to increase their volatility for GC analysis. Several AOAC approved methods for are available for specific oils. FAMES are saponified triacylglycerols and phospholipids to form methylated fatty acids (FAME). Gas is used to separate fatty acids at elevated temperatures through a column (Fig.7). The peaks in the chromatograph are compared to known standards to identify each fatty acid component and the area under the peak used to calculate the amount of individual fatty acids in the sample. (Pike 2003) It should be noted however that not all *cis*- and *trans*- isomers have been successfully separated although the geometrical



isomerization of some fatty acids such as  $\alpha$ -LA have been investigated (Wolff 1993).



**Figure 7: Gas chromatograph of fatty acids found in Menhaden fish oil**

#### 1.5.4 Radical Scavenging Capacity Assays and TPC of Foods

Multiple assays to measure the radical scavenging activity,  $\text{Fe}^{++}$  chelating capacity, and total phenolic content have been proposed for food analysis. Antioxidant activity is generally correlated with the quenching of free radical species or free radical initiators by either proton or electron transferring reactions. Hydrogen atom transfer reaction assays measure antioxidants that can donate a

hydrogen to quench the free radical while converting to a semi-stable oxidized state themselves. These reactions include but are not limited to: oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), inhibited oxygen uptake (IOU), inhibition of linoleic oxidation, and inhibition of low density lipoproteins (LDL) oxidation. (Liu and others 2002, Ozkan and others 2004, Cushnie and Lamb 2005, Huang and others 2005) In general, free radical quenching by antioxidants that donate a hydrogen are measured kinetically by the reduction of a generated free radical over time. In contrast, electron transfer reactions including but not limited to: trolox equivalent antioxidant capacity (TEAC), ferric ion reducing antioxidant parameter (FRAP), copper (II) reduction capacity, diphenyl-1-picrylhydrazyl (DPPH), and total phenolic content measured utilizing Folin-Ciocalteu reagent are generally measured at a specific endpoint. In these assays the antioxidant is generally oxidized, reducing an agent within the solution whereby the change in absorbance of the solution versus the antioxidant concentration generates a linear correlation by which the proportional amount of unknown antioxidant may be determined, provided that the antioxidant capacity is the same as the reduce capacity of the tested material. Other reactive oxygen and nitrogen species (ROS/RNS) such as: superoxide anion ( $O_2^{\cdot -}$ ), hydrogen peroxide ( $H_2O_2$ ), peroxy radicals ( $ROO^{\cdot}$ ), hydroxyl radical ( $HO^{\cdot}$ ), singlet oxygen ( $^1O_2$ ), and peroxynitrite ( $ONOO^{\cdot}$ ) are also biologically relevant and have individual assays, however similar to the hydrogen atom transfer and electron transfer reactions, multiple assays are required to measure the antioxidants true capacity against the different free radicals.

Important for this experiment are antioxidants, generally hydrogen donating molecules such as vitamin E, capable of combating peroxy radicals to prevent lipid oxidation. (Huang and others 2005)

### 1.6.0 Introduction to Food Microorganisms

#### 1.6.1 Microbial Related Food Quality Concerns

In 1995, 27% of the United States edible food supply was wasted partially due to foods discarded due to spoilage. While fresh fruits and vegetables were responsible for the majority, 6.8 billion pounds or 7.1% came from fats and oils while an additional 8.5% was from meat, poultry, and fish - all products subject to lipid oxidation and microbial degradation and spoilage (Kantor et. al., 1997). This amounts to an estimated economic loss of \$31 billion in foods annually (Kaferstein 2002). A reduction in food losses due to spoilage is therefore critical to maintaining an abundant food supply for a growing population.

#### 1.6.2 Microbial Related Food Safety Concerns

“Safe food” means foods are free from biological contamination, hazardous chemicals, and injurious physical objects. In the United States, the Centers for Disease Control and Prevention (CDC) estimates that foodborne diseases are responsible for approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths annually (1999). Among the 931 estimated annual deaths associated with foodborne transmission, bacteria are responsible for

72%, parasites 21%, and viruses 7%. Six pathogens in particular: *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *E. coli* 0157:H7 (3%), are responsible for >90% of food-related deaths (Mead and others 1999).

### 1.6.3 Classes of Microorganisms

The study of microbiology encompasses bacteria, algae, fungi, viruses, and protozoa. Bacteria are small, single cell membrane bound organisms which lack a true cell nucleus. Common distinguishers in bacteria are cell wall and shape. Gram-positive bacteria are bacteria with cell walls, and gram-negative bacteria lack cell walls. Two of the most common shapes are cocci (round) and rod shape. In contrast, some algae may be single-celled while others are larger multicellular organisms, however, all algae have a well defined cell nucleus and numerous membrane-bound intracellular structures some of which are capable of photosynthesis to produce energy internally. Fungi, like algae, may be small and single-celled, or macroscopic multicellular organisms with the primary difference being that fungi are stationary and algae are motile. Viruses are the smallest organism studied and are comprised of only nucleic acids and a few proteins. In order to replicate they must use a host cell, however some may remain dormant outside of a host. Protozoa are sometimes considered animal like and are heterotrophic, mostly motile, unicellular organisms with a true nucleus, membrane-bound organelles, and a pellicle for protection. Most microorganisms perform beneficial activities for humans and the environment, however some are

pathogenic and their presence requires special attention as they may induce unwanted spoilage and/or disease if present in foods. (Black 1996)

### 1.7.0 Bacteria in Food Products

#### 1.7.1 Fermented Food Products and Probiotics

Generally, microbes in food products are generally a concern however food fermentation and probiotics actually use microbes in foods intentionally. Fermented food products have been traced back in human civilization 7,000 years to ancient Babylon (Battcock and Azam-Ali 1999). In contrast, the use of probiotics goes back only to 1974 in animal husbandry. Since then interest has continued to grow as 60 literature reviews were published in 2005 and legislation is pending to regulate probiotic related health claims (Montville and Winkowski 1997, Marco and others 2006). “Fermentation is the slow decomposition of organic substances by micro-organisms or complex nitrogenous substances (enzymes) from plants or animals (Battcock and Azam-Ali 1998).” Fermented foods come from a variety of sources including fruits and vegetables, dairy, meat, poultry and fish, as well as grains in different areas of the world. In addition to simple preservation, fermented products; reduce waste due to food spoilage, impart flavor, have medical usage, and provide jobs and are important to many countries economy (Battcock and Azam-Ali 1999). Probiotics are a subset of fermented products which by definition are, “living micro-organisms, which, upon ingestion in sufficient numbers, exert health benefits” (Ezendam and Loveren 2006). There are multiple health benefits from probiotic consumption

including; the prevention or amelioration of diarrhea, increase disease resistance, and beneficial effects on gastrointestinal diseases, ulcerative colitis, lactose malabsorption, and allergies have been proposed although the complete mechanism(s) are not fully understood (Chermesh and Eliakim 2006, Ezendam and Loveren 2006, Bai and others 2006). Data suggests that probiotic bacteria compete with pathogenic bacteria for nutrients and adhesion altering the intestinal micro-flora (Chermesh and Eliakim 2006). Some probiotics belonging to the Lactobacilli family produce bacteriocins and lactic and acetic acid which act as antibiotics against pathogens (Chermesh and Eliakim 2006). Others found that administering *Bifidobacterium longum* inhibited the activation of NF- $\kappa$ B, the same mechanism as other effective synthetic drugs used to reduce inflammation in ulcerative colitis and related diseases (Bai and others 2006). A prebiotic effect using fructo-oligosaccharides that increased fecal *Bifidobacteria* and indicated reduced disease activity was also proposed (Korzenick and Podolsky 2006). To date, the basic requirements for probiotics is that (1) they be effective in that they are not digested by enteric or pancreatic enzymes, gastric acid and bile (2) they have the ability to prevent the adherence, establishment and/or replication of pathogens in the gastrointestinal tract, and (3) they are safe for consumption (Chermesh and Eliakim 2006).

#### 1.6.4 Intrinsic Growth Factors

Intrinsic growth factors are the inherent characteristics of a food that affect their microbiology. These include water-activity ( $A_w$ ), pH, oxidation-reduction

potential (Eh), physical structure, the presence of naturally occurring compounds that influence microbial growth, and compounds added as preservatives. The  $A_w$  of a food is defined as the vapor pressure of water in a food divided by the vapor pressure of pure water at the same temperature, thus the  $A_w$  of pure water is 1.0. The  $A_w$  of foods ranges from approximately 0.10 – 0.97 in crackers and fresh fruits respectively. This controls the type of microbial growth in a food item as the minimal  $A_w$  for most bacteria is 0.91, yeasts 0.88, and molds 0.80. Likewise the pH of food items ranges from approximately 1.9 to 7.6 in fruits and egg albumin respectively. The optimal pH range for most bacteria is 6.5 to 7.0, similar to the pH of most meat and dairy products, however fermented meat and dairy products have a lower pH due to acid production by bacteria. Oxidation-reduction potential is measured in millivolts (mV) and is expressed by the symbol Eh and ranges from +820 mV in  $O_2$  to -420 in  $H_2$ . Bacterial “aerobic” requirements are defined by (1) anaerobes which require a negative Eh value (2) microaerophilic which require a slightly negative Eh value (3) facultative anaerobes which grow in either a positive or negative Eh value and (4) aerobes which require a positive Eh, the presence of  $O_2$ . Thus, different bacteria grow preferentially in foods with Eh values that meet their oxygen requirements. The physical structure of foods such as the shell of an egg, fruit peel or rind, and animal skin also protect against microbial growth, however the freeze-thawing process ruptures food cells encouraging bacterial growth by spilling nutrients out of cell membranes and making them available for bacteria growth. Antibacterial compounds such as benzoic acid may occur naturally foods such as cranberries,

plums, prunes, and some spices such as cinnamon and cloves. Benzoic acid and sodium benzoate are also the first synthetic antimicrobial compounds permitted by the U.S. Food and Drug Administration for utilization in foods. (Davidson 1997, Ozkan and others 2004)

### 1.6.5 Extrinsic Growth Factors

Extrinsic growth factors are external factors that affect microbial growth in food including temperature, relative humidity, and atmosphere (gas composition). There are four general temperature growth categories of bacteria; psychrophiles, psychrotrophs, mesophiles, and thermophiles. These classes are based on their optimal growth at different temperatures. Psychrophiles grow at a range of 0°C to 25°C with optimal growth occurring at 15°C. Psychrotrophs such as *Listeria monocytogenes* can also slowly grow at 0°C with an optimal growth of 25°C, however they may also grow up to temperatures of 40°C. Mesophiles such as *Escherichia coli* grow at temperatures from 20°C to 45 °C while thermophiles grow at higher temperatures, generally 40°C to 70°C. The movement of gaseous water from foods to the air and vice versa is the second extrinsic factor that may be controlled to reduce microbial growth. The equilibrium relative humidity (ERH%) of air surrounding a product is related to the  $a_w$  of food products by the equation  $ERH\% = a_w \times 100$ . Atmosphere may be controlled to prevent microbial growth by altering the air pressure and composition. The reduction of air pressure from 1 bar for normal air to 0.3-0.4 bar in vacuum packaging reduces available oxygen inhibiting growth. Likewise, modified atmosphere packaging (MAP) and



modified storage atmosphere also reduce the available oxygen content to 2-5% and increase the carbon dioxide or nitrogen level to reduce the respiration rate of fresh produce and inhibit aerobic microbial spoilage. (Farkas 1997)

### 1.8.0 Methods of Food Preservation

#### 1.8.1 General Considerations

Food preservation depends on a microbes natural resistance, initial number of microorganisms, microbe growth rate, microbe-microbe interactions, cellular composition, and microbe status. Spore forming cells have an advantage in that spores may survive processes under which viable cells would be destroyed and thus are naturally resistant. The initial number of microbes may also be controlled in foods by preventing contamination. Logarithmic microbial growth may be decreased by proper storage conditions, and industrial hazard analysis critical control points (HACCP) should be implemented when possible to eliminate pathogenic bacteria. In some foods, such as fermented foods, bacteria will compete with one another and those that form byproducts that lower the pH may form an environment in food products that inhibits other bacteria from growing. In addition, not all bacteria are formed with the same defense mechanisms so resistance to antimicrobials under the same environmental conditions is different for different types and strains of bacteria. (Davidson and Harrison 2002) Lastly, the ability for a microbe to survive and proliferate during processing and storage is dependent upon its health status prior to treatment.

