University of Alberta

Oxidative Stability of Omega-3 Polyunsaturated Fatty Acids Enriched Eggs

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Food Science and Technology

Department of Agricultural, Food and Nutritional Sciences

Fall 2009
Edmonton, Alberta

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DEDICATION

This thesis is dedicated to my affectionate grandmother, Genqin Miao,

(February 10th 1929 - August 7th 2009).

ABSTRACT

Omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs are an increasingly important contributor to the egg industry due to the health benefits of n-3 PUFA. During storage and cooking, n-3 PUFA could undergo oxidation and further cause cholesterol oxidation. This study examined stability of n-3 PUFA enriched eggs fortified with antioxidants (vitamin E or organic Selenium [Sel-Plex] or both) following storage and cooking.

Total n-3 PUFA was not affected by cooking and storage, although long chain n-3 PUFA concentration decreased. Overall, both vitamin E and Sel-Plex decreased oxidation of yolk lipids and cholesterol. Cooking increased the lipid and cholesterol oxidation. Four weeks of storage did not affect the cholesterol oxidation products (COPs) content, but increased lipid oxidation. It is possible to make the n-3 PUFA in enriched eggs more stable with dietary antioxidants.

ACKNOWLEDGMENTS

This project was generously supported by Food Safety Initiative under the Canadian Food Safety and Quality Program, with additional support from O & T Farms and Alltech Inc. The financial and moral support provided by Food Safety Initiative, AFNS Entrance Scholarship and the Pacific Egg and Poultry Scholarship Program is deeply appreciated.

I would like to extend my sincere thank you to my supervisors, Dr. Robert Renema and Dr. Jianping Wu for their guidance, support and constant help in every area of my Masters degree throughout the past two years. As well, thank you to Dr. Martin Zuidhof, for his contribution of statistical analysis and constant support which was essential to my success, Dr. Mirko Betti, for his valuable inputs in the project.

Special thanks to Yan Xu, for his contribution of experimental methods and always be the best support for trouble shooting in lab.

I would also like to thank my partner and best friend, Tulia Perez, for all of hard working hours and fun hours we spent together in the past two years.

Deeply thanks to my very important friends, Kaustav Majumder, for always be a severe criticizer to my work and be a reliable supporter in my life, Sandeep Nain, for all of his help in my study, lab work and daily life in Edmonton.

Thank you to all of the people who helped me in the lab and at the PRC. Specifically, Kelvin Lien, Gary Sedgwick, Chris Ouellette, Lyle Bouvier, Felicity Dennis, Nigel Davidson, Shawn Rankin, Jordana Williams, Dileep AO, Shensheng Shen, Sunjong You, Bo Lei and other staffs, post-doctors and students of the University of Alberta Poultry Research Centre.

I would also like to thank the following people for their support and encouragement: Brittany Dyck, Alana Jin, and Qin Liu.

Lastly, thank you to the following people for their deeply love and amazingly support in my life as always: my boyfriend, Ning Xu, my parents, Bicheng Ren and Min Li, my grandparents, Wensan Li and Yongying Zhan and other family members.

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β-CE - β-cholestanetriol

α-CE - α-cholestanetriol

***OH -** hydroxyl

19-OH - 19-hydroxycholesterol

25-OH - 25-hydroxycholesterol

25-OH - 25-hydroxycholesterol

7α-OH - 7-α-hydroxycholesterol

7β-OH - 7-β-hydroxycholesterol

7-KC - 7-keto cholesterol

AHA - American Heart Association

CAT - catalase

CHD - coronary hearth diseases

CLA - conjugated linoleic acid

COPs - cholesterol oxidation products

CT - cholestanetriol

CVD - cardiovascular diseases

DHA - docosahexaenoic acid

DPA - docosapentaenoic acid

EFA- essential fatty acids

EPA - eicosapentaenoic acid

GSH-Px - glutathione peroxidase

H_2O_2 - hydrogen peroxide

HE x HSe - high vitamin E and high selenium treatment.

HE x LSe - high vitamin E and low selenium treatment

HNO_2 - nitrous acid

HOCl - hypochlorous

HOO* - hydroperoxyl

L*- lipid radical

LA - linoleic acid

LDL - low density lipoprotein

LE x HSe - low vitamin E and high selenium treatment.

LE x LSe - low vitamin E and low selenium treatment.

LNA - linolenic acids

LOO*- lipid peroxyl radical

LOOH - stable hydroperoxide

MA - malonaldehyde

MDA - malondialdehyde

n-3 PUFA - omega-3 polyunsaturated fatty acid

n-6 PUFA - omega-6 polyunsaturated fatty acids

NO^- - nitroxyl anion

NO* - nitric oxide

NO_2^* - nitrogen dioxide

O_2* - superoxide

O_3 - ozone

ONOO - peroxynitrite

PL - phospholipids

PUFA - polyunsaturated fatty acids

PUFA - polyunsaturated fatty acids

RDA - Recommended Daily Allowances

RNS - reactive nitrogen species

RO* - alkoxyl

ROO* - peroxyl

ROS - Reactive oxygen species

SFA - saturated fatty acids

SS - sodium selenite

SY - Se-enriched yeast

TBARs - thiobarbituric reactive substances

TG – triglycerides

CHAPTER 1 LITERATURE REVIEW [1]

1.1 Introduction

The egg is well known as a nutritionally balanced, healthy part of the diet. Hence, it is an important part of daily human diet. Due to adverse publicity about saturated fats and cholesterol, egg consumption has suffered declines in per capita consumption in the past two decades. More recent scientific evidence defuses much of this concern and has led to more recent moderate increase in egg consumption. The introduction of the concept of enriched designer eggs has provided addition momentum.

The primary enriched egg driving the growth of the sector is omega-3 polyunsaturated fatty acid (**n-3 PUFA**) enriched eggs. This has been on the strength of the body of evidence about the potential health benefits of n-3 PUFA. A potential concern with increased n-3 PUFA content in food products is susceptibly to oxidation. Furthermore, the presence of a high concentration of polyunsaturated fatty acids (**PUFA**) could enhance cholesterol oxidation susceptibility during storage and cooking and produce cholesterol oxidation products (**COPs**) through the process called "co-oxidation" (Conchillo et al.,

[1] Portions of this chapter are derived from the contributions of Y. Ren to an invited book chapter (Ren, Y., Wu, J. and Renema, R.A. 2008. Nutritional and health benefits of eggs. In Guerrero-Legarreta, I., Mine, Y. and Hui, Y.H. (eds). Handbook of Poult. Sci. and Technology: Volume 1: Primary Processing. Blackwell-Wiley Publishing, New York, In press.)

2005). There is a growing need to characterize the degree of n-3 PUFA stability under storage and cooking conditions. Additionally, the potential of various antioxidants to further stabilize the n-3 PUFA of the egg can be explored.

1.2 Table Egg Production

1.2.1 Egg Production

In the past 35 years, global egg production has grown 203.2% whereas beef and pork production has grown only 57.6% and 186.4%, respectively (Windhorst, 2007). In 1970, beef production reached 38 million tons, while egg production was only 19 million tons. However, about 59 million tons of eggs were produced worldwide in 2005, which was similar to beef production. If the egg production could maintain the same growth rates, egg production will be higher than beef production in a few years.

The rank order of primary egg-producing countries has shuffled in the past 35 years due to shifts in production and consumption. In 1970, the USA was the greatest egg producer in the world, contributing over 20% of the global egg production (Windhorst, 2007). However, the USA's egg production has changed little in since then, contributing to a decrease in its contribution to global egg production to 9 % in 2005. During the same period, China increased egg production over twenty times, contributing to 41.1% of worldwide egg production in 2005. In 1970, the 10 primary egg-producing countries, which were the USA, USSR, Japan, China, Germany, UK, France, Italy, Spain and Poland, held 70.5% of global egg production. This group included only two developing countries. In

contrast, in 2005, six developing countries (China, India, Mexico, Brazil, Indonesia and Turkey) contributed more than two thirds of global egg production (Windhorst, 2007).

In developed countries, egg production is increasing faster than in developing countries, because the total requirement of protein yield is increasing more rapidly in developing countries than in developed countries. This phenomenon can be explained by the data of per capita consumption of eggs, which is a measure of total egg consumption divided by the total population. In the USA, the per capita egg consumption was steadily declining due to the health concerns, resulting the lowest per capita egg consumption was 233.9 in 1991. Since then, the per capita egg consumption was steadily increasing but very slow, and the recent per capita egg consumption was 259 in 2007. In contrast, the egg per capita consumption in China never declined and steadily increased in the past two decades. The egg per capita consumption in 2008 in China was 468. Furthermore, due to growth of population in developing countries, the egg production and consumption were higher in developing countries than developed countries (American Egg Board, 2008).

The changes in egg production in Canada from 1987 through 2003 are represented in Figure 1.1. The egg production was about 480 million dozen in 1988 and it declined until 1990 (Statistic Canada, 1978-2003). The lowest production was about 465 million dozen in 1990. In contrast, from 1991 to 1996, egg production was increased by approximately 20 million dozen. The high growth rate led to the expansion of egg production from 1996 to 2002. In Canada,

about 575 million dozen of eggs were produced in 2002, which was 109 million dozen more than the production in 1990. Historically, rapid increase in egg production has been made possible by improvement in mechanical equipment (Stadelman, 1995). For instance, the automatic caretaker can manage over 100,000 laying hens by controlling watering, feeding, cleaning, ventilation, humidity and egg handling. With this type of technology the manager or workers are needed in moving flocks into the facility, vaccine or egg packaging.

In Canada, the processed egg products include liquid whole egg, egg white, yolk, dried whole egg/egg white/yolk, frozen whole egg/egg white/yolk, hard boiled eggs and pickled hard boiled eggs. The annual egg broken in Canada was increasing from 5.8 million boxes to 10.6 million boxes in the past decade, the annual increase rate of breaking egg in Canada from 1997 to 2008 are demonstrated in Figure 1.2.

1.2.2 Consumer Demand and Enriched Eggs

Consumers have become more health conscious in recent years and have higher demands for quality and healthiness of their food choices. Many prefer food that is not only safe and healthy, but are also characterized by their contribution to healthy growth and the reduced risk of specific chronic diseases such as cardiovascular diseases (**CVD**) and diabetes. Due to these costumers' demand, different types of functional foods have been launched into the market. Omega-3 polyunsaturated fatty acid (**n-3 PUFA**) enriched foods fall into this category. A strong body of research has defined the benefits of n-3 PUFA for

decreased risk of CVD, inflammation and several cancers, as well as for improved infant brain development. The recommended minimum n-3 PUFA intake is 1.6 g/day for men and 1.1 g/day for women (American Heart Association, 2007). To meet these requirements, including n-3 PUFA enriched eggs into daily diet is one way to increase n-3 PUFA intake. Commercial n-3 PUFA eggs were first introduced to the public in 1997. After eleven years, n-3 PUFA enriched eggs represent 12% of eggs marketed in Canada (Egg Farmers of Canada, 2008). These products and their variants are taking up more store shelf space in recent years, which is driven by increasing consumer demands of n-3 PUFA enriched eggs as well as eggs with multiple enrichments (ex: vitamins, selenium, specific n-3 PUFA variants such as DHA).

1.3 Egg Composition and Nutritional Value

1.3.1 Composition of Table Eggs

Eggs consist of approximately 9.5 % egg shell, 63 % egg white and 27.5 % yolk. The composition of a typical 55g White Leghorn egg is presented in Table 1.1. The main components are water (74 %), protein (12.8 %), lipids (11.8%), and small amounts of carbohydrates and minerals. The proteins are distributed in the egg white (50%) and the egg yolk (40%), and small proportion in the egg shell and shell membrane. The lipids are exclusively found in yolk, mainly in form of lipoproteins. The yolk comprises 48% water, 16% protein, 32.6% fat and some minerals and vitamins. The white consists of 88% water, 10% protein and some

minerals. The amount of lipid in the egg white is negligible (0.01%) compared with the amount present in the yolk (Powrie and Nakai, 1986). Several minerals have also been found in eggs, most of them in egg shell; approximately 98% of the shell consists of calcium carbonate. Because lipids only appear in yolk, enriched eggs through modification of n-3 PUFA only affect the egg yolk.