## 1.8.2 Physical Approach

Principal physical means of food preservation are accomplished by controlling temperature, available water, controlled atmosphere, canning and radiation. High temperatures may be used to pasteurize or sterilize food products. The aim of pasteurization is to kill the non-spore forming pathogenic bacteria, 99% spoilage bacteria, and inactivate enzymes. Sterilization is the elimination of all microbes; however commercial sterility requires that a product be free of pathogens and shelf stable and therefore dormant spores may still exist in the product. Refrigeration, temperatures of slightly above freezing (4°C), is suitable for short preservation periods, however bacteria may still slowly replicate and therefore freezing (-10°C) is required to preserve foods for longer periods of time. It is significant to note that while low temperatures slow bacterial growth, they do not eradicate bacteria and bacterial growth is stimulated upon thawing. (Montville and Matthews 2005).

Similarly, removing available water from a food source will slow the growth, but not kill existing bacteria. Several methods are currently in use to remove 90% or more water from food products including sun drying, high temperatures with controlled atmosphere, the addition of compounds such as sugar and salt that alter the osmotic pressure, and lyophilization (freeze drying). Controlled atmosphere is accomplished by reducing oxygen levels to 2-5% and increasing carbon dioxide levels to 8-10% to successfully slow the respiration rate of fresh fruits and vegetables as well as psychrotrophic spoilage bacteria. (Montville and Matthews 2005) Likewise, modified atmospheric packaging

reduces the air pressure to 0.3-0.4 bars, reducing the available oxygen required for fresh foods to respire. It is significant to note that low oxygen conditions such as controlled atmospheres, modified atmospheric packaging, and canning have a risk of anaerobic pathogens such as *Clostridium botulinum* and secondary safety measures such as low temperatures may be required. Canning is the most common methods of food preservation and is the first methods discussed to, if done properly; destroy present bacteria and their spores. Canning is accomplished by combining moist heat and high pressure. Recently irradiation technology has made ionizing radiation a useful method for eliminating bacteria. Gamma rays produced by cobalt-60 or cesium-137 penetrate further than nonionizing (UV) radiation and are bactericidal. (Black 1996)

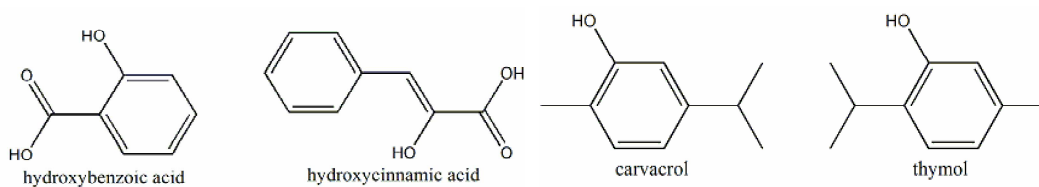
### 1.8.3 Chemical Approach

Chemical food preservatives added as antimicrobial ingredients are classified by the FDA as processing aids, secondary direct food additives, or direct food additives (Crozier-Dodson 2004). Most antimicrobial preservatives are bacteriostatic and inhibit bacterial growth while some are bactericidal and kill present bacteria. In 1991 37.5 million kg of synthetic antioxidants were used in the United States alone with an estimated increase to 47.3 million kg in 2000 comprised primarily of propionates, sorbates, and benzoates. This, despite consumer trends favoring “natural” foods containing fewer “chemical additives. Therefore, some fruits and spices such as oregano, cilantro, green tea, cranberry, and grape pomace among others have been analyzed for naturally occurring antimicrobial activity (Gill and others 2002, An and others 2004, Liu and others

2004, Ozkan and others 2004). The challenge associated with natural preservatives is to add them in an effective dose without altering the organoleptic, sensory, and nutritional properties of food items. (Brul and Coote 1999, Davidson & Harrison 2002)

### 1.8.4 Phytochemicals as Natural Antimicrobials

Multiple studies have been conducted to analyze the effectiveness of natural antimicrobials, and to isolate specific phytochemicals responsible for their antibacterial or bacteriocidal effects. Dietary phytochemicals are classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds (Liu 2004). The majority of studies conducted on phytochemicals have attempted to correlate antimicrobial activity with phenolics, specifically phenolic acids and flavanoids (Basile and others 1999, Rauha and others 2000, Ahn and others 2004, An and others 2004, Ozkan and others 2004, Cushnie and Lamb 2005, Proestos and others 2005, Proestos and others 2006).



**Figure 8: Hydroxybenzoic & hydrocinnamic acids**

**Figure 9: Carvacrol and thymol, two antimicrobial monoterpenes**

Phenolic acids are classified as hydroxybenzoic acids and hydroxycinnamic acids (Figure 8). Terpenes, which are phenolic in nature, are the primary antimicrobials in essential oil of spices (Davidson 2002). Likewise, the antifungal, insecticidal, and antimicrobial efficacy of plant essential oils is dependent upon their chemical composition, specifically two isomeric phenolic monoterpenes carvacrol and thymol shown in figure 9 (Chorianopoulos and others 2004). There are likely multiple mechanisms by which different phenolic compounds inhibit growth. Studies suggests that the majority of mechanisms concern phenolic ability to disrupt the cytoplasmic membrane causing cellular components to leak out, deplete cells of energy, inhibit active transport of nutrients, and ATPase inhibition, as well the removal of protons (the breakdown products of organic acids inside the cell) can deplete the cell of energy (Brul and Coote 1999, Davidson 2002). Other research suggests that phenolic acids work as antioxidants through their oxidation reduction (redox) capacity which allows them to act as reducing agents, hydrogen donors, and quench oxygen radicals as well as their ability to chelate transition metals (Pietta 2000 and Fernandez and Lopez 2005). Further research in this area is required to identify natural sources from which the phenolic concentration required for bacteriocidal activity is not above the tolerable threshold level and would avoid significantly alter the sensory properties of food products (Brul and Coote 1999).

The second class of phenolics known for their antibacterial activity is flavanoids. The general structure of flavanoids and flavonoid classifications (flavonols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoids) are

depicted in figure 10 (Liu 2004). Flavanoids may work by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and interfering with energy metabolism (Basile and others 1999, Cushnie and Lamb 2005). In addition, flavanoids may inhibit the free radical process and chelate transition metals providing a preservative effect as well as potential pharmacological activity *in vivo* (Pereira and others 2003).

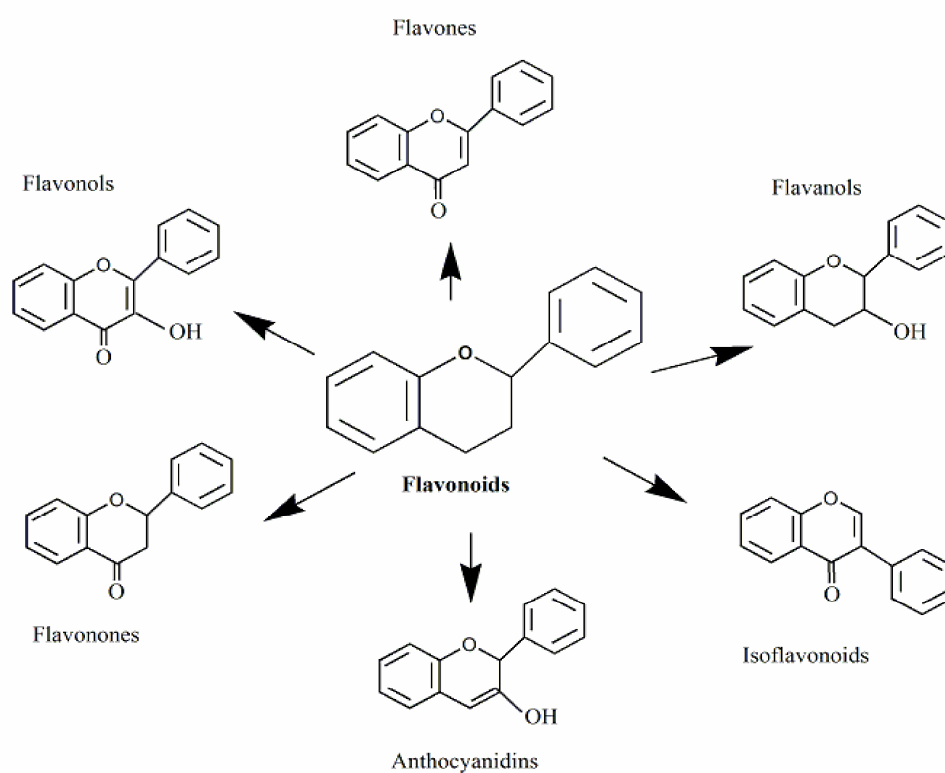


Figure 10: Class structure of selected flavanoids (Liu 2004)

### 1.8.5 Objectives to Identify Natural Preservatives

Multiple studies are necessary to determine the efficacy of a preservative. To analyze a protective effect against spoilage the inhibition of lipid oxidation in fish oil was analyzed, as well as the maintenance of health essential fatty acids to ensure nutrient preservation. Food quality and safety may also relate to the microbial load in a food product. Therefore bacteriocidal and antibacterial activity of both gram negative and gram positive species is important to analyze the capacity to avoid food-borne illness from pathogenic strains and accelerated spoilage from non pathogenic stereotypes. The activity of these two assays may be correlated to their radical scavenging and total phenolic content. Measuring these capacities may be helpful in identifying the mechanisms of food preservation and safety, as well as add to the beneficial effect that the addition of antioxidant based preservatives may provide the consumer. In addition, it is important that a preservative not alter the sensory properties of a product, specifically visibly, and therefore the impact of extract addition to fish oil will assist in the development of good natural antioxidants for target products. The data from this research may lead to novel natural food preservatives and improve the quality, safety, and nutritional value of food products.