1.3.2 Nutritional Value of Eggs

Since the main pupose of an egg is to support the development of a chick, it contains an excellent balance of nutrients. As mentioned before, there are four major nutritional components in eggs: protein, lipids, and all the necessary vitamins and minerals. The nutrient content of different raw egg components are presented in Table 1.2. All the fatty acids and cholesterol are found in yolk, approximately equal amount of amino acids are found in yolk and white. Except the three fat-soluble vitamins (A, D, E), which are only present in the yolk; other vitamins are present either in the white and the yolk. There are similar amounts of trace minerals present in the yolk and white, sometimes in combination with proteins and lipids (American Egg Board, 2008).

1.4 Egg Fatty Acid Composition and Stability

1.4.1 Lipids in Eggs

The lipids in eggs include triglycerides, phospholipids, cholesterol, cerebroside and some other minor lipids. Lipids are the major components in yolk, representing 60% of the yolk on dry weight basis. Almost all of the sterol found in

egg yolk is cholesterol. Cholesterol contributes to about 1.6% of raw egg yolk and about 5% of egg yolk lipids. Free cholesterol makes up approximately 84% of the total cholesterol with the remaining 16% being cholesterol ester (Cotterill and Glauert, 1979).

In 1970s, American Heart Association (**AHA**) recommended that people should limit their daily cholesterol consumption, and egg was ranked as one of the cholesterol-rich foods and the risk factor of coronary heart disease (**CHD**) (Elkin 2006; Herron and Fernandez, 2004). Brown and Schrader (1990) indicated that the definition of cholesterol information is a major reason for the continuing decline in USA egg consumption based on time-series data. They constructed a cholesterol information index to examine the impact of cholesterol information on egg consumption and found that egg consumption per person was negatively affected by the diffusion of cholesterol information from 1955 to 1987.

In contrast, many current studies did not support this hypothesis since the cholesterol metabolism is complicated in the human system and the absorption rate of cholesterol is varible. Human diet is not the only factor which determines the level of cholesterol in blood (Further details presented in section 1.5.3).

1.4.2 Cooking Effects on Lipid Profile and Stability

Autooxidation of yolk lipid may start during cooking due to high temperature and exposure in oxygen. Yang et al. (2003) indicated that boiling did not affect conjugated linoleic acid (CLA) content in eggs, while frying (40 s, 160 to180 °C) decreased CLA content in eggs from 4.3 to 3.9 mg/g. Similarly,

Cortinas et al. (2003) reported that boiling did not significantly affect fatty acid composition, as total n-3 PUFA in scrambled egg were only numerically reduced compared to fresh eggs. Cooking had a significant effect on oxidative stability measurement by thiobarbituric reactive substances (**TBARs**), with scrambled egg having the highest TBARs value (710.72 ng MDA/g dry matter) compared to boiled (494.55 ng MDA/g dry matter) or raw eggs (138.47 ng MDA/g dry matter). Clearly, frying can cause breakdown of n-3 PUFA and induce the lipid oxidation. In comparison, boiling led to less damage to n-3 PUFA due to the relative lower cooking temperature in egg yolk and also likely due to the protective buffer to the yolk provided by the shell and albumen.

1.4.3 Storage Effects on Egg Quality, Lipid Profile and Stability

Egg quality is an important factor in the marketing of eggs. Good interior quality (membrane strength and albumen quality) and shell quality are very important in the supermarket as well as egg processor. Better shell and internal quality are linked to better separation of components without cross contamination of yolk and albumen, particularly when producing albumen products. Genetic selection program in laying hens have included considerable emphasis on the improvements of these traits.

The process of egg storage can alter internal characteristics. Loss of water and carbon dioxide, and a subsequent increase in the pH of the albumen, which can cause the albumen break down, reduce egg quality (Tona et al., 2001). Jones and Musgrove (2005) examined difference factors of egg quality following on 0 to

10 weeks storage (-4 °C). They found albumen height and Haugh Unit decreased significantly ($P < 0.001$ and $P < 0.0001$, respectively) during storage, and there were no differences during storage for shell or vitelline membrane strength.

Cherian et al. (1996) found up to 40 days of storage at 4 °C did not affect fatty acid profile or TBARs values. Similar results have been proven by Marshall et al. (1994). In contrast, Cherian et al. (2007) reported storage significantly decreased total n-3 PUFA and increased TBARs values with 60 d of storage at 4 °C. Moreover, Maziar et al. (2008) found that TBARs values were increased during 14 d of storage under different storage temperature (4, 23, 27, or 31°C), with higher TBARs values associated with the higher storage temperatures.

Phospholipids and proteins are interwoven in the exterior surface of low density lipoprotein (**LDL**). The compact surface layer can partially exclude O_2 from lipid core of the particle, thus impact oxidation. Therefore, the structural configuration of yolk phospholipids may have contributed to reduce oxidation of long chain n-3 PUFA (Cherian, 2007).

1.5 Designer Egg

A “Designer Egg” was first mentioned by Dr. Jeong Sim and his colleagues in 1990s, and referred to n-3 PUFA enriched eggs. Other available “Designer Egg” products on the market include enriched vitamins (Michella and Slaugh, 2000), lutein (Leeson and Caston, 2004), or selenium, conjugated linoleic acid (**CLA**) enrichment (Van Elswyk, 1997), reducing yolk cholesterol (Sim and

Sunwoo, 2006), as well as various combinations of these enrichment ingredients.

1.5.1 Lutein Enriched Eggs

Lutein is an antioxidant which limits the oxidative stress in tissues that result from metabolism (Rapp et al., 2000). Lutein and zeaxanthin are able to absorb blue light that strikes the retina which is thought to initiate degeneration of the delicate surface membrane (Landrum and Bone, 2001). The daily intake of lutein and zeaxanthin in North Americans is less than 1 mg/d (Landrum and Bone, 2001), which is much less than the recommended preventive level of 6-10 ppm per day (Grando et al., 2003).

With lutein-enriched eggs, Lesson and Caston (2004) reported that it is possible to increase egg yolk lutein 5 to 8 times above regular concentrations, representing an additional 1.5 to 2 mg contribution to lutein daily intake. Although general egg quality was not affected, egg yolk color changed significantly up to a dietary lutein supplementation of 250 ppm. The conversion efficiency of lutein from feed to eggs is approximately 10% with 125 ppm in the diet, declining to 2 to 3% once the hen supplement level reaches 500 ppm. This transfer efficiency was decreased when flaxseed was added to the diet.

1.5.2 CLA Enriched Eggs

Conjugated linoleic acid (CLA) is the name given to a group of positional and geometrical isomers of 18-carbon unsaturated fatty acids with two conjugated double bonds (unlike linoleic acid, which has a non-conjugated diene).

During an investigation of the carcinogenic properties of grilled beef, some fatty acids present were actually found to be anticarcinogenic. Since then, CLAs have been shown to have antiadipogenic, anticarcinogenic, antiatherogenic, antidiabetogenic and anti-inflammatory properties (Wahle et al., 2004; Bhattacharya et al., 2006). Furthermore, individual isomers of CLA have distinct effects on tumorigenesis and lipid metabolism.

Previous studies have shown that concentration of CLA in the yolk lipids linearly increases as dietary CLA increases. Maximum CLA concentrations in the yolk lipids of hens fed 0.5, 2.5, or 5.0% CLA occurred 11 d after the start of the experiment and were 0.82, 5.82, and 11.20% of the total fatty acids respectively (Chamruspollert and Sell 1999). Feeding 5.0% CLA decreased feed intake but did not affect rate of egg production, weight of eggs, albumen, or yolk.

1.5.3 Egg with Reduced Cholesterol Content

Coronary heart disease (CHD) is the leading cause of death in the United States and most developed countries (American Heart Association, 2009). A 1% reduction in plasma cholesterol can lead to 2% of reduction in CHD risk (Lipid Research Clinics Program, 1984). In a typical western diet, approximately 30% of the cholesterol intake comes from table eggs (Kritchevsky and Kritchevsky 2000). Therefore, in the 1979's, American Heart Association (**AHA**) recommended that people should limit their daily egg intake in order to reduce the risk of CHD (Elkin, 2006). However, cholesterol metabolism is highly variable in the human which affects absorption rate. Not only diet, but also other factors such as physical

activity, age and gender, heredity also affect to blood cholesterol (Elkin and Yan, 1999). Howell et al. (1997) found no significant correlation between dietary cholesterol intake level and plasma cholesterol level. Another study indicated that consumption of one egg per day did not have a substantial overall impact on the risk of coronary heart disease and stroke (Hu et al., 2001).

The negative public perception about egg cholesterol levels cannot be changed easily, and consumption of eggs has been increasing very slowly. In 2000, AHA and National Cholesterol Education Program (NCEP) suggested a healthy daily cholesterol intake of less than 300 mg/day. Since cholesterol content in a typical large egg is 213 mg, therefore, consume one egg per day is safe (USDA, 1991); efforts continue to lower egg cholesterol content through genetic selection, nutritional alternative and anti-cholesterol drug addition have been pursued extensively to reduce egg cholesterol levels (Elkin 2006).

1.5.4 Omega-3 Polyunsaturated Fatty Acids Enriched Eggs

1.5.4.1 Benefit of n-3 PUFA

The n-3 PUFA are part of PUFA which have the first carbon-carbon double bond at the third carbon position counting from the omega end of the carbon chain. Important n-3 PUFA are derived from fish oils, such as, eicosapentaenoic acid (**EPA,** 20:5 n-3), docosapentaenoic acid (**DPA**, 22:5 n-3), and docosahexaenoic acid (**DHA**, 22:6 n-3) (González-Esquerra and Leeson, 2000), another important n-3 PUFA, LNA can be extracted from plant oils (Plourde and Cunnane 2007).

Benefits of n-3 PUFA enrichment include decreased risk of CVD, minimize

the hazard of inflammation diseases and several cancers, lower blood triglyceride levels, improved development and function of infant brain, and essential for normal growth and development of good vision. The recommended n-3 PUFA intake is 1.6 g/day for men and 1.1 g/day for women (American Heart Association. 2007).

1.5.4.2 n-6 to n-3 Ratio

Both linoleic acid (**LA**) and linolenic acid (**LNA**) are essential fatty acids, which mammals cannot make, and must be obtained in the diet. Both essential fatty acids can metabolize to long chain fatty acids through elongation and desaturation, with LA converted to arachidonic acid (**AA**, 20:4 n-6) and LNA to EPA and DHA. AA is then a substrate for conversion to the eicosanoids of the series 2 type products (leukotriene B_4, prostaglandin E_2 and thromboxane A_2) which have strong pro-inflammation and pro-aggregation actions. In contrast, the EPA and DHA produce series 3 type products (leukotriene B_5, prostaglandin E_3 and thromboxane A_3), which have anti-inflammation and weak aggregation effects (Drackley, 2000). There is a competition between omega-6 polyunsaturated fatty acids (**n-6 PUFA**) and n-3 PUFA for the desaturation enzymes (Δ4, Δ5 and Δ6 desaturases). Both Δ4 and Δ6 desaturases work more efficiency with n-3 PUFA than n-6 PUFA. Despite this, a high intake of LA will interfere with the desaturation and elongation of LNA. Increased intake of one essential fatty acids will decrease the elongation-desaturation products of the other one.

The typical western diet is characterized by high saturated fat and n-6 PUFA intake, and low n-3 PUFA. The n-6 to n-3 ratio in the chicken egg has

increased dramatically from 1.3 under wild conditions to 15:1 to 16.7:1 under a standard US diet (Simopoulos, 2008). These diets are corn based and typically use corn oil as the fat source. Corn is the primary dietary source of n-6 PUFA in the western diet. Since the ratio between n-6 PUFA and n-3 PUFA in eggs can be easily manipulated through diet enrichment, development of n-3 PUFA enriched eggs can contribute to an improved balance in the ratio of n-6 PUFA and n-3 PUFA in the poultry diet and subsequencely the human diet.

1.5.4.3. Dietary Sources

Egg lipid profile is influenced by the lipid composition of the hen diet. While the proportion of fat in the lipid classes is less pliable due to the need of the hen to support the chick embryos development that would normally occur, yolk fatty acid composition correlated well to dietary fatty acid composition. Flaxseed or flaxseed oil is widely used in poultry egg and meat enrichment due to its high content of LNA (50-60%) (Plourde and Cunnane, 2007). Other n-3 PUFA sources include fish oil (especially menhaden oil), marine algae, and canola oil (González-Esquerra and Leeson, 2000). A list of fats and oils used in poultry feeds and fatty acid composition is presented in Table 1.3. The fatty acids composition of major n-3 PUFA sources are summarized in Table 1.4.

It is generally accepted that the amount of LNA in yolk increases linearly with the diet level of n-3 PUFA up to 10% of flaxseed. However, the conversion of EPA and DHA from LNA does not increase proportionally with LNA increase (Aymond and Van Elswyk, 1995). Furthermore, the use of higher levels of flaxseed in the diet is limited due to the presence of antinutritional factors such as

mucilage, linatine, trypsin inhibitors and phytic acid (Bhatty 1995). Schumann et al. (2003) indicated that the hens fed on 100 g/kg flaxseed diets had lower body weight, liver weight, liver dry matter and fat content, and plasma TG concentrations compared to hens fed the control diet without flaxseed. (Flaxseed can reduce the availability of minerals and also inhibit the activity of proteolitic enzymes) (Ravidaran et al., 1995).

Hens fed with flaxseed enriched diet have a relatively low conversion rate from LNA into DHA or EPA (Aymond and Van Elswyk, 1995). The conversion efficiency from LNA to EPA and DHA in humans (males) is reported to be less than 10% (Emken et al., 1994). Elderly people, hypertensive individuals and some diabetics have a more limited capacity to synthesized EPA and DHA from LNA. Therefore, fish oil which already contains more of long chain n-3 PUFA would be an important addition to the diet (Emken et al., 1994).

Menhaden oil is the most popular fish oil as a source to enrich eggs with long chain n-3 PUFA (EPA, DPA, DHA). Generally, a linear response of DHA content to feeding levels of menhaden oil is observed (González-Esquerra and Leeson, 2000). Oh et al. (1991) reported that the n-3 PUFA in eggs (60 g) contain 760 mg of DHA and EPA by feeding 10% fish oil. Hargis et al. (1991) observed that adding 3% of menhaden oil in diet could slightly increase EPA to approximately 30 mg/yolk compared to DHA at 180 mg/yolk. Compared with flaxseed, eggs enriched with fish oil contain more DHA and EPA, which are thought to have a higher bioavailability than LNA in humans (Simopoulus, 2000). However, menhaden oil supplementation can more easily cause off-flavour in the

eggs (Amini and Ruiz-Ferria 2007).

Marine algae are an efficient dietary alternative to current long chain n-3 PUFA sources. Herber and Van Elswyk (1996) found that marine algae contained about 11.2% of long chain n-3 PUFA on a dry matter basis. It was also found that the presence of marine algae carotenoids may enhance the oxidative stability of n-3 PUFA enriched eggs (Herber and Van Elswyk, 1998). However, they have been difficult to include in poultry diet on a long term basis at high enough levels to properly enrich eggs.

Canola and rapeseed contain about 42% oil, of which about 12% is LNA (Table 1.4). Brettschneider et al. (1995) reported that total n-3 PUFA in eggs were 127 mg and 159 mg supplemented by 15 % and 30 % canola seeds, respectively. However, the transfer efficiency of the LNA from the diet to the eggs was lower in canola seed diet compared with that of flaxseed diet.

1.5.4.4 Impact on Egg Quality and Sensory Quality

Basmacioglu et al. (2003) reported that there was no effect of supplying 1.5% fish oil (**FO**); 4.32% flaxseed (**FS**); 1.5% FO + 4.32% FS; and 8.64% FS in hen diet on egg quality criteria such as egg weight, yolk weight, yolk ratio, albumen height, albumen ratio, shell weight, shell ratio, shell strength or shell thickness. However, supplemental flaxseed in a high dose may cause a decrease in egg quality. Even 10 % flaxseed supplementation decreased yolk weight and increased albumen percentages (Novak and Scheideler, 2001). This is an inclusion rate that would be typical in n-3 PUFA enriched eggs.

The stability of n-3 PUFA enriched egg is important for maintaining egg

quality, since supplementation of n-3 PUFA for laying hen may affect egg sensory quality. Leeson et al. (1998) found that panelists were able to distinguish between eggs from hens fed 10% flaxseed or a control diet. Van Elswyk (1997) indicated that trained flavor panelists could not accept the fishy taste eggs from fed with 30 g/kg menhaden oil. Egg sensory quality need to be maintained to provide a better n-3 PUFA enriched egg for the public.

1.5.4.5 Stability of n-3 PUFA Enriched Eggs

Since the degree of unsaturation (number of double bonds) is associated with the susceptibility of lipid oxidation; the presence of high amount of n-3 PUFA in eggs carries potential risk of lipid oxidation, leading to the development of off flavors. This process can be reduced by adding dietary antioxidants. Vitamin E and Selenium are key components of the antioxidant defence system to reduce lipid peroxidation. Surai (2006) summarized the advantage of enriched egg with antioxidants as followings: decrease susceptibility to lipid peroxidation; prevention formation of fishy taste; designer eggs serving as a good source of antioxidants in human diet.

Vitamin E is the common biological chain-break antioxidant, found in food in the forms of tocopherols and tocotrienols, each containing four isomers. Thurman and Mooradian (1997) indicated that the alpha-tocopherol isomer is the only form of vitamin E that the human body can use. While the body can absorb both natural and synthetic forms of alpha-tocopherol, natural forms have higher bioavailability than synthetic ones. Vitamin E is essential nutrient because it cannot be synthesized in the human body and must be contained in diet (Thurman

and Mooradian, 1997). Major vitamin E sources are vegetable oils and some other plant-derived foods. Vitamin E deficiency is associated with a development of a range of specific diseases involving major tissues of the organism including immune system incompetence, impairment of lipid metabolism, fertility problems and increased susceptibility to common and specific diseases (Machlin, 1991).

Vitamin E is widely used to improve the oxidative stability, because vitamin E is more vulnerable to be oxidized than lipid molecular during food processing or storage, so vitamin E “sacrifices” itself to protect the lipid molecule.

Egg production, vitelline membrane strength, yolk and albumen height, and foam stability was significant improved by adding dietary vitamin E (60 IU vitamin E/kg feed) (Kirunda et al., 2001). Moreover, Kucuk et al. (2003) indicated supplement of dietary vitamin E or vitamin C or both significantly improve laying hen performance in the cold environment.

Vitamin E in the hen diet increases the content of vitamin E in the egg yolk in a dose-dependent manner (Grobas et al., 2002). Similarly to many different nutrients, transfer efficiency of vitamin E decreases with increasing levels of dietary vitamin E in the diet. Galobart et al. (2001) presented the alpha-tocopherol transfer efficiency from feed to egg were 41.8% to 26% when vitamin E was added 52mg/kg to 200 mg/kg in feed. Leeson et al. (1998) recommended the level of feed dietary vitamin E should contain 100 IU per kg for commercial n-3 PUFA egg production.

Selenium is necessary to reduce the oxidative damage of cell membrane of animals and humans because selenium is an essential part of antioxidant enzyme

glutathione peroxidase (**GSH-Px**), which is involved in cellular antioxidant protection (Arthur, 1997). Adequate selenium consumption in the human can improve function of immunoregulation, protect cells from damage of oxidative stress, improve sperm quality, and reduce the risk of CHD, several cancers and inflammatory disease (Dvorska et al., 2006; Surai, 2006).

Selenium can be obtained in either inorganic or organic form. High levels of inorganic dietary selenium is toxic for animals. Attia et al. (2004) fed hens three level of selenium (0, 0.5 and 1 ppm) and found that the body weight, egg production, egg weight and feed conversion ratio decreased when the dietary selenium level increased. Furthermore, egg quality, such as egg shell weight, egg shell thickness and egg breaking strength were also significantly reduced by supplementing 0.5 and 1 ppm dietary Se to the diet, and hen mortality increased in the 1 ppm treatment. This led to recommendation of upper limit of 0.5 ppm Se in the diet.

The tolerance for organic selenium is much greater. Inorganic selenium (sodium selenite and sodium selanate) has lower transfer efficiency to eggs than that of organic selenium (selenomethionine). At a dietary inclusion rate of 0.5 ppm selenium, egg selenium is 0.4 mg/egg with an organic selenium source, compared to 0.2 mg/egg for an inorganic source (Paton et al., 2002). Rutz et al. (2004) found that the supplementation of organic selenium to layer diets significantly improved egg production, egg weight, feed conversion ratio, albumen height and specific gravity. In addition, egg-shell weight and shell thickness were increased by the combination of organic selenium, organic zinc

and organic manganese. Compared to the inorganic selenium (sodium selenite), organic selenium (Se-enriched yeast) contributed to a higher selenium content in eggs and increased egg weight (Payne et al., 2005). There was no initial difference in albumen height between those two treatments, but albumen height was higher in selenium yeast eggs after storage at 22.2 °C (Payne et al., 2005).

1.6 Oxidation and Anti-oxidation

1.6.1 Antioxidant : prooxidant Balance

In the animal body, there is an equilibrium antioxidant defence system and the generation of free radicals. The normal antioxidant-prooxidant balance is illustrated in Fig 1.3. Factors affecting the normal antioxidant defense system include proper diets, optimum environment condition, and prevention of disease, appropriate amount of Mn, Cu, Zn, and Se.

Oxidative stress mainly results from the overproduction of free radicals. The oxidative stress conditions can be divided into three categories and include nutritional factors, environmental factors and internal factors (Surai, 1999)

The most important source of stress conditions is nutritional factors, which include presence of toxic compounds, deficiency of antioxidants, deficiency of Zn, Cu, high level of PUFAs, and overload of Fe. Undesirable environmental factors will cause oxidative stress as well, such as high temperature and humidity, hyperoxia, radiation. Bacterial, and diseases as well as allergies are internal sources of oxidative stress (Surai, 1999).

In commercial poultry production, there are timepoints where oxidative stress is increased. Surai (2006) identified increasing interval between egg being laid and cooling down for storage, high storage temperature, carbon dioxide concentration and humidity during storage and hatching, delay in collection of chicks from incubator, disease challenge, mycotoxins in the feed, oxidized fat in the diet, vitamin A excess in the diet, as some key sources of oxidative stress. Late incubation is particularly stressful; broiler embryos quadruple in weight during the final week of incubation.

1.6.2 Oxidation in Food and Animal

1.6.2.1 Free Radicals

Free radicals are highly unstable and reactive due to the presence of one or more unpaired atomic electrons. Free radicals damage biologically important molecules such as DNA, protein, lipid and carbohydrate. Reactive oxygen species (**ROS**) and reactive nitrogen species (**RNS**) are the two major groups of free radicals (Halliwell and Gutteridge, 1999). Reactive oxygen species include superoxide (**O_2***) alkoxyl (**RO***), hydroxyl (***OH**), hydroperoxyl (**HOO***), peroxyl (**ROO***), hydrogen peroxide (**H_2O_2**), hypochlorous (**HOCl**), ozone (**O_3**) and singlet oxygen ($^1O_2$). Reactive nitrogen species include nitric oxide (**NO***), nitrogen dioxide (**NO_2^***), peroxynitrite (**ONOO**), nitroxyl anion (**NO^-**) and nitrous acid (**HNO_2**).

Superoxide is the main free radical produced in biological systems during normal respiration in mitochondria. In the human body, about 1.72 kg of O_2^* is

produced per year (Halliwell, 1994). Superoxide is not extremely dangerous and it does not cross the lipid membrane bilayer. However, the O_2^* is the precursor of other ROS and RNS. For example, by donating an electron, O_2^* can react with Fe^{3+}/Cu^{2+} and produce H_2O_2 and *OH. The hydroxyl radical (*OH) is the most reactive species, it can damage any biological molecule, and is a trigger of the chain reaction of lipid peroxidation (Surai, 2006).

1.6.2.2 Lipid Oxidation

The most important effect on cellular metabolism of free radicals is their involvement in lipid peroxidation reactions (Surai, 2006). There are three phases in lipid oxidation reaction: initiation, propagation and termination. For example, a lipid molecule (**LH**) is attacked by *OH:

Initiation: $LH + *OH \longrightarrow L* + H_2O$

Propagation: $L* + O_2 \longrightarrow LOO*$

Termination: $LOO* + LH \longrightarrow LOOH + L*$

During the initiation phase, lipid radical (**L***) is formed after LH is attacked by the reaction initiator (hydroxyl radical, radiation etc.). The lipid peroxyl radical (**LOO***) is produced after L* reacts with oxygen (**O_2**), causing the unstable radical L* to become the highly reactive radical LOO*. The LOO* attacks peroxidazable materiel, producing a stable hydroperoxide (**LOOH**) and a new lipid radical (L*) that continue the reaction cycle. The lipid oxidation chain reaction can therefore lead to a substantial amount of damage to the cell.