## 1.9.0 References

- Ahn, J., Grun, I.U., Mustapha, A. Antimicrobial and antioxidant activities of natural extracts *in vitro* and in ground beef. *J. of Food Protection*. 67(1):148-155 (2004).
- An, B.J., Kwak, J.H., Son, J.H., Park, J.M., Lee, J.Y., Jo, C., Byun, M.W. Biological and antimicrobial activity of irradiated green tea phenolics. *Food Chemistry*. 88: 549-555 (2004).
- Arcella, D., Le Donne, C., Leclercq, C. Dietary exposure to chemicals within the process of risk assessment: possible applications to substances that may cause allergic reactions. *Proceeding of the Nutrition Society*. 64: 418-425 (2005).
- Bai, A.P., Ouyang, Q., Xiao, X.R., Li, S.F. Probiotics modulate inflammatory cytokine secretion from inflamed mucosa in active ulcerative colitis. *International Journal of Clinical Practice* 60(3):284-288 (2006).
- Basile, A., Giordano, S., Lopez-Saez, J.A., Cobianchi, R.C. Antibacterial activity of pure flavanoids isolated from mosses. *Phytochemistry*. 52:1479-1482 (1999).
- Battcock, M., Azam-Ali, S. (1999) Fermented fruits and vegetables. A global perspective. *Intermediate Technology*. Warwickshire, UK: FAO Agricultural Service Bulletin No. 134.  
<http://www.fao.org/docrep/x0560e/x0560e00.htm>. Access date March 20, 2006.
- Black JG. 1996. Scope and history of microbiology. In: Black JG, editor. *Microbiology principles and applications*, 3<sup>rd</sup>. Edition. Upper Saddle River, New Jersey: Prentice-Hall, Inc. P. 0-25.
- Brown, A. Fats and Oils. (2000) In: Brown, A., editor. *Understanding Food Principles and Preparation*. Belmont, CA: Wadsworth/Thomson Learning. P 187-207.
- Brul, S., Coote, P., Preservative agents in foods: Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology*. 50: 1-17 (1999).
- Caterina, R.D., Zampolli, A., Turco, S.D., Madonna, R., Massaro, M., Nutritional mechanisms that influence cardiovascular disease. *Am J Clin Nutr*. 83: 421-6 (2006).
- Chermesh, I., Eliakim, R., Probiotics and the gastrointestinal tract: Where are we in 2005? *World J Gastroenterol*. 12(6):853-857 (2006).
- Chorianopoulos, N., Kalpoutzakis, E., Aligiannis, N., Mitaku, S., Nychas, G-J., Haroutounian, S.A. Essential oils of *Satureja*, *Origanum*, and *Thymus*,



- species: chemical composition and antibacterial activities against foodborne pathogens. *J. Agric. Food Chem.* 52: 8261-8267 (2004).
- Clifton, P.M., Keogh, J.B., Noakes, M. *Trans* fatty acids in adipose tissue and the food supply are associated with myocardial infarction. *J. Nutr.* 134: 874-79 (2004).
- Crozier-Dodson, B.A. Carter, M., Zheng, Z., An overview of antimicrobial ingredients. *Food Safety Magazine.* December 2004/January 2005.
- Cushnie, T., Lamb, A.J. Antimicrobial activity of flavanoids. *Inter. J. of Antimicrobial Agents.* 26:343-356 (2005).
- Davidson P.M. (1997) Chemical preservatives and natural antimicrobial compounds. In: *Food microbiology: Fundamentals and frontiers.* Washington, DC: American Society for Microbiology. P 520-556.
- Davidson, M.P., Harrison, M.A. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. *Food Technology.* 56(11):69-78 (2002).
- Ezendam, J., and Loveren, H. Probiotics: Immunomodulation and evaluation of safety and efficacy. *Nutrition Reviews.* 64(1): 1-14 (2006).
- Fahy, E., Subramaniam, S., Brown, A.H., Glass, C.K., Merrill, A.H., Murphy, R.C., Raetz, C.R-H., Russell, D.W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., Meer, G., VanNieuwenhze, M.S., White, S.H., Witztum, J.L., Dennis, E.A. A comprehensive classification system for lipids. *J. of Lipid Research.* 46:839-861 (2005).
- Fairweather, F.A., Food additives and cancer. *Proc. Nutr. Soc.* 40:21-30. (1981).
- Farkas, J. (1997) Physical methods of food preservation. In: Doyle, M.P., Beuchat, L.R., Montville, T.J., editors. *Food microbiology: Fundamentals and frontiers.* Washington, DC: American Society for Microbiology. P 497-519.
- Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Perez-Alvarez, J.A., Kuri, V. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science.* 69:371-380 (2005).
- Frankel, E.N., Volatile lipid oxidation products. *Prog. Lipid Res.* 22:1-33 (1982).
- Frankel, E.N., Antioxidants in lipid foods and their impact on food quality. *Food Chemistry.* 57(1): 51-55 (1996).
- Gill, A.O., Delaquis, P., Russo, P., Holley, R.A. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *International J. of Food Microbiology.* 73(1):83-92 (2002).
- Gropper, S.S., Smith, J.L., Groff, J.L. 2004. Lipids. In: Gropper, S.S., Smith, J.L., Groff, J.L., editors. *Advanced Nutrition and Human Metabolism.* Belmont, CA: Thomson Wadsworth. P 128-171.

- Harnack, L.J., Jeffery, R.W., Boutelle, K.N. Temporal trends in energy intake in the United States: an ecological perspective. *Am J Clin Nutr.* 71:1478-84. (2000).
- Harbige, L.S. Fatty acids, the immune response, and autoimmunity: A question of n-6 essentiality and the balance between n-6 and n-3. *Lipids.* 38(4):323-335 (2003).
- Huang, D., Boxin, O., Prior, R.L., The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53: 1841-1856 (2005).
- Kaferstein, F.K. 2002. Food Safety – a worldwide public health issue. Actions to reverse the upward curve of foodborne illness. In: Smulders, J.M., Collins, J.D., editors. Safety assurance during food processing. Food safety assurance and veterinary public health, vol. 2. Amstelveen, Netherlands: Wageningen Academic Publishers. P 19-31.
- Kantor, L.S., Lipton, K., Manchester, A., Oliveira, V., Estimating and addressing America's food losses. *Food Review.* January-April (1997).
- Kris-Etherton, P.M., Taylor, D.S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R.L., Zhao, G., Etherton, T.D. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr.* 71:179S-188S (2000).
- Kris-Etherton, P.M., Harris W.S., Appel L.J., Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Arterioscler Thromb Vasc Biol.* 23: 20-31 (2003).
- Kolakowska A Sikorski ZE. 2003. The role of lipids in food quality. In: Sikorski ZE, Kolakowska A, editors. Chemical and functional properties of food lipids. Boca Raton, Florida: CRC Press. P 1-8.
- Korzenik, J.R., Podolsky, D.K. Evolving knowledge and therapy of inflammatory bowel disease. *Nature Reviews.* 5:197-209 (2006).
- Lindsay, R.C. (1996) Food Additives. In: Fennema O.R., editor, Food Chemistry 3<sup>rd</sup>. Edition. New York, New York: Marcel Dekker, Inc. P:768-821.
- Liu, M., Wallin, R., Wallmon, A., Saldeen, T., Mixed tocopherols have a stronger inhibitory effect on lipid peroxidation than  $\alpha$ -tocopherol alone. *J. of Cardiovascular Pharmacology.* 39:714-721 (2002).
- Liu, R.H., Potential synergy of phytochemicals in cancer prevention: Mechanism of action. American Society for Nutritional Sciences. 3479S-3485S (2004).
- Marco, M.L., Pavan, S., Kleerebezem, M. Towards understanding molecular modes of probiotic action. *Current Opinion in Biotechnology.* 17:1-7 (2006).
- Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., Food-related illness and death in the United States. *Center for Disease Control (CDC) Emerging Infectious Diseases.*

5(5): 607-625 (1999)

<http://www.cdc.gov/ncidod/eid/vol5no5/mead.htm> Access date March 20, 2006.

- Miljanovic, B., Trivedi K.A., Dana M.R., Gilbard J.P., Buring J.E., Schaumberg D.A., Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr.* 82:887-93 (2005).
- Min, D.B., Boff, J.M. (2003) Crude Fat Analysis. In: Nielsen, S.S., editor Food Analysis 3<sup>rd</sup>. Edition. West Lafayette, Indiana: Kluwer Academic/Plenum Publishers. P113-130.
- Montville, T.J. 1997. Principles which influence microbial growth, survival, and death in foods. In: Food microbiology: Fundamentals and frontiers. Washington, DC: American Society for Microbiology. P 13-29.
- Montville, T.J., Matthews K.R. 2005. Factors that influence microbes in foods. In: Montville TJ, Matthews KR, editors, Food microbiology: An introduction. Washington, DC: American Society for Microbiology. P 11-26.
- Montville, T.J., Winkowski, K., (1997) Biologically based preservation systems and probiotic bacteria. In: Doyle, M.P., Beuchat, L.R., Montville, T.J., editors. Food microbiology: Fundamentals and frontiers. Washington, DC: American Society for Microbiology. P 557-578.
- National Institute of Medicine of the National Academies. 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, D.C.: The National Academies Press.  
<http://www.nap.edu/openbook/0309085373/html/R1.html>. Access date April 10, 2006.
- Nawar WW. 1996. Lipids. In: Fennema OR, editor. Food Chemistry, 3<sup>rd</sup>. Edition. New York, New York: Marcel Dekker, Inc. P: 225-320.
- Nettis, E., Colanardi, M.C., Ferrannini, A., Tursi, A. Sodium benzoate-induced repeated episodes of acute urticaria/angio-oedema: randomized controlled trial. *British Journal of Dermatology.* 151:898-902 (2004).
- Newton, I.S. (2001) Long-chain fatty acids in health and nutrition. In: Shahidi, F., Finley, J.W., editors. Omega-3 fatty acids chemistry, nutrition, and health effects. Washington, DC: Oxford University Press. P: 14-27.
- Nichols DS, Sanderson K. 2003. The nomenclature, structure, and properties of food lipids. In: Sikorski ZE, Kolakowska A, editors. Chemical and functional properties of food lipids. Boca Raton, Florida: CRC Press. P 29-60.
- National Institutes of Health. 1998. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. National Institutes of Health Publication No. 98-4083.

[www.nhlbi.nih.gov/guidelines/obesity/ob\\_gins.pdf](http://www.nhlbi.nih.gov/guidelines/obesity/ob_gins.pdf). Access date March 15, 2006.

- O'Brien, R.D. 2004 Fats and Oils: Formulating and Processing for Applications. 2<sup>nd</sup> ed. Boca Raton, FL: CRC Press.
- Ohm, V.A., Stockmann, H., Schwarz K., The more – The better? Estimating the inhibitory activity of alpha-tocopherol towards lipid oxidation. *Journal of Plant Physiology*. 162: 785-89. (2005).
- Ozkan, G., Sagdic, O., Bayder, N.G., Kurumahmutoglu, Z. Antibacterial activities and total phenolic contents of grape pomace extracts. *J Sci Food Agric*. 84:1807-1811 (2004).
- Pereira, A.L., Cesquini, M., Tomizawa, E., Torsoni, M.A., Ogo, S.H. The beneficial effect of three flavonols against oxidative damage. *J. of Food Biochemistry*. 27:141-152 (2003).
- Pietta PG. 2000. Flavanoids as antioxidants. *J Nat Prod*. 63: 1035-1042.
- Pike, O.A. (2003) Fat Characterization. In: Nielsen, S.S., editor Food Analysis 3<sup>rd</sup> Edition. West Lafayette, Indiana: Kluwer Academic/Plenum Publishers. P227-246.
- Proestos, C., Chorianopoulos, N., Nychas, G-J. E., Komaitis, M. RP-HPLC analysis of the phenolic compounds of plant extracts, investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem*. 53:1190-1195 (2005).
- Proestos, C., Boziaris, I.S., Nychas, G-J.E., Komaitis, M. Analysis of flavanoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chemistry*. 95:664-671 (2006).
- Raatz, S.K., Bibus, D., Thomas, W., Kris-Etherton, P. Total fat intake modifies plasma fatty acid composition in humans. *J. Nutr*. 131:231-234. (2001).
- Rauha, J.P., Remes, S., Heinonem, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H., Vuorela, P., Antimicrobial effects of Finnish plant extracts containing flavanoids and other phenolic compounds. *Inter. J. of Food Microbiology*. 56:3-12 (2000).
- Rees, D. Miles, E.S., Banerjee, T., Wells, S.J., Roynette C.E., Wahle, K.W.J., Calder, P.C. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr*. 83:331-342 (2006).
- Roche, H.M. Unsaturated fatty acids. *Proceedings of the Nutrition Society*. 58:397-401 (1999).
- Roche, H.M., Terres, A.M., Black, I.B., Gibney, M.J., Kelleher, D. Fatty acids and epithelial permeability: effect on conjugated linoleic acid in Caco-2 cells. *Gut*. 48:797-802 (2001).