Long chain PUFA (EPA, DPA, DHA, and AA) are present in phospholipids,

which are the major component of cell membrane. Within the cell membrane, the long chain PUFA contribute to the maintenance of the basic membrane properties such as fluidity and permeability. If DHA and AA are attacked by free radicals, an oxidation chain reaction is triggered inside the membrane. Fluidity and permeability of the membrane will be affected. Damage of cell membrane can lead to the apoptosis of the cell (Ames, 2003; Diplock, 1991). Damage to the n-3 PUFA in triglyceride fraction is less catastrophic because the triglyceride are more likely to be the main form of lipid rather than a structural lipid. In chicken meat, the LNA from flaxseed has been reported to associate closely with the triglyceride fraction while the EPA, DPA and DHA were primarily in the phospholipids fraction of the muscle fat (Betti et al., 2009)

1.6.2.3 Cholesterol Oxidation

Similarly to the n-3 PUFA oxidation reaction, cholesterol oxidation is also initiated by the attack of free radicals (Kubow, 1993). The major COPs found in foods are 7-α-hydroxycholesterol (**7α-OH**), 7-β-hydroxycholesterol **(7β-OH)**, β-cholestanetriol (**β-CE**), α-cholestanetriol (**α-CE**), 25-hydroxycholesterol (**25-OH**), cholestanetriol (**CT**) and 7-keto cholesterol (**7-KC**) (Larkeson et al., 2000). Tai et al. (2000) indicated that heating, and storage time are the major factors for formation of COPs in egg and egg products. For instance, Kou and Holmes (1985) failed to detect 25-OH in fresh egg yolk and yolk powder; however, after heating at 110 ºC for 4 days, a significant amount of 25-OH was found in yolk powder. Likewise, Nourooz-Zadeh and Appelqvist (1987) initially found no COPs in fresh or freeze dried egg yolks, but this increased after one year

of storage to 36 ppm concentration of total COPs. One of the COPs, 7-KC, inhibits the activity of HMG-CoA reductase, rate-limiting enzyme for cholesterol biosynthesis in eggs. However, Vargas and Naber (1984) found that 7-KC did not lead to any significant change in yolk cholesterol level, although 43% of the activity of 3-hydroxy-3-methylglutaryl-Coenzyme A (**HMG-CoA**) reductase was reduced.

Cholesterol oxidation products are linked to the propagation of CVD. The COPs can build up and cause apoptosis in the vascular cells. They are consistently found within the walls of major arteries and in the lesions of atherosclerosis. Lizard et al. (1999) found that a final concentration of 20 to 30 μM or above is considered as toxic for 7β-OH, whereas 40 to 60 μM for 7-KC on human culture smooth muscle cells (SMC). In another study, the apoptosis occurred in a human monocytic cell line after treatment of 25-OH or 7β-OH at a final concentrations above 20 to 30 (Aupeix et al., 1995). Furthermore, the presence of 25-OH could highly inhibit the T-Cell response to different stimuli, and the apoptosis process was accelerated (Christ et al., 1993).

1.6.3 Three Levels of Antioxidant Defence

Living in an oxygen-rich and nitrogen-rich environment, animals have had to adapt through development of an antioxidant system to protect against oxidative damage (Halliwell, 1994). Four types of natural antioxidants have been recognized in the animal body: natural fat-soluble antioxidants (vitamin A,E,C, carotenoids), water–soluble antioxidants (ascorbic acids, uric acids, taurine),

antioxidant enzymes (glutathione peroxidase [**GSH-Px**], catalase [**CAT**], and superoxide dismutase [**SOD**]) and the thiol redox system (consisting of glutathione system and thioredoxin system). These systems cope with most oxidation challenges through a range of mechanisms.

1.6.3.1 Antioxidant Enzymes Complexes

Three antioxidant enzymes (SOD, GSH-Px and CAT) and metal-binding proteins constitute the first level antioxidant defence. Since the O* is the major free radical in cellular physiology condition in cell, the SOD is working as the major antioxidant in the first level of antioxidant defence (Surai, 1999), the reaction is follows:

$$2O_2^* + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Then, the hydrogen peroxide (H_2O_2) which is also one of the active ROS, can be detoxified to water by GSH-Px and CAT as follows:

$$H_2O_2 + 2GSH \xrightarrow{GSH-Px} GSSG + 2H_2O$$

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Since the GSH-Px has much higher affinity for H_2O_2 than CAT (Chaudiere and Ferrari-Illiou, 1999) and wider distribution in cell (CAT is located mainly in peroxisomes), GSH-Px is the major enzyme to remove H_2O_2 from cell.

The decomposition of LOOH can also accelerate by transition of metal iron into the cytotoxic products such as aldehydes, alkoxyl radicals. Even worse, highly active free radial (LOO*) can be produced during metal iron transportation:

$$LOOH + Fe^{2+} \longrightarrow LO^{*} + Fe^{3+} + OH^{-}$$

$$LOOH + Fe^{3+} \longrightarrow LOO^{*} + Fe^{2+} + H^{+}$$

Therefore, the metal-binding proteins, such as transferrin, lacoferrin, and haptoglobin, can "catch" the free metal irons and reduce the oxidation stress (Halliwell et al., 1999).

1.6.3.2 Chain Break Antioxidants

Some free radicals escape the first level of antioxidant defence. The second level of antioxidant defence consist of chain-break antioxidants, such as vitamin A,E,C ascorbic acid, and uric acid. During the propagation phase of lipid oxidation, the chain-break antioxidants are more vulnerable to attack than lipid molecules. The chain-break antioxidants inhibit peroxidation by keeping the oxidation chain reaction as short as possible (Gutteridge and Halliwell, 1990). As an example, the antioxidant defence of vitamin E (tocopherol, **Toc**), the major chain-break antioxidant follows:

$$LOO^{*} + Toc \longrightarrow Toc^{*} + LOOH$$

However, LOOH is produced in this reaction, and LOOH is toxic and can impair membrane structure and function if it is not removed from the cell (Gutteridge and Halliwell, 1990), The LOOH is removed in the same way as H_2O_2. Becasue CAT is unable to detoxify LOOH, the Se-dependent GSH-Px (Se-GSH-Px) converts LOOH to non-reactive products (Brigelius-Flohe, 1999) as follow:

$$LOOH + 2GSH \xrightarrow{GSH-Px} LOH \text{ (non-tocix)} + H_2O + GSSG$$

Thus, vitamin E performs half of the job in preventing lipid peroxidation, and Se-GSH-Px completes the process.

1.6.3.3 Cellular Repair Mechanism

If the first two levels of antioxidant defence are not able to stop ROS and RNS from damaging on lipid, proteins and DNA, the third level of antioxidant defence, which consisting of lipases, peptidases, proteases and other enzymes (DNA repair enzyme, ligase etc.) will eliminate the damaged the molecules or repair them.

Several experiments on animal and human have shown that selenomethionine can directly and indirectly effect DNA integrity and repair by enhancing repair complex formation of DNA repaire enzymes (Waters et al., 2003, Mukherjee et al., 2001).

1.7 Research Objectives and Project Design

Eggs can be enriched with n-3 PUFA through alterations to the hen diet. However, work on the stability of enriched products is sporadic and has primarily been done on raw products. Little has been done to evaluate risks associated with enrichment. One such risk is the phenomenon of co-oxidation, where the egg enrichment of n-3 PUFA can lead to an increase of COPs compounds that have been indicated to have adverse biological effects, such as mutagenicity, carcinogenicity, angiotoxicity, cytotoxicity, atherogenicity, atherosclerosis, cell membrane damage and inhibition of cholesterol biosynthesis. These processes

accelerate during cooking.

While vitamin E can reduce this effect, increasing antioxidant protection in the eggs through other means has not been well investigated. Organic selenium (selenomethionine) is absorbed well and increases GSH-Px (antioxidant protection pathway) activity in cells. Unlike some other natural antioxidants, it has demonstrated effect on cell membrane strength, resulting in improved egg and meat quality. The combined effects of Vitamin E and selenium on reducing co-oxidation is unique and could lead to effective means to protect the functional capacity of n-3 PUFA products.

This project was designed to assess the stability of n-3 PUFA enriched eggs and the formation of potential hazardous compounds associate with lipid oxidation. To mitigate these associated problems we used n-3 PUFA enriched diet fortified with antioxidants (Vitamin E, or Sel-Plex, or both).

Objectives: In this project, we employed employ GC and HPLC methodologies to determine the concentration of n-3 PUFA and genotoxic products (of PUFA origin and of cholesterol origin) in eggs. We planned to:

(a) Study the stability and fate of n-3 PUFA in feed enriched n-3 PUFA eggs under simulated retail storage condition and varied cooking methods.

(b) Determine the n-3 PUFA and cholesterol-oxidized genotoxic compounds formed during storage and cooking.

(c) Determine the degree to which antioxidants (vitamin E and Sel-Plex) in feed prevent the production of genotoxic compounds through the inhibition of oxidation of n-3 PUFA and cholesterol during storage and cooking.

The goal of this project was to work towards the improvement of enriched egg products while at the same time contributing to customer safety. The means to establish and protect the health benefits of n-3 PUFA enriched products is a key part of the provision of ‘food-for-health’ products.

Table 1.1 Composition of a table egg [a]

Egg component Percentage	Percentage
Entire egg	
Water	73.6
Solids	26.4
Organic matter	25.6
Proteins	12.8
Lipids	11.8
Carbohydrates	1
Inorganic matter	0.8
Fractions in the yolk	
Water	48
Solids	51.3
Organic matter	50.2
Proteins	16.6
Lipids	32.6
Carbohydrates	1
Inorganic matter	1.1
Fractions in the albumen (White)	
Water	87.9
Solids	12.2
Organic matter	11.6
Proteins	10.6
Lipids	Trace
Carbohydrates	0.9
Inorganic matter	0.6

[a] Adapted from Board (1969).

Table 1.2 The nutrient composition of the edible portion of fresh raw hen's egg and egg components [a].

Nutrient (unit)	Whole egg	Egg White	Egg Yolk
Proximate			
Water	37.66	29.33	8.1
Food energy—calories.	75	17	59
Protein (N x 6.25)--g.	6.25	3.52	2.78
Total lipid--g.	5.01	--	5.12
Total carbohydrate--g.	0.61	0.34	0.3
Ash--g.	0.47	0.21	0.29
Lipids			
Fatty acids as triglycerides--g.	4.327	--	4.428
Saturated--total	1.55	--	1.586
8:0 Caprylic	0.002	--	0.002
10:0 Capric	0.002	--	0.002
12:0 Lauric	0.002	--	0.002
14:0 Myristic	0.017	--	0.017
16:0 Palmitic	1.113	--	1.139
18:0 Stearic	0.392	--	0.401
20:0 Arachidic	0.02	--	0.02
Monounsaturated--total	1.905	--	1.949
14:1 Myristoleic	0.005	--	0.005
16:1 Palmitoleic	0.149	--	0.152
18:1 Oleic	1.736	--	1.776
20:1 Eicosenoic	0.014	--	0.014
22:1 Erucic	0.002	--	0.002
Polyunsaturated--total	0.682	--	0.698
18:2 Linoleic	0.574	--	0.587
18:3 Linolenic	0.017	--	0.017
20:4 Arachidonic	0.071	--	0.073
20:5 Eicosapentaenoic	0.002	--	0.002
22:6 Docosahexaenoic	0.018	--	0.019
Cholesterol--mg.	213	--	213
Lecithin--g.	1.15	--	1.11
Cephalin--g.	0.23	--	0.219
Vitamins			
A--IU	317	--	323
D--IU**	24.5	--	24.5
E--mg.	0.7	--	0.7
B12--mcg.	0.5	0.07	0.52
Biotin--mcg.	9.98	2.34	7.58
Choline--mg.	215.06	0.42	216
Folic Acid (Folacin)--mcg	23	1	24
Inositol--mg.	5.39	1.38	3.95
Niacin--mg (B3)	0.037	0.031	0.002
Pantothenic acid--mg	0.627	0.04	0.632

Pyridoxine (B6)--mg.	0.07	0.001	0.065
Riboflavin (B2)--mg.	0.254	0.151	0.106
Thiamine (B1)--mg.	0.031	0.002	0.028
Minerals--mg.			
Calcium	25	2	23
Chlorin	87.1	60	27.1
Copper	0.007	0.002	0.004
lodine	0.024	0.001	0.022
Iron	0.72	0.01	0.59
Magnesium	5	4	1
Manganese	0.012	0.001	0.012
Phosphorus	89	4	81
Potassium	60	48	16
Sodium	63	55	7
Sulfur	82	56	25
Zinc	0.55	--	0.52
Amino Acids--g.			
Alanine	0.348	0.203	0.143
Arginine	0.375	0.191	0.199
Aspartic acid	0.628	0.358	0.272
Cystine	0.145	0.091	0.05
Glutamic acid	0.816	0.467	0.353
Glycine	0.21	0.123	0.086
Histidine	0.148	0.079	0.072
Isoleucine	0.341	0.199	0.141
Leucine	0.534	0.296	0.244
Lysine	0.449	0.239	0.221
Methionine	0.195	0.121	0.069
Phenylalanine	0.332	0.205	0.119
Proline	0.249	0.137	0.116
Serine	0.465	0.242	0.238
Threonine	0.3	0.16	0.148
Tryptophan	0.076	0.043	0.033
Tyrosine	0.255	0.137	0.124
Valine	0.381	0.224	0.155

Assayed nutrient values for a large raw eggs based on 59 g, shell weight with 50 g, total liquid whole egg, 33.4 g. white and 16.6 g. yolk.

[a] Adapted from American Egg Board (2008) and Cotterill and Glauert (1979).

Table 1.3 Fatty acid composition of various oil sources used in poultry [a]

Fat Source	SFA [1]	PUFA [2]		MUFA [3]
		n-6 [4]	n-3 [5]	
Restaurant grease	21.4	23.3	2.6	52.4
Canola oil	7	22	10	61
Flaxseed oil	10	17	55	18
Safflower oil	10	76	Trace	14
Sunflower oil	12	71	1	16
Corn oil	13	57	1	29
Soybean oil	15	54	8	23
Cottonseed oil	27	54	Trace	19
Beef tallow	48	2	1	49
Palm oil	51	10	Trace	39
Fish oil	16.8	10.9	26.4	41.5
Menhaden fish oil	26.9	2.2	29.5	25

[a] Adapted from Cherian 2007. Values reported as percentages (weight of total fatty acids) and subject to change due to differences in batch, cultivars or processing method used.

[1] SFA = saturated fatty acids

[2] PUFA = polyunsaturated fatty acids

[3] MUFA = monounsaturated fatty acids

[4] n-6 = omega-6 polyunsaturated fatty acids

[5] n-3 = omega-3 polyunsaturated fatty acids

Table 1.4 The composition of dietary sources of omega-3 polyunsaturated fatty acids (n-3 PUFA). (% of total polyunsaturated fatty acids)

Source	LNA [1]	EPA [2]	DPA [3]	DHA [4]
Flaxseed oil [a]	53.5 %	_	_	_
Menhaden oil [a]	0.3 %	11.0 %	1.9 %	9.1 %
Marine algae [b]	_	_	3.8 %	7.4%
Canola oil [b]	12.0 %	_	_	_

[a] National Research Council (1993)
[b] Herber and Van Elswyk (1996)
[1] LNA = alpha-linolenic acid, 18:3 n-3
[2] EPA = eicosapentaenoic acid, 20:5 n-3
[3] DPA = docosapentaenoic acid, 22:5 n-3
[4] DHA = docosahexaenoic acid, 22:6n-3

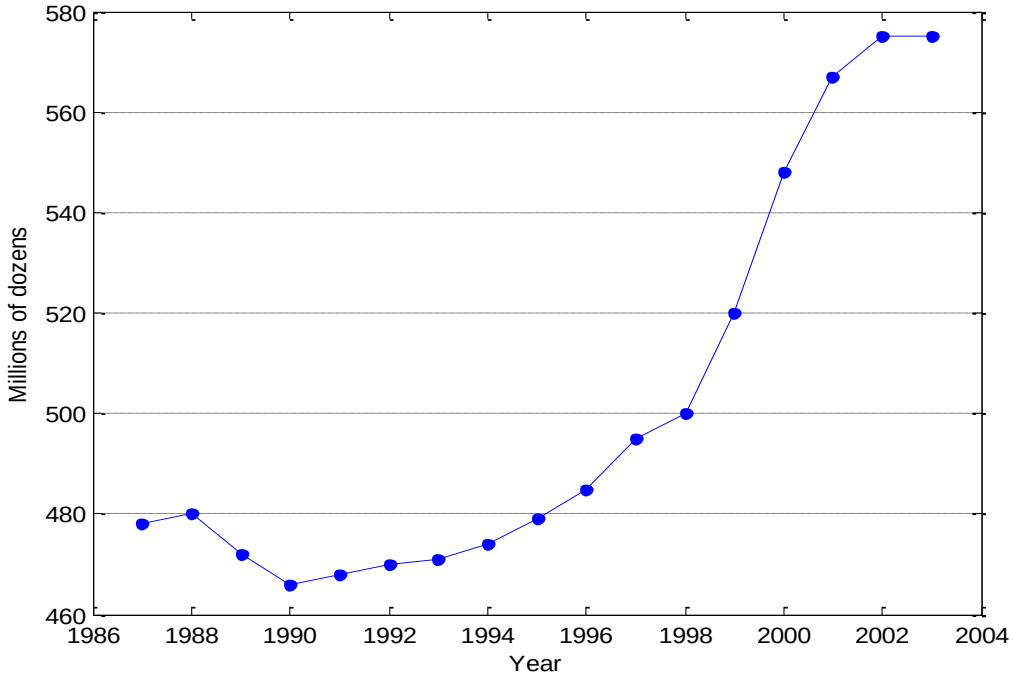

Figure 1.1 Production of eggs in Canada, 1987-2003
Source: Egg Production, Statistics Canada (1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003).

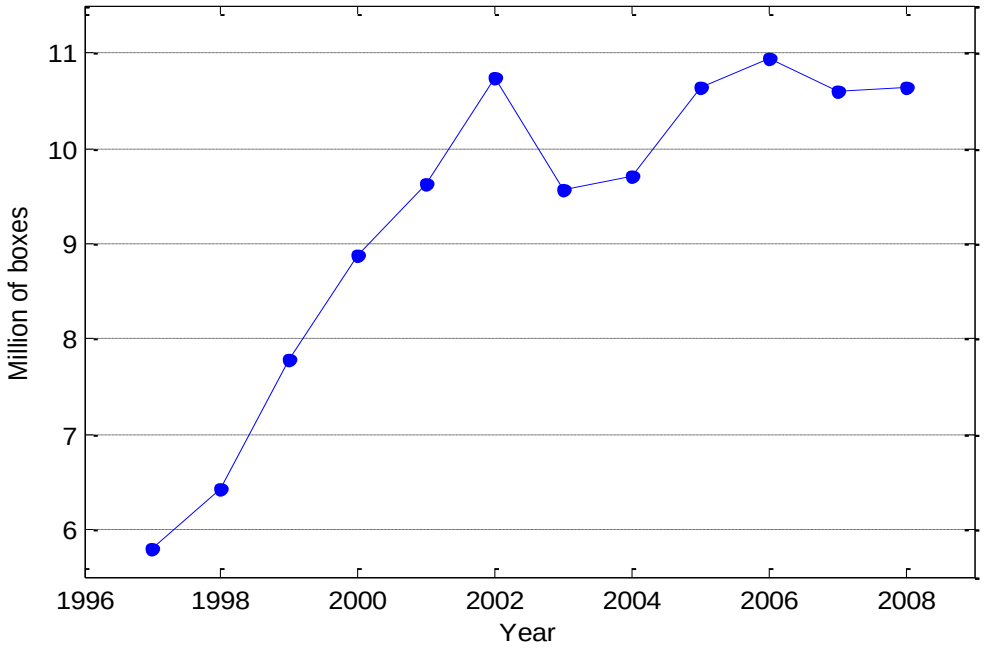

Figure 1.2 Annual Egg broken in Canada. (In box of 15 dozen)
Source: Processed Egg Production, Agriculture and Agri-Food Canada (1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008).

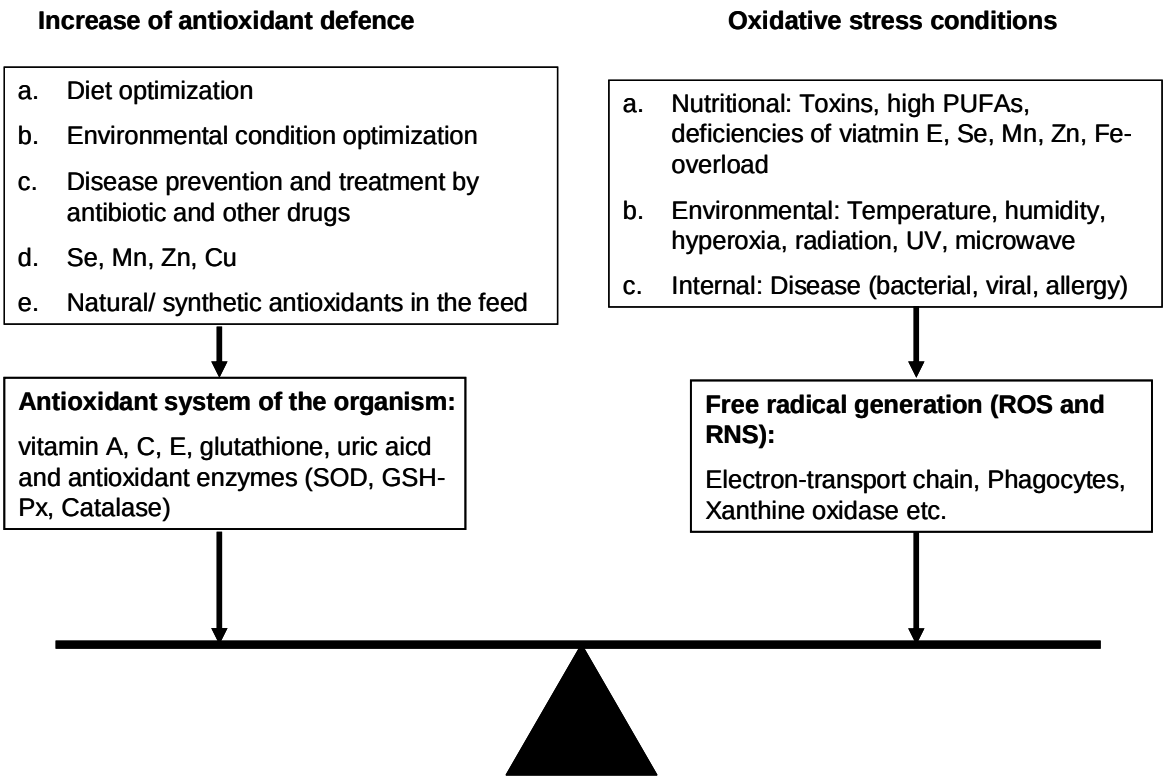

Figure 1.3 Antioxidant-prooxidant balance in the organism[a].
[a] Adapted from Surai (1999)

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CHAPTER 2 OXIDATIVE STABILITY OF OMEGA-3 FATTY ACIDS IN ENRICHED EGGS

2.1 Introduction

Omega-3 polyunsaturated fatty acids (**n-3 PUFA**), mainly eicosapentaenoic acid (**EPA,** 20:5 n-3), docosapentaenoic acid (**DPA**, 22:5 n-3), docosahexaenoic acid (**DHA**, 22:6n-3) and alpha-linolenic acid (**LNA**, 18:3 n-3), reduce the risk of cardiovascular diseases and certain forms of cancer, as well as improved brain development and function (Simopoulos, 2000; Damsgaard et al., 2007). Recommended minimal intake level of n-3 PUFA is 1.6 g/day for men and 1.1 g/day for women; a deficiency of n-3 PUFAs can lead to symptoms including cardiovascular disorders, hypertension, more rapid aging, acne, and other conditions (American Heart Association. 2007). Omega-3 polyunsaturated fatty acids deficiency is calculated to cause 72,000 to 96,000 preventable deaths annually in just the USA (Nutraceuticals World, 2009). While omega-6 polyunsaturated fatty acids (**n-6 PUFA**) are also an important part of diet, daily need are more than met through sources like corn oil.