- Scrimgeour, C., Chemistry of Fatty Acids. In: Shahidi, F. Baileys Industrial Oil and Fat Products, 6<sup>th</sup>. Edition (2005).
- Simopoulos, A.P., Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. *Food Reviews International*. 2004. 20(1):77-90.
- Singh, U. Devaraj, S. and Jialal, I., Vitamin E, Oxidative Stress, and Inflammation. *Annul. Rev. Nutr.* 2005. 25:151-74.
- Stansby, M.E. Schlenk, H., Gruger, E.H. (1990) Fatty acid composition of fish. In: Stansby, M.E., editor. Fish oils in nutrition. New York, New York: Van Nostrand Reinhold. P 6-39.
- Stender, S., Dyerberg, J. Influence of *trans* fatty acids on health. *Ann. Nutr. Metab.* 48:61-66. (2004).
- Timbo, B., Koehler, K.M., Wolyniak, C., Klontz, K.C., Sulfites – A food and drug administration review of recalls and reported adverse events. *J. of Food Protection*. 67(8):1806-11. (2004).
- Tollefson, L., Monitoring adverse reactions to food additives in the U.S. Food and Drug Administration. *Regulatory Toxicology and Pharmacology*. 8:438-446 (1988).
- Tucker, J.M., Townsend, D.M. Alpha-tocopherol: roles in prevention and therapy of human disease. *Biomedicine and Pharmacotherapy*. 59:380-387 (2005).
- U. S. Department of Health and Human Services and U.S. Department of Agriculture. *Dietary Guidelines for Americans*, 2005. 6<sup>th</sup>. Edition, Washington, DC: US Government Printing Office, January 2005.
- Weiss, L.A., Barrett-Connor, E., Muhlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. *Am J Clin Nutr.* 81:934-948 (2005).
- Wijendran, V., Hayes, K.C. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annual Review of Nutrition*. 24:597-615 (2004).
- World Health Organization. (2002) WHO global strategy for food safety: safer food for better health. Geneva, Switzerland: WHO <http://www.who.int/fsf>. Access date March 10, 2006.
- Wolff, R.L., Heat-Induced isomerization of linoleic acid: Effect of temperature and heating time on the appearance of individual isomers. *J. Am. Oil Chem. Soc.* 70:425-430 (1993).
- Wolff, R.L., Nour, M., Bayard, C.C. Participation of the cis-12 ethylenic bond to cis-trans isomerization of the cis-9 and cis-15 ethylenic bonds in heated  $\alpha$ -Linolenic acid. *J. Am. Oil Chem. Soc.* 73(3): 327-332. (1996)
- Woodside, J.V., Kronhout, D. Fatty acids and CHD. *Proceedings of the Nutrition Society*. 64:554-564 (2005).

- Wuthrich, B., Adverse reactions to food additives. *Annals of Allergy*. (71): Oct. (1993).
- Xia, S.H., Wang, J., Kang, J.X. Decreased n-6/n-3 fatty acid ratio reduces the invasive potential of human lung cancer cells by downregulation of cell adhesion/invasion-related genes. *Carcinogenesis*. 26(4):779-784 (2005).
- Yamashita, T., Oda, E., Sano, T., Yamashita, T., Ijiru, Y., Giddings, J.C., Yamamoto, J. Varying the ratio of dietary n-6/n-3 polyunsaturated fatty acid alters the tendency to thrombosis and progress of atherosclerosis in apoE<sup>-/-</sup>LDLR<sup>-/-</sup> double knockout mouse. *Thrombosis Research*. 116:393-401 (2005).
- Yehuda S., Rabinovitz S., Mostofsky D.I. Essential fatty acids and the brain: From infancy to aging. *Neurobiology of Aging*. 26S: S98-S102 (2005).
- Zamaria, N., Alteration of polyunsaturated fatty acid status and metabolism in health and disease. *Reprod Nutr. Dev*. 44:273-282 (2004).
- Zuberbier, T., Pfrommer, C., Specht, K., Vieths, S., Basti-Borrmann, R., Worm, M., Henz, B. Aromatic components of food as novel eliciting factors of pseudoallergic reactions in chronic urticaria. *J Allergy Clin Immunol*. Feb: 343-348 (2002).

## Chapter 2: Inhibitory Effect of Selected Spice and Fruit Seed Extracts on Lipid Oxidation in Fish Oil and Their Radical Scavenging and Antimicrobial Properties.

### 2.1.0 Introduction

Lipid oxidation is a critical problem during food processing, distribution, storage, and consumption as it decreases food quality, stability, safety, and nutritive value. Antioxidants have been utilized to prevent oxidative damage to food components and prevent off-flavor development in food products (Yu and others 2002a). It is widely accepted that lipid oxidation in food products is a free radical mediated oxidative chain reaction involving three general phases: initiation, propagation, and termination. Antioxidants may suppress the initiation step and/or discontinue the propagation steps by quenching the radicals in the system, leading to the termination of oxidative radical chain reactions (Yu and others 2002a; Matthaus 2002; Athukorala and others 2003). Antioxidants may also prevent the oxidation of protein in meat and other food products that contain high concentrations of prooxidants such as heme, transition metals and polyunsaturated fatty acids (Aligiannis and others 2003; Viljanen and others 2004b). Recently, the demand for novel natural antioxidants has greatly increased, primarily because of the possible side effects of synthetic antioxidants and the potential health beneficial effects of natural antioxidants (Athukorala and others 2003; Aligiannis and others 2003). A number of studies have been

conducted to discover and develop natural antioxidants from agricultural products traditionally used for human consumption such as cereal grains, herbs, fruits, vegetables, marine red algae, and edible seeds and their fractions (Guleria and others 1983; Velioglu and others 1998; Athukorala and others 2003; Ramadan and others 2003; Zhou and others 2004; Rey and others 2005; and Yu and others 2005a). A few natural antioxidants such as rosemary extracts have been successfully developed for commercial utilization (Yu and others 2002a; Fernandez-Lopez and others 2005). Novel antioxidative preservatives with different physicochemical properties are needed for diversified food systems because the physical and chemical nature of a selected food system may require an antioxidative preservative with different physicochemical properties (Frankel and others 1996).

It is also well recognized that food-borne pathogens are major concerns of food safety (Buzby and others 1996). They are responsible for approximately 76 million cases of food-borne illness, 325,000 hospitalizations, and 5,000 deaths in the U.S. annually

(<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5115a3.htm>). The estimated annual cost of the top five bacterial pathogens, including *Campylobacter*, *Salmonella* (nontyphoidal serotypes only), *Escherichia coli* (*E. coli*) 0157 and non-0157 STEC, and *Listeria monocytogenes* (*L. monocytogenes*), is \$6.9 billion (<http://www.ers.usda.gov/briefing/FoodborneDisease/>). In a recent study, grape pomace extract was shown to have antibacterial activity against thirteen different bacteria (Ozkan and others 2004). The antimicrobial activity of natural extracts is



closely linked with their polyphenolic content (Ahn and others 2003). Therefore, grape and other fruit seed extracts rich in phenolics may serve as potential natural antimicrobial agents.

A recent study in our laboratory showed that 100% ethanol and 50% acetone extracts of black raspberry seed flours can directly react with and quench DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals (Parry and Yu, 2004). Phenolics were also detected in the seed flour extracts at a level of 26.7 and 45.6 mg gallic acid equivalent per gram of flour for the ethanol and 50% acetone extracts, respectively (Parry and Yu 2004). Furthermore, about 60-70 % of the total extractable phenolic compounds in grapes are located in the seeds (Shi and others 2003). These data suggested the potential of fruit seed flour extracts in reducing lipid oxidation and the risk of foodborne illness caused by pathogens. Fruit seeds are by-products from fruit processing, and seed flours are the by-products from seed oil production. The amount of caneberry seeds from Oregon and Washington seedless processing in 2003 alone was estimated 180,000 kg (Bushman and others 2004). Developing natural food preservatives with both antioxidant and antimicrobial capacities from these fruit seeds may improve food quality, safety, and nutritional value, while enhancing the profitability of fruit production and processing industries. This also may benefit human health (Yu and others 2002a; Ahn and others 2003; Fernandez-Lopez and others 2005).

The present study was conducted to evaluate the selected edible seed extracts for their capacities of 1) inhibiting lipid oxidation in fish oil; 2) preserving EPA and DHA in fish oil; 3) suppressing pathogenic bacteria; and 4)

directly reacting with and quenching DPPH radicals and peroxy radicals (ORAC). In addition, total phenolic contents and effects of these seed extracts on oil color were also examined. The data from this research may lead to novel natural food preservatives and improve the quality, safety, and nutritional value of food products.

### 2.2.0 Materials and Methods

#### 2.2.1 Materials & Preparation of Antioxidant Extracts

Cumun-3 parsley (C3P, *Petroselinum crispum*), green river parsley (GRP, *Petroselinum crispum*), black currant (BC, *Ribes nigium*), Chardonnay grape (Ch, *Vitis vinifera*), Pinot noir grape (PN, *Vitis vinifera*), and black raspberry (*Rubus occidentalis*) seed flours were obtained from Botanical Oil Innovations, Inc. (Spooner, Wisconsin), while cranberry seeds (Cr, *Vaccinium macrocarpon*) were provided by Decas Cranberry Products Inc (Wareham, Massachusetts). Unstabilized Menhaden Fish oil was donated by Omega Protein (Reedville, VA). Mixed tocopherols, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (AAPH), disodium ethylenediaminetetracetate, and 2,2'-bipyridyl gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). Brain heart infusion agar (BHI) was purchased from Fischer Scientific (Difco, Detroit, MI) and bacteria were freezer stock strains of *E. coli* ATCC25922 and *L. monocytogenes* ATCC 19114. All other

chemicals and solvents were of the highest commercial grade and used without further purification.

Seed flours and whole seeds were ground to a 20 mesh using a Micromill manufactured by Bel-Art Products (Pequannock, NJ). Ten grams of ground seed flour or meal was extracted with about 170 mL of 100% ethanol utilizing the Soxhlet extractor for 3 h. After measuring the final volume, the ethanol extracts were kept in the dark under nitrogen at ambient temperature until further analysis. The concentration of each ethanol extract was calculated and expressed as the equivalents of starting material in mg per mL extract.

### 2.2.2 Oxidative Stability Index (OSI)

The stabilization of Manhedan fish oil by each seed flour and meal extracts was determined by OSI using a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland). The oxidation was carried out with 6 mL of fish oil containing different levels of seed flour extract at 80 °C with air flow of 7 L/h (Chen and Ho 1997; Yu and others 2002b). The two dose levels were tested for each antioxidant extract, and tocopherol was used as the positive control. Fish oil containing no antioxidant was included as the negative control (the blank) to calculate the % Extension Time. For each dose level, a known volume of selected ethanol extract was mixed in the fish oil, and the ethanol was removed below 35 °C under reduced pressure using a rotary evaporator to obtain the testing oil sample containing known amount of antioxidants. For the negative control (blank) oil sample, the same volume of ethanol was mixed in and evaporated from

the oil under the same experimental conditions. The low dose of antioxidants was 5.7, 11.7, 6.6, 7.4, 5.8, 7.1, and 6.5 mg seed flour/meal equivalent per mL of fish oil for cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot nior grape and black raspberry seed flour and cranberry seed meal extracts, respectively. The high dose of antioxidants was 12.9, 26.3, 14.9, 16.7, 13.0, 16.0, and 14.5 mg seed flour equivalent per mL of fish oil for cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour and cranberry seed meal extracts, respectively. Triplicate assays of the low dose and high dose and duplicate assays of the negative control were conducted. The results were expressed as the % Extension Time and calculated as:

$$\% \text{ Extension Time} = \frac{(OSI_{\text{sample}} - OSI_{\text{negative-control}})}{OSI_{\text{negative-control}}} \times 100\%$$

where the OSI is defined as the hours required for an oil sample to develop measurable rancidity.

### 2.2.3 Fatty Acid Composition

Fish oil samples containing different antioxidant extracts were collected after oxidation reaction at 80 °C with an air flow rate of 7 L/h for 4.5 hours. These oil samples were analyzed and compared with the original fish oil (the blank) for their fatty acid composition. Relative concentrations of DHA, EPA, and total n-3 poly-unsaturated fatty acid (PUFA) were of primary concern. Fatty acid methyl esters (FAME) were prepared from the oil samples and analyzed by GC according to a laboratory protocol (Yu and others 2002c). GC analysis was

conducted using a Shimadzu GC-2010 with a FID and a Shimadzu AOC-20i autosampler (Shimadzu, Columbia, MD). A fused silica capillary column SP<sup>TM</sup>-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco (Bellefonte, PA, U.S.A.) was used with helium as the carrier gas at a flow rate of 0.8 mL/min. Injection volume was 1 μL at a split ratio of 10/1. Initial oven temperature was 142 °C and increased 6 °C/min to 184 °C and held for 3 min, then increased 6 °C/min to 244 °C. Identification of the individual fatty acids was accomplished by comparing GC retention time with fatty acid standard reference mixes GLC-84 and GLC-76 as well as unoxidized fish oil. All samples were analyzed in triplicate. Quantification was based on the area under individual fatty acid peak and the total area of all fatty acid peaks. Results were reported as g fatty acid per 100 g total fatty acids.