There are various n-3 PUFA enriched food products available on the market, ranging from eggs to yogurt, bread and even drinks. Compared to other foods, eggs are highly digestible for humans and an efficient “carrier” for enrichment nutrients. So, n-3 PUFA enriched eggs are an effective method to enhance n-3 PUFA intake without significant dietary change. Enriched egg products continue

to expand market share globally.

The unsaturated bonds of both n-3 PUFA and n-6 PUFA are susceptible to oxidation (Conchillo et al., 2005). The relatively high concentration of unsaturated fatty acids in enriched egg products makes these products susceptible to oxidative damage during cooking preparations and storage. Lipid oxidation yields free radials in the cell, and free radicals can cause more oxidation damage as chain reactions and trigger secondary oxidation reactions. Some secondary oxidation products of lipid are toxic chemicals from lipids, including malonaldehyde (**MA**) and other dicarbonyl compounds (Kazutoshi and Takayuki, 2004).

Oxidation of n-3 PUFA (particularly susceptible to oxidative damage during cooking) not only produces genotoxic compounds (causing cell and DNA damage), but also accelerate the formation of cholesterol oxidation products (**COPs**), which have been shown to be responsible for the proatherogenic action of cholesterol (Kubow, 1993).

Vitamin E has traditionally been added to diets to help stabilize n-3 PUFA in storage, but little work has been done to test the vitamin E impact on stability under different cooking methods. Organic selenium (selenomethionine) is an essential part of a variety of selenoproteins, such as glutothione peroxidase **(GSH-Px).** Selenomethionine increased activity of GSH-Px, which is an important antioxidant complex (Surai, 1999). Vitamin E works better with GSH-Px, because GSH-Px continues the work of vitamin E by detoxifying hydroperoxides (Surai et al., 2001). The combination of vitamin E and organic Se

could therefore be an effective antioxidant for n-3 PUFA in enriched eggs and egg products during storage. In the current study, the stability of n-3 PUFA enriched eggs fortified with antioxidants (vitamin E or organic Selenium [Sel-Plex] or both) was tested.

Therefore, the objectives of the current study were to determine the effect of dietary vitamin E or Sel-Plex or both on (1) the stability and fate of n-3 PUFA in n-3 PUFA enriched eggs under simulated retail storage conditions and varied cooking methods, (2) the PUFA- and cholesterol-oxidized genotoxic compounds formed during storage and cooking, and (3) if a combination of vitamin E and Sel-Plex works better than individual ones in stabilization of n-3 PUFA in enriched eggs.

2.2 Materials and Methods

2.2.1 Animals and Dietary Treatments

A total of 120 laying hens (White Leghorn, 37 weeks old) were randomly divided in 4 groups of 30 (housed in pairs). Omega-3 polyunsaturated fatty acids enriched diets were fed to all hens (18.7% Crude Protein; 3,000Kcal/kg), and included 17% LinPRO (50:50 extruded mixed flaxseed and field peas, O & T Farm, Regina, SA, Canada). There were 2 antioxidants (vitamin E and selenomethionine source [Sel-Plex]), both of which were provided at a low (50 IU/kg vitamin E and 0.1 mg/kg Se as sodium selenite) and high (200 IU/kg vitamin E and 0.3 mg/kg Sel-Plex) level, in a 2 x 2 factorial arrangement. The

four diets were as follows:

1) control diet: base diet only, which contain 50 IU/kg Vitamin E and 0.1 mg/kg Se as sodium selenite (low vitamin E+ low selenium, **LE x LSe**)

2) vitamin E diet: base diet + 200 IU/kg vitamin E supplement (high vitamin E+ low selenium, **HE x LSe**)

3) selenium diet: base diet + 0.3 mg/kg Sel-Plex, (low vitamin E + high selenium, **LE x HSe**)

4) vitamin E + selenium: base diet + 200 IU/kg Vit E and 0.3 mg/kg Sel-Plex, (high vitamin E + high selenium, **HE x HSe**).

The diets were presented in Table 2.1. Following 28 d of feeding the experimental diets, 238 eggs were collected and equally divided into 4 pools per treatment. Following 0 or 28 d of simulated retail storage (4 ºC, in shell inside Styrofoam cartons), half of the eggs from each pool were cooked using home-style cooking methods (raw / boiled / fried), then frozen at -20 ºC until analysis.

2.2.2 Egg Traits

At the start and end the end of the trial, total egg and component weights were measured. The analyzed egg traits included egg weight, specific gravity, yolk weight, albumen height, shell think and shell weight. Daily egg production was recoreded throughout the study.

2.2.3 Sample Preparation

To prepare boiled eggs, eggs were placed in single layer in a saucepan, with at least one inch of cold water over tops of shells. After boiling 10 min, eggs were placed under running water (room temperature) for 5 min. Then, yolks were separated from albumen and packaged using a vacuum packager (Food Saver® Vac 1200, 110V). To prepare fried eggs, a non-stick frying pan (Multi-cuisine SK200 non-stick frying pan, Black&Decker, MD.) was preheated for 30 min (177 °C/350 °F) and approximately 15 g of yolk were oil-free fried for 80 s (40 s each side) and vacuum packaged. To prepare raw yolk samples, yolk were separated from albumen and vacuum packaged. All egg samples were freeze dried and stored in sealed container at -20 °C pending further analysis.

2.2.4 Lipid Extraction

Total lipids were extracted from the fine powder of raw, boiled and fried yolk samples by direct chloroform extraction method (Mirjana, 1992). Around 0.5 g of powder sample was weighed, and 10 mL of chloroform (Fisher Scientific, Ottawa, ON, CA) was added to dissolve the yolk lipid completely, sample placed in chloroform for more than 16 h. Then, 10 mL of Hexane (Fisher Scientific, Ottawa, ON, CA) was added; after centrifugation at 3000 x *g* for 5 min, 10 mL of supernatant was transferred into a pre weighed scintillation vial, and dried under nitrogen (2h, 55 °C).

2.2.5 Analysis of Fatty Acid Composition

Dry yolk lipid was redissolved in a known quantity of chloroform to a final lipid concentration of 0.2 g/ mL. Fifty µL of lipid-chloroform solution was then divertised using 2 mL of methylating reagent (Methanolic HCl, 1N, Sigma, Oakvilly, ON, CA) in a water bath at 60 °C for 60 min. After cooling to room temperature, 100 µL of water, a known amount of internal standard (heptadecanoic acid, 17:0, Sigma, Oakvilly, ON, CA) and 5 mL of hexane was added, mixed thoroughly, and centrifuged at 1500 x *g* for 3 min. The top hexane layer was transferred to a clean test tube containing a pinch of anhydrous sodium hydroxide in order to remove water from hexane completely. After the third centrifugation (3 min, 1500 x *g*), 1 mL of hexane solution was transferred to gas chromatograph (**GC**) vial and 1 µL of hexane was injected to GC for analysis. Fatty acid composition was determined with a gas chromatograph (Model 3400, Varian, Palo Alto, CA,USA), equipped with a flame ionization detector and a SGE BP20 capillary column (30m x 0.25 mm ID x 0.25 µm film thickness; Scientific Instrument Services Inc., Ringoes, NJ, USA). Operating conditions for the GC were the following: held 0.2 min at 50 °C, then increased to 120 °C at a rate of 20 °C/min for 5 min. The temperature continued increasing at a rate of 10 °C/min until the final temperature (230 °C) was reached, then maintained at 230 °C till the end of total running time (30.2 min). A Cool-on-Column injection method was used, with an initial and final injector temperature (CO_2) of 60 °C (0.2 min) and 230 °C (28 min) respectively, increasing at a rate of 150 °C/min. The temperature of the detector was 240 °C and the column head pressure of the carrier gas

(helium) was 25 PSI. The fatty acid peak integration was performed using the Galaxie Chromatography Data System (Varian). Samples were analyzed in duplicate.

2.2.6 Cholesterol Oxidation Products (COPs)

2.2.6.1 Extraction and Purification of COPs.

Extraction of COPs was performed according to the method described by Guardiola et al. (1995) with slight modifications. COPs extraction and purification procedure included four steps: lipid extraction (Folch et al., 1957), cold saponification, cartridge purification, and silanization. About 0.5 g of dried yolk was weighed into a 50 mL screwed test tube, where 50 µg of 19-hydroxycholesterol (**19-OH**) (Steraloids, Inc. PO Box 689 Newport, RI 02840 USA) was added as internal standard. Then, 16 mL of folch solution was added and kept the samples in dark for overnight at 21 °C to extract lipid and COPs. After filtering through Whatman No. 1 filter paper, the residue in the test tube was extracted for the second time using 8 mL of folch solution. Combining the filtrates, sodium chloride solution (0.88%, w/v) was mixed and centrifuged at 2200 x *g* for 15 min. The bottom layer (organic layer) was transferred into a scintillation vial to dry in nitrogen. Cold saponification was done by following the procedures described by Guardiola et al. (1995). The organic extract obtained from the saponification was re-dissolved in 5mL of hexane and applied to the silica cartridge (Sep-pack, Vac 6cc, 1g, SPE, Waters, Millpore, Bedford MA, USA). In the cartridge purification, gradually increasing polarity solvent mixtures (10 mL

of 95/5 hexane/ether, 30 mL of 90/10 hexane/ether and 10 mL of 80/20 hexane/ether) were applied to remove the interference compounds and finally 10 mL of acetone/methanol (60/20) was used to obtain the COPs portion. Then, acetone/methanol (60/20) was collected and dried under Nitrogen (1h, 40 °C). The final residue was redissolved in 50 µL of anhydrous pyridine and 50 µL of Sylon BTZ (Supelco Inc., Supelco Park, Bellefonte, PA 16823, USA) to complete the silanization reaction for 20 min at 21 °C. Silanization reaction is done to produce silyl derivatives which are stable at -20 °C for several days (Guardiola et al., 1995; Pie et al., 1990).

2.2.6.2 GC-MS and GC Analysis of COPs.

Identification of 7 major COPs in sample was performed according to the method of Lee et al. (2006) by gas chromatography mass spectroscopy (**GC-MS**) (PerkinElmer, Clarus® 600, Waltham, USA). The seven COPs include 7-α-hydroxycholesterol (**7α-OH**), 7-β-hydroxycholesterol (**7β-OH**), β-cholestanetriol (**β-CE**), α-cholestanetriol (**α-CE**), 25-hydroxycholesterol (**25-OH**), cholestanetriol (**CT**) and 7-keto cholesterol (**7-KC**) as well as internal standard 19-OH, Quantification of COPs was done using a DB5-column (Agilent J&W GC Columns, 300 Laurier Blvd, Brockville, Ontario K6V 5W1, Canada) coupled with a gas chromatograph (Model 3400, Varian, 3120 Hansen Way, Palo Alto, CA 94304-1030, USA). A flame ionized detector was used for COP quantification. Helium was used as a carrier gas, and carbon dioxide as a cooling gas. The initial column temperature was 70 °C holding for 0.20 min, then temperature was increasing at an rate of 20 °C/min until the final temperature of

250 °C was reached and hold for 2 min. Temperature continued increasing at a rate of 15 °C/min until the final temperature of 280 °C was reached and then held for 17 min. Injector and detector temperatures were 90 °C and 300 °C, respectively. The head pressure of the column was set at 22-23 PSI.

2.2.7 The Thiobarbituric Acid Reactive Substances

The thiobarbituric acid reactive substances (**TBARs**) test was performed according to the method of Slater and Sawyer (1971). Two gram of yolk samples were weighed into screw tap tubes, 4 mL of 1.15% KCl was immediately added and the mixture was homogenized for 30s using a polytron homogenizer (Brinkmann Instruments, Rexdale, Ontario), and then 1 mL of 80mM Tris/maleate buffer solution and 4 mL of TCA-TBA-HCl (15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid; this solution was heated to completely dissolve the TBA) were added, mixed rapidly, and filtered through #1 Whatman Filter Paper. The filtrate was incubated in the dark from 15 to 17 h at 21 °C. After incubation, absorbance at 532 nm was recorded spectrophotometrically (UV/VIS Spectophotometer). An extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ was used to estimate the malondialdehyde (**MDA**) values (Slater and Sawyer, 1971).