#### 2.2.4 DPPH Radical Scavenging Activity

Free radical scavenging capacity of each seed meal extract was estimated following a previously reported procedure using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) (Yu and others 2002a). Briefly, 800 μL of freshly made 200 μM DPPH<sup>•</sup> working solution was added in 800 μL of seed meal extract to start the radical-antioxidant reaction. The 200 μM DPPH<sup>•</sup> working solution was freshly prepared from a 1 mM DPPH<sup>•</sup> stock solution in ethanol and used within 8 h. The absorbance at 517 nm was determined by spectroscopy and compared against a blank of pure ethanol at 0.5, 1, 5, 10, 15 and 20 minutes of

reaction to estimate the amount of DPPH radicals quenched. The initial concentration was 100  $\mu\text{M}$  for DPPH $^{\bullet}$ . The assay was conducted in triplicate.

### 2.2.5 Oxygen Radical Absorbing Capacity (ORAC)

ORAC was conducted for 100 % ethanol extracts of the seed flours and meal using fluorescein (FL) as the fluorescent probe and a Victor<sup>3</sup> multilabel plate reader (PerkinElmer, Turku, Finland) following an assay previously described (Moore and others 2005). Trolox standard was dissolved in 100% ethanol while all other reagents were prepared in 75 mM phosphate buffer (pH 7.4). A final volume of 280  $\mu\text{L}$  of each testing mixture was comprised of 225  $\mu\text{L}$   $8.16 \times 10^{-8}$  M fluorescein (FL) solution, 30  $\mu\text{L}$  of sample, standard or ethanol for the blank, and 25  $\mu\text{L}$  of 0.36 M 2,2-azobis (2-amidino-propane) dihydrochloride (AAPH), a free radical generator. The reaction mixture containing the FL and other agents was preheated for 20 minutes at 37 °C prior to the addition of the AAPH solution. The fluorescence of each reaction mixture was recorded every minute for 45 minutes at 37°C with the excitation and emission wavelengths at 485 nm and 535 nm, respectively. Trolox equivalents (TE) were calculated using the relative area under the curve for samples using a standard curve prepared using trolox under the same conditions. Results were expressed as  $\mu\text{moles}$  of TE per gram seed flours or meal.

### 2.2.6 Total Phenolic Content (TPC)

The TPC of each seed meal extract was measured using Folin-Ciocalteu reagent according to laboratory protocol (Yu and others 2002a). Briefly, the

reaction mixture contained 100  $\mu$ L of seed flour/meal extract, 500  $\mu$ L of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate and 1.5 mL pure water. After two hours of reaction at ambient temperature, a spectrophotometer was used to read the absorbance at 765 nm. Samples were conducted in triplicate and a gallic acid standard curve was conducted in duplicate. TPC was calculated and reported as gallic acid equivalents (GAE).

### 2.2.7 Antibacterial Activity

Antibacterial activity of these seed flour and meal extracts were tested using *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 19114 according to the established laboratory protocols (Zhao and others 2001). *E. coli* was streaked on Luria-Bertani (LB; Difco, Cockeysville, MD) agar plate and *L. monocytogenes* on Brain Heart Infusion (BHI; Difco) agar plate from freezer stocks. A single colony was picked from each plate and subcultured on LB and BHI plates, respectively, and incubated overnight at 35 °C to purify. Purified overnight cultures were resuspended in 5 mL of saline and the optical density was adjusted to 0.09 (*E. coli*) and 0.06 (*L. monocytogenes*), which resulted in a bacterial concentration of approximately  $10^8$  CFU/mL. Bacteria suspensions were diluted 100 times with saline to obtain the working bacterial solution. A volume of 200  $\mu$ L each ethanol extract was mixed with 3.8 mL of bacteria cell suspension ( $10^6$  CFU/mL) followed by 15 hours of incubation at 35 °C (*E. coli*) or 4 °C (*L. monocytogenes*). The final concentration was 2.6, 5.3, 3.0, 3.3, 2.6, and 3.2 mg seed flour equivalent/mL for the cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour

extracts, respectively in the assay culture. Bacterial survival was measured by viable cell counting on LB agar for *E. coli* and BHI agar for *L. monocytogenes* after incubation using the serial dilution method (Marino and others 2001). Triplicate samples were prepared for each flour and meal extract. Equal volume of ethanol was used in the negative control. The antibacterial activity of the flour and meal extracts was noted as the bacterial survival rates after incubation calculated as:

$$\text{Survival rate} = \frac{\text{Cell number in the treatment}}{\text{Cell number in the control}} \times 100\%.$$

### 2.2.8 Color Measurement

Ten mL fish oil containing certain level of each seed meal extract was analyzed for color. Hunter color values were obtained using a HunterLab Labscan spectrophotometer (Model 45/0; Reston, VA, U.S.A.) with a setting of D65 (daylight 65° illuminant/10° observer) (Yu and others 2002a). Four measurements were recorded for each concentration of selected seed flour and meal extract. The tested concentrations of each extracts were summarized in Table 4.

### 2.2.9 Statistical Analysis

Data were reported as mean and standard deviation for triplicate measurements. Analysis of variance and Tukey's honestly significant difference tests were conducted (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc.,



Chicago, IL) to determine differences among means. Statistical significance was declared at  $P < 0.05$ .

### 2.3.0 Results and Discussion

Cumun-3 parsley (C3P), black currant (BC), green river parsley (GRP), Chardonnay grape (Ch), Pinot noir grape (PN), black raspberry (BR), and cranberry (Cr) seed oils are commercially available as specialty edible seed oils, and the seed flours from oil production are treated as wastes. Developing natural food preservatives from these edible seed flours and meal may improve the profitability of the oil processing industry and benefit the growers and food processing industries. Developing natural food preservatives from these edible seed flours and meal may also improve food safety and consumer acceptability of food products.

Also noted was that ethanol extracts were used in the present study, although our previous study showed that the 50% acetone extract of black raspberry seed flour had much stronger free radical scavenging ability and higher TPC content than that of its ethanol extract (Parry and Yu 2004). This is because the ethanol extracts are more practical for commercial scale production. Ethanol may be reused, which reduces the environmental concern and overall cost on per unit of preservative basis. The solvent composition does not change during extraction and the extract quality and consistency may be better controlled.

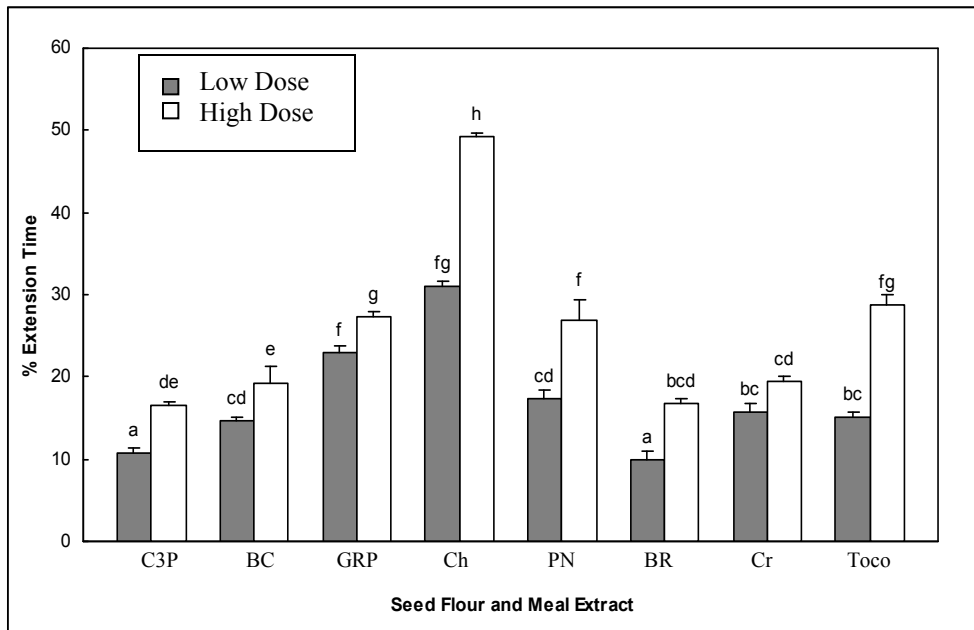
### 2.3.1 Lipid Stability and Maintenance of Essential Fatty Acids

This study examined the potential of the selected seed flour and meal extracts for their potential to inhibit lipid oxidation and preserve n-3 PUFA in fish oil. The oxidative stability index (OSI) measures the secondary products of lipid peroxidation by total volatile carbonyl compounds. OSI is the time required for an oil to develop measurable rancidity. A larger OSI value is generally associated with a longer shelf life. With the Rancimat instrument, heating and forced air flow accelerates lipid peroxidation, and results in elevated formation of volatile carbonyl compounds including aldehydes and small molecular weight carboxylic acids. The aldehydes may be further oxidized in air to carboxylic acids. These volatile compounds are collected in the pure water and increase the conductivity of water. The water conductivity, which is proportional to total volatile secondary products from lipid peroxidation, is measured to reflect the degree of lipid peroxidation using the Rancimat instrument.

As illustrated in figure 11, all tested seed flour and meal extracts were found to dose dependently inhibit lipid oxidation in the fish oil. C3P, BC, GRP, Ch, PN, and BR represent fish oil containing cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, respectively. Cr and Toco are fish oil samples containing cranberry seed meal extract and the mixed tocopherol. The solid columns represent low dose of antioxidants at 5.7, 11.7, 6.6, 7.4, 5.8, 7.1, and 6.5 mg seed flour/meal equivalent per mL of fish oil for cumun-3 parsley, black currant, green river

parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour and cranberry seed meal extracts, respectively. The open columns represent the high dose of antioxidants at 12.9, 26.3, 14.9, 16.7, 13.0, 16.0, and 14.5 mg seed flour equivalent per mL of fish oil for cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour and cranberry seed meal extracts, respectively. The solid and open columns labels as Toco represent fish oils samples containing 60 and 130 ppm mixed tocopherols, respectively.

The greatest OSI of 2.85 h was detected in the fish oil sample containing 16.7 mg Chardonnay grape seed flour equivalents/mL, which represents about 50% extension of OSI comparing to the fish oil containing no antioxidants (Figure 11). Also noted was that the Chardonnay grape seed flour extract at a level of 7.4 mg flour equivalents/mL exhibited same power in suppressing lipid oxidation in fish oil as 130 ppm mixed tocopherols. In addition, green river parsley and Pinot noir grape seed flour extracts exhibited the same inhibitory ability against lipid oxidation in fish oils at concentrations of 15 and 16 mg flour equivalents/mL, respectively, as that observed for 130 ppm mixed tocopherols (Figure 11). These data suggest the potential of developing natural antioxidative preservatives from edible seeds and their fractions, especially Chardonnay grape seed flour and maybe Pinot noir grape and green river parsley seed flours. It needs to be pointed out that OSI is a measurement of oil stability under accelerated conditions, which may not truly reflect the common storage condition of oils. (Pike 2003)



**Figure 11: OSI extension of Manhedan fish oil by the selected seed flour and meal extracts.** Dark bars represent fish oil stabilized by the low dose of antioxidants which was 5.7, 11.7, 6.6, 7.4, 5.8, 7.1, and 6.5 mg seed flour/meal equivalent per mL of fish oil for cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot nior grape and black raspberry seed flour and cranberry seed meal extracts, respectively. Light bars represent the high dose of antioxidants which was 12.9, 26.3, 14.9, 16.7, 13.0, 16.0, and 14.5 mg seed flour equivalent per mL of fish oil for cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour and cranberry seed meal extracts, respectively. Triplicate assays of the low dose and high dose and duplicate assays of the negative control were conducted. The results were expressed as the % Extension Time and calculated as the induction point of the samples minus the induction point of the ethanol control divided by the ethanol control multiplied by one hundred. Vertical bars represent the standard deviation (n = 3). Values marked by the same letter are not significantly different (P < 0.05).