2.2.8 Vitamin E Analysis

Vitamin E in egg yolk was extracted according to the method described by Abdollahi et al. (1993). Two grams of fresh yolk sample were weighed into a 50

mL screw-capped tube, 4 mL of water added and homogenized with a polytron homogenizer for 30s at a high speed. Then 3 mL of methanol and 4 mL saturated methanolic KOH were added (4 mL, 33%, w/v), placed in a 70 °C oven for 16 h for complete saponification. After cooling to 21 °C, 5 mL of acetone/chloroform (3:7, v/v) were added, shaken for 2 min, and centrifuged at 22 °C, 3000 x *g* for 15 min. The supernatant was collected and dried under nitrogen with constant temperature of 40 °C. The residue was re-dissolved with 1 mL of methanol and filtered through 0.45 μm filter for HPLC analysis.

HPLC analysis was performed according to Drotleff and Ternes (1999) on a SUPELCOSIL^{TM} LC-18, 3 μm (15 cm x 4.6 mm) column (3050 Spruce St., St. Louis, MO 63103, USA) equipped with the high-performance liquid chromatography (**HPLC**; Varian 9010 HPLC system). The injection volume was 10 μL. The column was eluted by a mixture of 75 % of acetonitrile and 25 % of methanol for 14 min, and 100% isopropanol for 6 min at a flow rate of 1 mL/min. The elution was monitored by fluorescence detector (Shimadze Corparation, RF-335, Japan) with emission wavelength of 330 nm and excitation wavelength of 295 nm. An external calibration curve by plotting five concentrations of alpha-tocopherol against areas was applied for quantification.

2.2.9 Selenium Determination

The Selenium content was determined by using inductively coupled plasma mass spectrometry (**ICP-MS**) (Perkin Elmer's Elan 6000 instrument, Perkin Elmer Life And Analytical Sciences, Inc., 940 Winter Street, Waltham,

Massachusetts 02451 USA). Four concentrations were used for the standard calibration curve (0.005, 0.010, 0.020 ppm). Samples were analyzed in triplicate.

2.2.10 Statistical Analysis

All data were subjected to a 2 x 2 x 2 x 3 factorial ANOVA to establish the significant differences between the 2 vitamin E levels, 2 Sel-Plex levels, 2 storage periods, and 3 cooking methods. The Mixed procedure of SAS (SAS System, 2002, Cary, NC, USA) was used. Means were separated using Tukey adjustment procedure of SAS. To study relationships among the content of vitamin E, Se, fatty acids, COPs and MDA, the data were submitted to correlation analysis in SAS (SAS System, 2002, Cary, NC, USA). Unless otherwise stated, significance was assessed at the $P<0.05$ level.

2.3 Results and Discussion

2.3.1 Egg Traits

Egg production as well as egg weight, specific gravity, yolk weight, albumen height, shell thick, and shell weight were not affected by the dietary treatments. At the end of the dietary treatment, when eggs were sampled for the current study, the mean of egg weight was 61.3 g and yolk represented 28.2 % of total egg weight. The lack of dietary effect on egg traits means that any subsequent treatment-based difference were due to diet rather than the egg size or component weight (Data presented in Appendix Table A.6).

2.3.2 Fatty Acid Profile

2.3.2.1 Fatty Acid Composition

Total fat content was not significantly affected by cooking or simulated retail storage (mean = 31.2% of yolk weight). The main fatty acids identified were 14:0, 16:0, 18:0, 16:1, 18:1 n-7, 18:1 n-9, 18:2 n-6, 18:3 n-3, 18:3 n-6, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:5 n-3 and 22:6 n-3. Egg yolks contained predominantly oleic acid (18:1 n-9, 39%) followed by linoleic acid (**LA**, 18:2 n-6, 15%, [data not shown]).

2.3.2.2 Cooking Effect

Saturated fatty acids (**SFA**) and monounsaturated fatty acids (**MUFA**) levels were very consistent during cooking, with the exception of n-6 PUFA in boiled eggs where a significant increase occurred (Table 2.2). The mean values of n-6 to n-3 ratio were higher (1.99) in the fried eggs than those of in boiled eggs and raw eggs (1.94 and 1.93, respectively), which are very close to the recommended ratio of 2/1 (Eaton et al., 1996). In comparison to the typical n-6 to n-3 ratio (15/1 to 16.7/1) in western diet, the ratio in current study is much lower (Simopoulos, 2008). The LNA was not affected in most treatments. However, there was a significant interaction in the HE x LSe treatment, where the LNA concentration was 6.6 g/100g compared to an average of 6.0 g/100g total fatty acid in raw and fried samples. This effect was lost when compared at the main effect level (Table 2.2).

Boiling had less effect on the composition of the fatty acids than did frying. Similarly, Cortinas et al. (2003) reported that boiling did not significantly affect fatty acids composition, the total n-3 PUFA in scrambled egg numerically reduced compare to fresh eggs. This is probably because the yolk temperature in boiling is lower than that of frying. The presence of albumen and shell surrounds the yolk, which might prevent the interaction of oxygen and light with the lipids during boiling, whereas a direct contact with oxygen and light is inevitable in frying. Therefore, level of n-3 PUFA was higher in boiled eggs than in the fried eggs.

2.3.2.3 Storage Effect

Level of the two essential fatty acids (**EFA**), LNA (approximately 65% of total n-3 PUFA) and LA (approximately 90 % of total n-6 PUFA) were not affected by 28 d of storage (Table 2.2). Similarly, Ahn at el. (1995) reported that LNA and LA in eggs were stable over 49 d of storage at 4 °C. However, long chain PUFA (EPA, DPA, DHA and arachidonic acid [**AA**, 20:4 n-6]) were decreased by 8.74%, 12.0%, 6.74% and 4.72 % respectively. The loss of n-3 PUFA was relatively more than the loss of n-6 PUFA, therefore, the n-6 to n-3 ratio increased from 1.93 to 1.99 after 28 d of storage (Table 2.2).

2.3.2.4 Antioxidant Effect

The content of n-3 PUFA and n-6 PUFA were not significantly affected by vitamin E and Sel-Plex (Table 2.2), which was consistent with the results of Qi and Sim (1998) who reported that vitamin E (tocopherol) supplementation did not affect the n-3 PUFA level in n-3 PUFA enriched eggs. Although, similar results

were reported by Galobart et al. (2001) at the supplemental vitamin E level of 50 mg/kg, they absorbed a decrease in LNA, EPA, DPA, DHA, and total n-3 PUFA content were observed at the supplemental vitamin E level of 200 mg/kg. In the current study, supplemental vitamin E did not significantly affect the contents of LNA and LA, whereas the long chain PUFA (EPA, DPA, DHA, and AA) decreased at the higher level of vitamin E (Table 2.2). Earlier research reported that vitamin E at higher doses can decrease the content of n-3 PUFA in eggs by interfering in the intestinal absorption and transportation of long-chain n-3 PUFA (Meluzzi et al., 1999). It was also suggested that a high does of vitamin E (>200 IU/kg) could act as a pro-oxidant in eggs (Meluzzi et al., 2000).

2.3.3 Contents of Vitamin E and Se

Cooking reduced the content of vitamin E (Table 2.3). The content of vitamin E in boiled and fried samples was 75.52 mg/g and 54.22 mg/g, cooperating to 21% and 44% decrease, compared to raw (96.83 mg/g). Storage of 28 d reduced vitamin E by 16%. This result agreed with Murcia et al. (1999), they found that a 22% reduction of vitamin E in boiled eggs (boiled for 10 min) and 51 % reduction of vitamin E in scramble eggs.

Vitamin E, which acts by quenching lipid peroxide radicals, is the major chain-breaking antioxidant in body tissues. Vitamin E is unstable in the presence of oxygen, heat, light, and radiation, which could be the reasons for the reduction of vitamin E observed during cooking (Murcia et al., 1999). While cooking, particularly frying, the yolk sample was exposed to heat and ambient light which

can both increase of susceptibility to oxidation.

Selenium is a trace element which is already present in the basal hen diet from the grains used and from the 0.1 ppm sodium selenite in the base ration. In the current study, an increase content of Se was observed in both eggs from high Sel-Plex dietary treatments (high Se and vitamin E + Se, 1.53 ppm and 1.48 ppm, respectively), whereas the Se content in control treatment was 1.36 ppm.

2.3.4 Cholesterol Oxidation Products

Oxidation of n-3 PUFA (particularly susceptible to oxidative damage during cooking) not only produces genotoxic compounds (causing cell and DNA damage), but also accelerate the formation of cholesterol oxidation products (**COPs**), which are responsible for the proatherogenic action of cholesterol (Kubow, 1993). Eggs are a rich source of cholesterol. The presence of a high concentration of PUFA in enriched eggs can enhance the susceptibility of cholesterol oxidation during storage and cooking through the process called "co-oxidation" (Galobart and Guardiola, 2002). Larkeson et al. (2000) pointed out that the major COPs in foods are 7α-OH, 7β-OH, β-CE, α-CE, 25-OH, CT and 7-KC. The COPs have been reported to have a potential hazard in human health. Cholesterol oxidation products are consistently found within walls of major arteries, mainly in characteristic lesions of atherosclerosis (Tai et al, 1999). Among the COPs, 7-KC is the most potent inhibitor of cellular proliferation, and 25-OH was an effective inducer for apoptosis (programmed cell death; Leonarduzzi et al., 2002).

2.3.4.1 Cooking Effect

The presence of three COPs in the egg samples, 7α-OH, 7β-OH and 7-KC, was identified by GC-MS and quantified by GC. The total content of COPs was affected by cooking methods. The fried samples had the highest total COPs compared to both boiled or raw samples (9.03 vs. 10.15 and 13.58 μg/g dry yolk, for raw, boiled and fried samples, respectively). The content of 7-KC was not affected by cooking, but the content of 7α-OH was increased in both boiled and fried samples compared to the control ($P<0.05$), and 7β-OH was increased by 2-fold compare to fried samples (Table 2.4). The dehydration reaction of cholesterol hydroperoxide produce 7-KC, 7α-OH, and 7β-OH. Among them, more 7α-OH and 7β-OH can be produced than 7-KC during a short period of heating. However, after a prolonged (more than 12h) heat treatment, 7-KC showed a sharp increase (Lee et al., 2006). Hence, a heat treatment extended over 24h exhibits the reverse effect. This phenomenon may be due to the reduction reaction proceeding faster than the dehydration reaction during the initial heating period (Lee et al., 2006).

A significant interaction was observed between dietary treatment and cooking methods. The highest amount of 7-KC was found in the fried egg yolk samples obtained from control (LE x LSe) compared to the other dietary treatments (2.754 vs. 0.995, 1.863, and 1.807 μg/g, for HE x LSe, LE x HSe and HE x HSe dietary treatment, respectively). Likewise, the highest amount of total COPs was detected in the control group (LE x LSe); (17.47 vs.10.01, 14.076 and 10.74 μg/g dry yolk, for HE x LSe, LE x HSe and HE x HSe dietary treatments

respectively). By comparing Figure 2.1 and Figure 2.2, it was observed that the antioxidant treatments in the fried group reduce total COPs and MDA amount. Without antioxidant treatment, "co-oxidation" occurred in the fried samples. However, the content of COPs was not affected by boiling. The lipid and cholesterol are located in yolk, which is rich in natural antioxidants such as tocopherols, cartenoids and metal ion chelators (phosvitin). Furthermore, yolk is surrounded by albumen and shell, which provide a physical buffer against atmospheric oxygen (Galobart and Guardiola, 2002).

2.3.4.2 Storage Effect

Twenty-eight days of storage did not affect COPs levels, with the exception of 7α-OH, where a significant decrease was observed after 28 d of storage (Table 2.4). Mazalli and Bragagnodo (2007) reported that the COPs content of spay-dried egg powder was not affected by 1 month of storage at 25 °C. They also found that the content of 7-KC, 7α-OH and 7β-OH increased rapidly at the 3rd month of storage and stayed consistent from the 3rd month to the 6th month at 25 °C. The current results suggested that simulated retail storage conditions (28 d in fridge at 4 °C) were relatively stable, which likely limited the formation of COPs by slowing down the speed of auto-oxidation and inhibiting the photo-oxidation of cholesterol.

2.3.4.3 Antioxidant Effect

Compared to the eggs from the control treatment, the addition of vitamin E and Sel-Plex into the hen's diet reduced total COPs formation in eggs by 12% and

13%, respectively (Table 2.4 and Figure 2.1). The results demonstrated an antioxidant effect of vitamin E and Sel-Plex in preventing lipid oxidation during cooking and storage, which concurred with the results of Winne and Dirinck(1996), and Li et al. (1996).