The Pinot Noir grape extract was superior to all other tested extracts in preserving important fatty acids from oxidative damages. The extract of the Pinot noir grape seed flour was able to significantly prevent oxidation of EPA, total n-3

fatty acids, and total PUFA in the fish oil under accelerated oxidation (Table 1). The extracts of cumun-3 parsley, black currant, Chardonnay grape, cranberry, and black raspberry seed flours and meal were also able to reduce the loss of EPA, and the extracts of black raspberry flour and cranberry seed meal showed significant capacity to preserve total PUFA in the fish oil under the accelerated oxidation conditions (Table 1). Longer-chain n-3 polyunsaturated fatty acids DHA (C22:6n-3) and EPA (C20:5n-3) are well known for their potential in reducing the risk of heart disease, cancer, hypertension, and autoimmune disorders (Connor 2000; Hung and others 2000; Parry and Yu 2004; Ruxton and others 2004). Other PUFA such as linoleic and  $\alpha$ -linolenic acids are also important for human health (Parker and other 2003). Preservation of special PUFA is critical to maintain the nutritional value of the oil (Athukorala and others 2003). The results from this study indicate that the capacity of an individual antioxidant preparation in preserving a selected fatty acid may differ to that in suppressing the overall lipid oxidation in the oil. The exact mechanism(s) underlying is not clear, but this observation may be partially explained by the different polarity of the antioxidants in each antioxidant preparations, which may lead to the different distribution of antioxidative components in the oil. This finding is important for creating optimal antioxidative food preservatives for minimized nutrient loss and rancidity development.

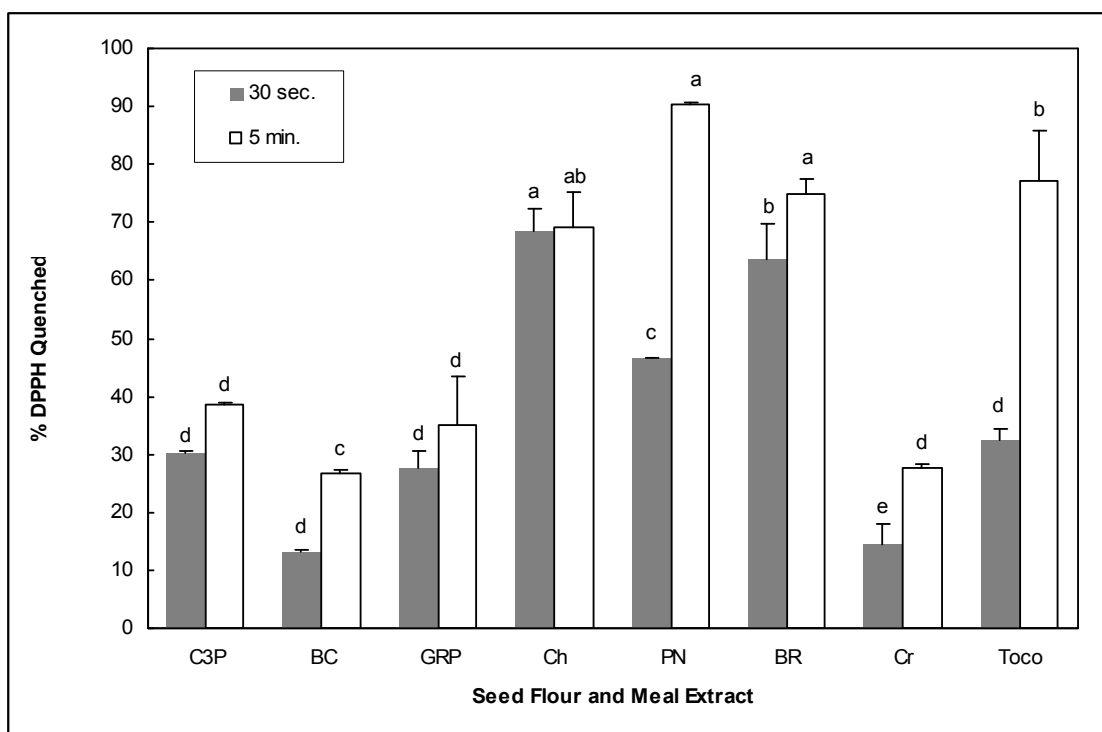
Fatty Acid	Fatty Acid Composition (g/100 g total fatty acids)									
	Blank	Control	C3P	BC	GRP	Ch	PN	BR	Cr	Toco
12:0	0.15 ± 0.01a	0.17 ± 0.00b	0.17 ± 0.00ab	0.16 ± 0.00ab	0.17 ± 0.01ab	0.17 ± 0.00ab	0.17 ± 0.00ab	0.17 ± 0.01b	0.18 ± 0.00b	0.16 ± 0.01ab
14:0	11.58 ± 0.21a	13.15 ± 0.05d	13.20 ± 0.05d	12.64 ± 0.06d	13.00 ± 0.08d	12.90 ± 0.02d	12.17 ± 0.08b	12.58 ± 0.20c	13.09 ± 0.08d	13.10 ± 0.02d
14:1	0.05 ± 0.00a	0.07 ± 0.00c	0.06 ± 0.00bc	0.06 ± 0.00bc	0.06 ± 0.00bc	0.06 ± 0.00bc	0.06 ± 0.00ab	0.06 ± 0.00bc	0.06 ± 0.00bc	0.06 ± 0.00bc
16:0	20.21 ± 0.01a	22.45 ± 0.06de	22.63 ± 0.06e	21.76 ± 0.11de	22.18 ± 0.18d	22.10 ± 0.03d	20.86 ± 0.16b	21.57 ± 0.32c	22.38 ± 0.14de	22.43 ± 0.11de
16:1	15.64 ± 0.64a	16.81 ± 0.03c	16.69 ± 0.02bc	16.38 ± 0.06bc	16.55 ± 0.21bc	16.32 ± 0.01bc	15.59 ± 0.06a	16.15 ± 0.19ab	16.63 ± 0.03bc	16.75 ± 0.03bc
18:0	3.43 ± 0.07a	3.88 ± 0.01cd	3.95 ± 0.01d	3.75 ± 0.01cd	3.76 ± 0.14bc	3.84 ± 0.01cd	3.64 ± 0.02b	3.76 ± 0.04bc	3.89 ± 0.02cd	3.93 ± 0.02d
18:1	7.11 ± 0.39a	8.38 ± 0.01cd	8.67 ± 0.00d	8.05 ± 0.03cd	8.39 ± 0.09cd	8.27 ± 0.01c	7.87 ± 0.05b	8.16 ± 0.13bc	8.35 ± 0.03cd	8.42 ± 0.04cd
18:2n-6	1.58 ± 0.00a	1.64 ± 0.01ab	1.73 ± 0.00bc	1.65 ± 0.01d	1.66 ± 0.01ab	1.80 ± 0.00cd	1.66 ± 0.01ab	1.74 ± 0.11bcd	1.71 ± 0.01bc	1.68 ± 0.01b
18:3n-3	1.67 ± 0.01cd	1.57 ± 0.00a	1.64 ± 0.01c	1.62 ± 0.01d	1.58 ± 0.01ab	1.64 ± 0.00c	1.74 ± 0.01f	1.76 ± 0.01f	1.71 ± 0.01e	1.61 ± 0.01b
20:0	0.14 ± 0.00a	0.16 ± 0.00d	0.16 ± 0.00d	0.15 ± 0.00cd	0.16 ± 0.01cd	0.16 ± 0.00cd	0.15 ± 0.00ab	0.15 ± 0.00bc	0.16 ± 0.00d	0.16 ± 0.00d
20:1	1.18 ± 0.04bcd	1.04 ± 0.00ab	1.01 ± 0.01a	1.08 ± 0.01a	1.02 ± 0.07a	1.04 ± 0.01a	1.21 ± 0.00cd	1.11 ± 0.10abc	1.29 ± 0.00d	1.08 ± 0.02ab
20:4n-6	1.24 ± 0.00b	1.13 ± 0.01ab	1.22 ± 0.00b	1.19 ± 0.00b	1.02 ± 0.21a	1.22 ± 0.01b	1.29 ± 0.00b	1.21 ± 0.01b	1.19 ± 0.01ab	1.19 ± 0.02ab
20:5n-3	21.02 ± 0.03f	18.38 ± 0.03a	19.06 ± 0.04cd	19.49 ± 0.10d	18.74 ± 0.14abc	19.84 ± 0.03e	21.79 ± 0.18g	19.55 ± 0.30e	18.83 ± 0.12bcd	18.63 ± 0.09ab
22:6n-3	15.01 ± 0.03c	11.17 ± 0.20ab	9.81 ± 0.15a	11.99 ± 0.26ab	11.71 ± 0.95b	10.64 ± 0.06ab	11.81 ± 0.54b	12.02 ± 1.17b	10.53 ± 0.38ab	10.80 ± 0.30ab
Sat	35.80 ± 0.26a	39.68 ± 0.26ef	40.07 ± 0.00f	39.78 ± 0.00e	39.71 ± 0.00e	39.11 ± 0.00d	37.07 ± 0.00b	37.61 ± 0.00c	39.95 ± 0.00ef	39.90 ± 0.00ef
MUFA	23.64 ± 0.29a	26.30 ± 0.29ef	26.44 ± 0.03f	26.03 ± 0.04de	26.02 ± 0.22de	25.70 ± 0.01cd	24.73 ± 0.08b	25.48 ± 0.21c	26.33 ± 0.01ef	26.31 ± 0.04ef
PUFA	40.56 ± 0.04ab	33.89 ± 0.04f	33.46 ± 0.11a	34.40 ± 0.16abc	34.71 ± 0.63bc	35.14 ± 0.04c	38.29 ± 0.34e	36.28 ± 0.76d	33.97 ± 0.24ab	33.90 ± 0.17ab
n-3	37.73 ± 0.04e	31.12 ± 0.04ab	30.51 ± 0.09a	31.35 ± 0.13ab	32.03 ± 0.68b	32.12 ± 0.03bc	35.34 ± 0.29d	33.33 ± 0.72c	31.07 ± 0.21ab	31.04 ± 0.16ab
n-6	2.82 ± 0.00abc	2.77 ± 0.00ab	2.95 ± 0.00bcd	3.05 ± 0.00d	2.68 ± 0.17a	3.02 ± 0.01cd	2.95 ± 0.01bcd	2.95 ± 0.10bcd	2.90 ± 0.02abcd	2.87 ± 0.02abcd
n-6/n-3	0.07 ± 0.00a	0.09 ± 0.00bc	0.10 ± 0.00c	0.10 ± 0.00c	0.08 ± 0.01ab	0.09 ± 0.00c	0.08 ± 0.00ab	0.09 ± 0.00bc	0.09 ± 0.00bc	0.09 ± 0.00bc

**Table 1: Preservation of PUFA in fish oil by selected seed flour and meal extracts.** Sat: saturated FAs; MUFA: monounsaturated FAs; PUFA: polyunsaturated FAs; n-3: n-3 PUFA; n-6: n-6 PUFA; n-6/n-3: ratio of polyunsaturated n-6 FAs to polyunsaturated n-3 FAs. Blank: fresh Menhadan fish oil, Control: oxidized fish oil oxidized without antioxidants, C3P, BC, GRP, Ch, PN, BR represent fish oil containing cumun-3 parsley, black currant, green river parsley, Chardonnay grape seed, Pinot Noir seed flour and cranberry seed meal extracts at concentrations of 12.9, 26.3, 14.9, 16.7, 13.0, 16.0, and 14.5 flour equivalents per mL oil respectively. Toco represents fish oil containing tocopherol at 130 ppm. Data expressed as mean ± standard deviation (n = 3). Values in the same row sharing the same letter are not significantly different (P < 0.05).

### 2.3.2 Free Radical Scavenging Properties

To further understand the mechanisms involved in their antioxidative actions, seed flour and meal extracts were evaluated for their free radical scavenging capacity against DPPH and peroxy radicals. All tested extracts were able to directly react with and quench DPPH radicals (Figure 12). C3P, BC, GRP, Ch, PN, and BR represent the cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, respectively, while Cr is the cranberry seed meal extract. Solid bars represent the % DPPH quenched at 30 seconds and transparent bars represent the % DPPH quenched at 5 minutes of antioxidant-radical reactions. The final concentration of antioxidants was 26 mg seed flour or meal equivalent/mL in the final antioxidant-radical reactions for all the tested seed flour and meal extracts.

Chardonnay grape seed extract exhibited the strongest DPPH<sup>•</sup> scavenging activity at 30 seconds of antioxidant-radical reactions. In contrast, Pinot noir grape seed flour extract had the highest DPPH<sup>•</sup> activity at 5 minutes of antioxidant-radical reactions followed by that of black raspberry, Chardonnay grape, cumun-3 parsley, green river parsley, black currant and cranberry seed flour or meal extracts. These data indicated that these seed flour extracts have different kinetic and thermodynamic properties in their reactions with DPPH free radicals. These extracts also exhibited significant oxygen radical absorbing capacities (ORAC) (Figure 13).

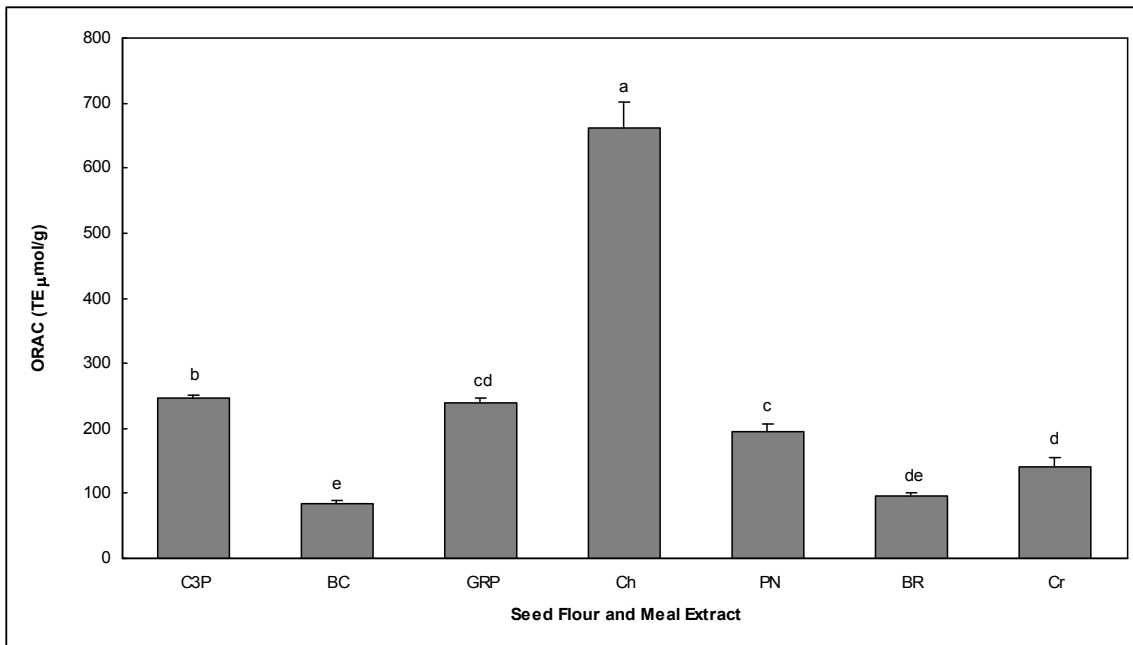


**Figure 12: DPPH radical scavenging capacity of the tested seed flour and meal extracts.** C3P, BC, GRP, Ch, PN, BR represent fish oil containing seed flour extracts at concentrations of 12.9, 26.3, 14.9, 16.7, 13.0, and 16.0 flour equivalents per mL oil respectively. Cr and Toco represent fish oil samples containing 14.5 meal equivalents per mL oil and 130 ppm respectively. Vertical bars represent the standard deviation (n = 3). Values marked by the same letter for the same reaction time are not significantly different ( $P < 0.05$ ).

The greatest ORAC value was 662  $\mu$ moles TE/g in Chardonnay grape seed flour under the experimental conditions, a 2.5 fold higher over the remaining seed flours and meal which varied from 246  $\mu$ moles TE/g in cumun-3 parsley seed flour to 85  $\mu$ moles TE/g in black currant seed flour. ORAC values from these seed flours and meals are higher than that of caneberries tested whole (Wada and Ou 2002). The results from this study are supported by the findings from a previous study that caneberry seeds may have substantial quantities of tocopherols (Bushman and others 2004) and grape seeds are widely recognized



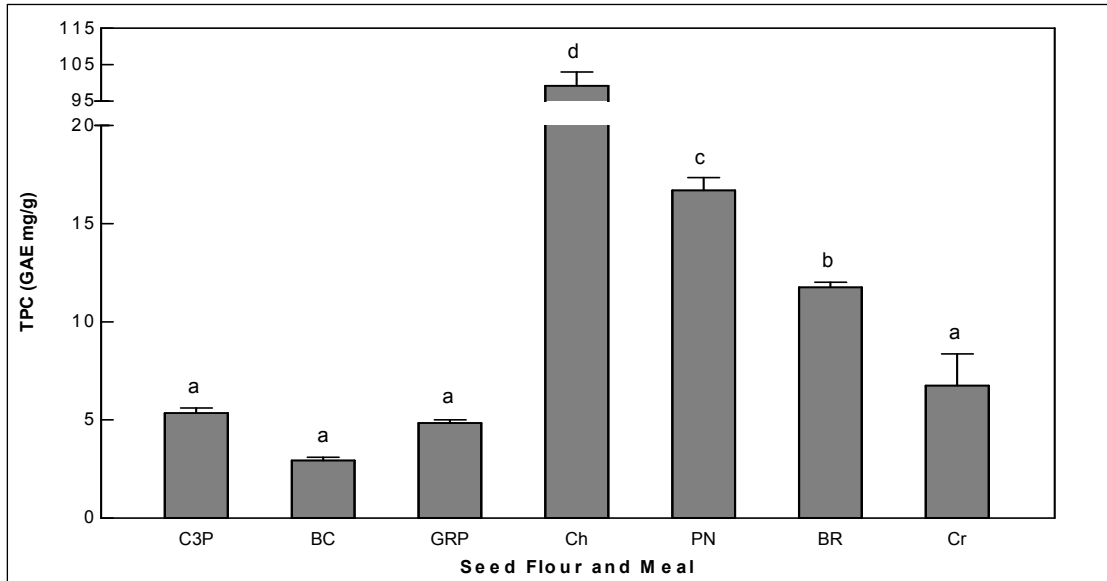
for their antioxidant properties as well as their free radical scavenging abilities (Shi and others 2003). Antioxidants may reduce the risk of cancer, cardiovascular disease, and dermal disorders (Pietta 2000; Yilmaz and Toledo 2004b). Grape skin and seed extracts are safe for consumption in the human diet and may be suitable for antioxidant dietary supplementation (Yilmaz and Toledo 2004a). Therefore, natural antioxidants may provide health benefits to consumers besides improving food quality and stability.



**Figure 13: Oxygen radical absorbing capacity of the selected seed flour and meal extracts.** Results are expressed as  $\mu\text{moles}$  trolox equivalent (TE) per gram of seed meal. C3P, BC, GRP, Ch, PN, BR represent fish oil containing 12.9, 26.3, 14.9, 16.7, 13.0, and 16.0 flour equivalents per mL oil respectively. Cr represents fish oil samples containing 14.5 meal equivalents per mL oil. Data are expressed as means  $\pm$  standard deviations ( $n = 3$ ). Values marked by the same letter are not significantly different ( $P < 0.05$ ).

### 2.3.3 Overall Antioxidant Activity

Total phenolic content (TPC) was evaluated for all tested seed flour and meal extracts because they are believed to contribute to the antioxidant activities. TPC analysis of seed meal extracts was in agreement with other antioxidant tests (Figure 14). C3P, BC, GRP, Ch, PN, BR, and Cr represent the cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, and cranberry seed meal respectively. Results are expressed as mg gallic acid equivalent per gram of seed flour or meal. Chardonnay seed flour with a TPC value of 99 mg gallic acid equivalents/g was substantially higher than the other samples tested on a per weight flour or meal basis although TPC was detected in all seed flour and meal samples.



**Figure 14: Total phenolic content of seed meal extracts.** C3P, BC, GRP, Ch, PN, BR, and Cr represent the cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, and cranberry seed meal respectively. Data are expressed in Gallic Acid Equivalents (GAE) as means  $\pm$  standard deviations ( $n = 3$ ). Values marked by the same letter in the same column are not significantly different ( $P < 0.05$ ).

### 2.3.4 Antimicrobial Activity

Extracts from botanicals have shown antimicrobial activity against various pathogenic microorganisms (Singh and others 2002). In the present study, the seed flour and meal extracts were evaluated using *E. coli* and *L. monocytogenes* for their potential in inhibiting foodborne pathogens (Table 3). All tested seed flour and meal extracts exhibited bactericidal activity against *E. coli* (expressed as ND for not detectable). C3P, BC, GRP, Ch, PN, and BR represent the cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts at concentrations of 2.6, 5.3, 3.0, 3.3, 2.6, and 3.2 mg seed flour equivalent/mL, respectively in the assay culture. Cr and Toco represent cranberry seed meal extract and tocopherol at levels of 2.9 mg seed meal equivalent/mL and 25 ppm (equivalent to about 1 mg  $\alpha$ -tocopherol/mL), respectively.

The antibacterial activity of seed meal extracts is noted as the bacterial survival rates after incubation. The lower the survival rate, the stronger the inhibition effect of the extract. Zero survival was observed in the *E. coli* cultures treated with black currant, Chardonnay grape, Pinot noir grape, black raspberry seed flour, and cranberry seed meal extracts. Also noted was that all the tested seed flour and meal extracts had antibacterial growth activity against *L. monocytogenes* under the 4°C experimental conditions, except the cumun-3 and green river parsley extracts, while none of the tested extracts had bacteriocidal activity under optimal growing conditions. Zero survival was observed in the *L.*

*monocytogenes* cultures treated with black currant, Chardonnay grape and Pinot noir grape seed flour extracts under the testing conditions. The cumun-3 and green river parsley extracts at concentrations of 2.6 and 3.0 mg seed meal equivalent/mL, respectively, had no bactericidal activity against *L. monocytogenes* (Table 3). In comparison, mixed tocopherol at a concentration of 25 ppm, which is equal to 1 mg  $\alpha$ -tocopherol per mL, had slight antiproliferation effect against *E. coli* and no inhibition against *L. monocytogenes*. These data suggest that these seed flour and meal extracts have potential applications as natural food preservatives to inhibit microbial growth and suppress lipid oxidation.

Antioxidant	Dose mg/mL	% Survival Rate (Mean $\pm$ SD)		
		<i>E. coli</i> 35°C	<i>L. monocytogenes</i> 35°C	<i>L. monocytogenes</i> 4°C
<b>C3P</b>	2.6	1.88 $\pm$ 0.00b	Approx. 100a	Approx. 100c
<b>BC</b>	5.3	ND a	Approx. 100a	0.03 $\pm$ 0.00a
<b>GRP</b>	3.0	1.00 $\pm$ 0.22a	Approx. 100a	Approx. 100c
<b>Ch</b>	3.3	ND a	Approx. 100a	ND a
<b>PN</b>	2.6	ND a	Approx. 100a	ND a
<b>BR</b>	3.2	ND a	Approx. 100a	1.43 $\pm$ 0.38b
<b>Cr</b>	2.9	ND a	Approx. 100a	2.86 $\pm$ 0.00b
<b>Toco</b>	25ppm	3.12 $\pm$ 0.00d	Approx. 100a	Approx. 100c

**Table 2: Antibacterial activity against *E. coli* & *L. monocytogenes***

C3P, BC, GRP, Ch, PN, and BR represent the cumun-3 parsley, black currant, green river parsley, chardonnay grape, pinot noir grape and black raspberry seed flour in mg seed flour equivalent/mL, respectively in the assay culture. Cr and Toco represent cranberry seed meal extract and tocopherol in mg seed meal equivalent/mL and ppm (equivalent to about 1 mg  $\alpha$ -tocopherol/mL), respectively. All extracts were applied in the final dose concentration listed in the second column. The antibacterial activity of seed meal extracts is noted as the bacterial survival rates after incubation. The lower the survival rate, the stronger the inhibition effect of the extract. Approximately 100% survival rate indicates that there was no inhibition effect. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

### 2.3.5 Color Analysis

Color is an important sensory property of food products. Most of the botanical extracts have dark color and may alter the color of some final food products. The degree of color alteration in food products depends on the color and level of the extract and the nature of the food matrix. It is important to evaluate these fruit seed flour and meal extracts in model food for their possible effects on food color. Color alteration due to adding these extracts in fish oil was measured by the Hunter L-(lightness), a-(redness), b-(yellowness) values (Table 4). C3P, BC, GRP, Ch, PN, and BR represent fish oil containing cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, respectively. Cr and Toco are fish oil samples containing cranberry seed meal extract and the mixed tocopherols. Hunter color values: L value measures lightness and varies from 100 for perfect white to zero for black; a value measures redness when positive, gray when zero, and greenness when negative; b value measures yellowness when positive, gray when zero, and blueness when negative.

Extracts had variable effects on color perception. Among the tested extracts, cranberry meal and black raspberry seed flour extracts had least influence on fish oil color. The rest of the fruit and spice seed flour extracts made the oil darker, and increased their greenness and blueness. These data may be used to select the food product category to obtain the desired preservation with less undesirable color alteration.

	Concentration	L	a	b
Blank	-	3.55 ± 0.43abc	-1.92 ± 0.13f	2.90 ± 0.55ab
C3P	5.73	5.36 ± 0.06ef	-3.31 ± 0.24bc	3.68 ± 0.10bcd
C3P	12.89	8.49 ± 0.14i	-4.93 ± 0.52a	7.17 ± 0.54h
BC	11.69	3.63 ± 0.12bc	-1.99 ± 0.13ef	3.13 ± 0.20ab
BC	26.31	3.35 ± 0.09ab	-1.87 ± 0.09fg	3.25 ± 0.16ab
GR	6.62	5.13 ± 0.47ef	-2.82 ± 0.42cd	5.28 ± 0.62fg
GR	14.89	7.87 ± 0.09h	-4.40 ± 0.10a	8.79 ± 0.13i
Ch	7.41	5.17 ± 0.04ef	-2.02 ± 0.12ef	4.57 ± 0.12ef
Ch	16.68	4.57 ± 0.03d	-1.18 ± 0.23g	4.27 ± 0.17d
PN	5.79	5.50 ± 0.07f	-2.59 ± 0.26de	4.20 ± 0.18de
PN	13.02	4.60 ± 0.12d	-2.05 ± 0.32ef	3.38 ± 0.26abc
BR	7.13	4.84 ± 0.08de	-2.82 ± 0.18cd	4.07 ± 0.03cde
BR	16.04	6.39 ± 0.11g	-3.63 ± 0.28b	5.77 ± 0.21g
Cr	6.46	3.63 ± 0.03bc	-1.91 ± 0.30f	2.74 ± 0.10a
Cr	14.53	3.95 ± 0.02c	-1.96 ± 0.27f	2.77 ± 0.10a
Toco	60 ppm	3.10 ± 0.08a	-2.03 ± 0.09ef	3.17 ± 0.18ab
Toco	130 ppm	3.07 ± 0.03a	-2.00 ± 0.15e	3.01 ± 0.18ab

**Table 3: Effect of tested seed flour and meal extracts on oil color**

C3P, BC, GRP, Ch, PN, and BR represent fish oil containing cumun-3 parsley, black currant, green river parsley, Chardonnay grape, Pinot noir grape and black raspberry seed flour extracts, respectively. The blank represents fish oil with no added preservatives. Extracts were added to fish oil by volume with final concentrations in the second column. Color is expressed in Hunterlab values where L-(lightness), a-(redness), b-(yellowness). Data are expressed as means ± standard deviations (n = 3). Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

## 2.4.0 Conclusion

Results from this study suggest that natural food preservatives may be developed from edible seed flour and meal. Further testing is required to analyze the specific components of these seed meals and flours to identify the components responsible for their food preservation capacity. Specifically, a high performance

liquid chromatography (HPLC) analysis of the individual phenolic acids may help to explain why the Chardonnay extract was kinetically superior in quenching the DPPH• radical and preferentially preserved the omega-6 FAs while the Pinot Noir extract was thermodynamically favored to quench the DPPH• radical and preferentially preserved the omega-3 FAs and overall was superior at retaining PUFA. This data suggests that a combination of spice and fruit seed extracts may be preferential. In addition, further testing is required to select the proper dose in different food models prior to application. The impact of spice and fruit extracts will most likely have a minimal impact on the sensory properties of complex food matrixes compared to that of fish oil. Overall, these natural food preservatives may stabilize food products, maintain essential fatty acids, and reduce pathogenic microbial load to improve food quality, stability, nutritional value, and safety, while benefiting fruit producing and processing industries.

## 2.5.0 References

- Ahn J, Grun IU, Mustapha A. 2003. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J Food Prot.* 67: 148-155.
- Aliyiannis N, Mitaku S, Tsitsa-Tsardis E, Harvala C, Tsaknis I, Lalas S, Haroutounian S. 2003. Methanolic extract of *Verbascum macrurum* as a source of natural preservative against oxidative rancidity. *J Agric Food Chem.* 51: 7308-7312.
- Athukorala Y, Lee K, Shahidi F, Heu MS, Kim H, Lee J, Jeon Y. 2003. Antioxidant efficacy of extracts of an edible red alga (*Grateloupia Filicina*) in linoleic acid and fish oil. *J Food Lipids.* 10: 313-327.
- Bushman BS, Phillips B, Isbell T, Ou B, Crane JM, Knapp SJ. 2004. Chemical composition of caneberry (*Rubus* spp.) seeds and oils and their antioxidant potential. *J Agric Food Chem.* 52: 7982-7987.
- Buzby JC, Roberts T, Jordan Lin CT, MacDonald JM. 1996. Bacterial foodborne disease: Medical costs and productivity losses. AER-741. Economic Research Service/USDA.
- Chen JH, Ho, CT. 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J Agric Food Chem.* 45: 2374-2378.
- Connor WE. 2000. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr* 71 (Suppl. 1): 171S-175S.
- Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Sci.* 69: 371-380.
- Frankel E, Huang S, Prior E. 1996. Evaluation of antioxidant activity of rosemary extracts, carnosol, and carnistic acid in bulk vegetable oils, and fish oil and their emulsions. *J Sci Food Agric.* 72: 201-208
- Guleria SPS, Vasudevan P, Madhok KL, Patwardhan SV. 1983. Use of tomato seed powder as antioxidant in butter and ghee. *J Food Sci & Technol.* 20: 79-80.
- Hung P, Gu JY, Kaku S, Yunoki S, Ohkura K, Ikeda I, Tachibana H, Sugano M, Yazawa K, Yamada K. 2000. Dietary effects of eicosapentaenoic acid and docosahexaenoic acid esters on lipid metabolism and immune parameters in Sprague-Dawley rats. *Biosci Biotechnol Biochem.* 64: 2588-2593.
- Marino M, Bersani C, Comi G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int J Food Microbiol.* 67: 187-195.



- Matthaus B. 2002. Antioxidant activity of extracts obtained from residues of different oilseeds. *J Agric Food Chem.* 50: 3444-3452.
- Moore J, Hao Z, Zhou K, Luther M, Costa J, Yu L. 2005. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland grown soft wheat. *J Agric Food Chem.* 53: 6649-6657.
- Ozkan G, Sagdic O, Baydar NG, Kurumahmutoglu Z. 2004. Antibacterial activities and total phenolic contents of grape pomace extracts. *J Sci Food Agric.* 84: 1807-1811.
- Parker TD, Adams DA, Zhou K, Harris M, Yu L. 2003. Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Food Chem and Toxicology.* 68(4): 1240-1243.
- Parry J, Yu L. 2004. Fatty acid content and antioxidant properties of cold-pressed black raspberry seed oil and meal. *J Food Sci.* 69: 189-193.
- Pietta PG. 2000. Flavanoids as antioxidants. *J Nat Prod.* 63: 1035-1042.
- Pike, O.A. (2003) Fat Characterization. In: Nielson, S.S., editor Food Analysis 3<sup>rd</sup> Edition. West Lafayette, Indiana: Kluwer Academic/Plenum Publishers. P227-246.
- Ramadan M, Kroh L, Morsel J. 2003. Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *J Agric Food Chem.* 51: 6961-6969.
- Rey AI, Hopia A, Kivikari R, Kahkonen. 2005. Use of natural food/plant extracts: cloudberry (*Rubus Chamaemorus*), beetroot (*Beet Vulgaris* "Vulgaris") or willow herb (*Epilobium angustifolium*) to reduce lipid oxidation of cooked pork patties. *Lebensm-Wiss –Technol.* 38: 363-370.
- Ruxton CHS, Reed SC, Simpson MJA, Millington KJ. 2004. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Dietet.* 17: 449-459.
- Shi J, Yu J, Pohorly JE, Kakuda Y. 2003. Polyphenolic in grape seeds-biochemistry and functionality. *J Med Food.* 6(4): 291-299.
- Singh G, Kapoor IPS, Pandey SK, Singh UK, Singh RK. 2002. Studies on essential oils: Part 10; Antibacterial activity of volatile oils of some spices. *Phytother Res.* 16: 680-682.
- Velioglu YS, Mazza G, Gao L, Oomah BD. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem.* 46: 4113-4117.
- Viljanen K, Kylli P, Kivikari R, Heinonen M. 2004. Inhibition of protein and lipid oxidation in liposomes by berry phenolics. *J Agric Food Chem.* 52: 7419-7424.

- Wada L, Ou B. 2002. Antioxidant activity of phenolic content of Oregon caneberrries. *J Agric Food Chem.* 50: 3495-3500.
- Yilmaz Y, Toledo R. 2004a. Health aspects of functional grape seed constituents. *Food Sci Tech.* 15: 422-433.
- Yilmaz Y, Toledo R. 2004b. Major flavanoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J Agric Food Chem.* 52: 255-260.
- Yu L, Scanlin L, Wilson J, Schmidt G. 2002a. Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. *J Food Sci.* 67: 582-585.
- Yu L, Haley S, Perret J, Harris M. 2002b. Antioxidant properties of extracts from hard winter wheat. *Food Chem.* 78: 457-461.
- Yu L, Adams DA, Gabel M. 2002c. Conjugated linoleic acid isomers differ in their free radical scavenging properties. *J Agric Food Chem.* 50: 4135-4140.
- Yu L, Zhou K, Parry J. 2005a. Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chem.* 91: 723-729.
- Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* Serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C. area. *Appl Environ Microbiol.* 67: 5431-5436.
- Zhou K, Su L, Yu L. 2004. Phytochemicals and antioxidant properties in wheat bran. *J Agric Food Chem.* 52: 6108-6114.