2.3.4.4 Toxic Levels of COPs

Cholesterol oxidation products are consistently found within the walls of major arteries, mainly in the characteristic lesions of atherosclerosis. Lizard et al. (1999) reported that the toxic concentration of 7-KC and 7β-OH are >40 and >20 μM respectively, by using human culture smooth muscle cells (**SMC**). In another study, the concentration of 25-OH or 7β-OH causing apoptosis was >20 μM on human monocytic cell (Aupiex et al., 1995). In the current study, the amount of 7-KC was approximately 0.028 μM per raw egg and approximately 0.037 μM per cooked egg. The amount of 7β-OH was 0.030 μM per raw egg and 0.073 μM per fried egg. The concentration of COPs in egg is below the toxic level, even though the egg contains a high level of cholesterol.

2.3.5 Thiobarbituric Reactive Acid Subtances (TBARs)

2.3.5.1 Cooking Effect

Frying the eggs triggered the generation of the MDA (2.02 ug/kg) compared to boiling (1.44 μg/kg) and raw egg yolks (0.56 μg/kg) (Table 2.5). Cortinas et al. (2003) reported that boiling and scrambling increased the TBARS

value by 2- and 9-fold, respectively. It is well established that oxidation was affected by heating temperature, duration of heating and light (Galobart and Guardiola, 2002; Maerker, 1987). The elevated TBARs level in fried eggs compare to boiled eggs was probably due to the exposure to a much higher cooking temperature. The surface temperature of the frying pan was 177 °C compare to approximately 98 °C in the boiled eggs (temperature lower than 100 °C due to the elevation of research site). Tai et al. (2000) indicated that frying could stimulate n-3 PUFA oxidation due to the presence of light, heat and oxygen through photo-oxidation and auto-oxidation.

2.3.5.2 Storage Effect

The content of MDA significantly increased after 28 d of storage (1.23 vs. 1.44 μg MDA/kg sample, for 0 day and 28 d storage, respectively) (Table 2.5). Similar result were reported by Cherian et al. (2007) and Mohiti-Asli et al. (2008) who observed an increase of TBARs value during storage, with the increase associated with storage temperature.

2.3.5.3 Antioxidant Effect

Addition of vitamin E or selenium in hen's diet significantly reduced the formation of MDA compared to the eggs from the control diet (LE x LSe) diet (Table 2.5). Several studies indicted that due to the antioxidant property of vitamin E and the role of Selenium in GSH-Px activity, additional vitamin E and Sel-Plex in poultry diets would protect the cells from lipid peroxidation damage, leading to a better oxidative stability of eggs during cooking and storage (Qi and

Sim, 1998; Mohiti-Asli et al., 2008). Vitamin E is the major chain break antioxidant, can break the oxidation chain at propagation phase. Selenium is an important part to the activity of GSH-Px, which is the major antioxidant to remove the reactive oxygen species (ROS) in the initiation phase.

The interaction between cooking methods and dietary treatments on TBARs was presented in Figure 2.2. The content of MDA in both raw and boiled samples was not affected by addition of antioxidants; however, in fried eggs, a greater than 40% reduction was observed for the antioxidant groups. The combination of vitamin E and Sel-Plex did not have additional benefits in terms of reducing the formation of MDA compare to supplying these feed additives alone (Figure 2.2).

2.3.6 Correlations

The content of n-3 PUFA content in egg yolk was negatively correlated with MDA content. For example, DPA, which is mainly found in membrane and prone to attack by free radicals, was correlated with the MDA ($r = -0.4733$, $P = 0.0195$; Figure 2.3). When oxidation occurred in yolk, lipids such as DPA are attacked by ROS and change form to other active radicals, such as lipid oxygen radicals (**L***) and peroxyl radicals (**LOO**); (Surai, 1999). Other oxidation reactions and secondary oxidation reactions would be trigged by those radicals. The LOO*, which was a highly reactive radical, can attack any available peroxidizable material producing hydroperoxide (**LOOH**). Therefore, the amount of MDA (secondary oxidation products) increased while DPA content decreased

(Surai, 2006).

Cholesterol oxidation would also be initiated and accelerated, due to the present of L* and LOO*. Theoretically, an increase of MDA content should associate with an increase of COPs concentration. In the current study, there was a positive correlation (r=0.7171, P<.0001) between COPs and MDA content (Figure 2.4). Few studies have reported that the formation of cholesterol oxides associated with the oxidation of PUFA in eggs (Wahle et al., 1993; Kim and Nawar, 1991; Galobart et al., 2002; Smith, 1987). Because cholesterol oxidation is greatly accelerated by the oxidation of coexisting highly unsaturated lipid, the fatty acid oxidation products attack the cholesterol portion of the cholesterol ester molecules and accelerate the cholesterol oxidation.

Cooking and storage increased the oxidative stress in yolk sample to stimulate the oxidation reaction. However, cooking and storage also caused some reduction of vitamin E content. Vitamin E is unstable in the presence of oxygen, light and even some unsaturated fats. In the current study, vitamin E content of the egg yolk was negatively correlated with the amount of oxidation products for the TBARs test (r = -0.7857, P <0.0001) and for the COPs test (r = -0.6565, P = 0.0005) (Figure 2.5). When the yolk sample contained a higher amount of vitamin E, protection of yolk lipid and cholesterol was better, and a relatively lower level of lipid oxidation and cholesterol oxidation was observed. More MDA and COPs were found in the low vitamin E content samples, indicating that the lipid and cholesterol oxidation reaction was stronger in those samples. Clearly, the antioxidant treatments improved the stability of the n-3 PUFA.

2.4 Conclusion

Antioxidants reduced yolk fatty acids from oxidative damage after cooking and storage by slowing the oxidation reaction. Cooking increased oxidation of n-3 PUFA and cholesterol in yolk. The amount of long chain n-3 PUFA also decreased after cooking, especially frying. Fried sample in LE x LSe treatment had the highest content of TBARs and COPs. Refrigerated storage of shell eggs had no effect on COPs after 28 d, but the amount of MDA increased after storage. Four weeks of storage also led to a reduction of long chain n-3 PUFA in yolk sample. It is possible to make the n-3 PUFA in enriched eggs more stable with combination of dietary antioxidants.

In the current study, n-3 PUFA enriched eggs was prepared under the simulated home cooking and storage conditions. Therefore, costumers can understand the actual amount of n-3 PUFA consumption and the intake of oxidation products. The results of the study indicate that the n-3 PUFA in enriched eggs was quite stable, maintaining the food value of n-3 PUFA enriched eggs during storage and home cooking.

Table 2.1 Diet composition (% of total ingredients) and nutrient content of base diet, high vitamin E diet, high selenium diet and high vitamin E plus selenium diet for White Leghorn.

Treatments	**LE x LSe**	**HE x LSe**	**LE x HSe**	**HE x HSe**
Ingredient Name				
Soybean meal deh - plant [1]	16.62	16.63	16.63	16.63
Wheat, hard, grain	50.87	50.88	50.88	50.88
Calcium carbonate	9.46	9.46	9.46	9.46
Dicalcium phosphate	1.61	1.601	1.61	1.61
Salt, plain (NaCl)	0.35	0.35	0.35	0.35
D,L - Methionine	0.13	0.13	0.13	0.13
L-Threonine	0.0002	0.0002	0.0002	0.0002
Linpro	17	17	17	17
Layer Vit/Min Premix[1]	0.5	0.5	0.5	0.5
Choline Chloride Premix[2]	0.5	0.5	0.5	0.5
Generic Enzyme[3]	0.05	0.05	0.05	0.05
Canola Oil	2.90	2.90	2.90	2.90
Vitamin E (IU/kg)	—	200	—	200
Sel-Plex 600 (g/kg)	—	—	0.5	0.5
Nutrient Analysis:				
Nutrient Name				
M.E. (kcal/kg)	3,000	3,000	3,000	3,000
Crude Protein, %	18.7	18.7	18.7	18.7
Crude FAT, %	7.48	7.48	7.48	7.48
Crude Fiber, %	2.94	2.94	2.94	2.94
Total Phosphorous, %	0.64	0.64	0.64	0.64
Available Phosphorous, %	0.4	0.4	0.4	0.4
Met + Cys, %	0.76	0.76	0.76	0.76
Methionine, %	0.42	0.42	0.42	0.42
Lysine, %	0.90	0.90	0.90	0.90
Tryptophan, %	0.26	0.26	0.26	0.26
Arginine, %	0.94	0.94	0.94	0.94

[1]Layer premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; and Fe, 100 mg.

[2]Provided choline chloride in the diet at a level of 100 mg/kg.

[3]Generic Enzyme: Avizyme 1302, Xylanase enzyme, Danisco Animal Nutrion, Marlborough, Wiltshire, UK.

LE x LSe = low vitamin E and low selenium treatment.

HE x LSe = high vitamin E and low selenium treatment.

LE x HSe = low vitamin E and high selenium treatment.

HE x HSe = high vitamin E and high selenium treatment.

Table 2.2 The fatty acids composition (g/100g of total fatty acids) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	14.42b	5.72b	1.68	0.24a	0.25a	2.28a	42.20	32.94	16.38b	8.49ab	1.93b
Boiled	16	14.87a	5.92a	1.65	0.22b	0.24a	2.29a	41.96	32.57	16.80a	8.67a	1.94b
Fried	16	14.77a	5.78ab	1.64	0.21b	0.21b	2.19b	41.54	33.37	16.69ab	8.40b	1.99a
SEM[4]		0.094	0.05	0.027	0.006	0.003	0.029	0.306	0.29	0.114	0.068	0.005
Storage (Mean)												
0day	24	14.62	5.78	1.70a	0.23a	0.25a	2.36a	41.70	33.07	16.61	8.63a	1.93b
28days	24	14.76	5.83	1.62b	0.21b	0.22b	2.14b	42.10	32.85	16.64	8.41b	1.99a
SEM		0.077	0.041	0.022	0.005	0.002	0.024	0.25	0.237	0.093	0.056	0.004
Vitamin E (Mean)												
High	24	14.75	5.82	1.62b	0.22	0.23b	2.24	41.88	32.97	16.64	8.51	1.96
Low	24	14.62	5.80	1.69a	0.23	0.24a	2.27	41.92	32.95	16.60	8.53	1.95
SEM		0.077	0.041	0.022	0.005	0.002	0.024	0.25	0.237	0.093	0.056	0.004
Selenium (Mean)												
High	24	14.62	5.78	1.63	0.22	0.23b	2.24	41.89	33.13	16.52	8.46	1.96
Low	24	14.75	5.83	1.68	0.23	0.24a	2.27	41.91	32.80	16.72	8.57	1.95
SEM		0.077	0.041	0.022	0.005	0.002	0.024	0.25	0.237	0.093	0.056	0.004
Source of Variation		**Probability**										
Cooking (C)		0.0066	0.0300	0.5866	0.0240	<.0001	0.0408	0.3245	0.1685	0.0411	0.0278	<.0001
Storage (S)		0.2049	0.3550	0.0181	0.0046	<.0001	<.0001	0.2654	0.5151	0.7965	0.0113	<.0001
Vitamin E (E)		0.2614	0.6669	0.0399	0.3639	0.0126	0.3416	0.9216	0.9592	0.7608	0.7758	0.1002
Selenium (Se)		0.2475	0.4057	0.1212	0.1187	0.0002	0.3077	0.9542	0.3339	0.1426	0.1768	0.5583
C*S		0.1832	0.0002	0.0049	0.4732	0.0249	0.0005	0.0197	0.0112	0.3812	0.2926	<.0001
E*Se		0.0011	<.0001	0.0244	0.1700	0.3955	0.0055	0.4812	0.1577	0.0197	<.0001	<.0001
E*C		0.5958	0.0028	0.0189	0.9181	0.2046	0.0963	0.1270	0.1026	0.3242	0.1902	<.0001
Se*C		0.1927	0.0717	0.2840	0.0460	0.0403	0.8613	0.6881	0.5025	0.3170	0.3011	0.3884

E*S	0.0834	0.0003	0.3421	0.6971	0.3413	0.0185	0.0829	0.0082	0.2132	0.0580	0.0046
Se*S	0.0304	0.1764	0.0371	0.7177	0.1192	0.0287	0.4288	0.0295	0.0215	0.0458	0.8893
E*C*S	0.3738	0.0431	0.3276	0.8479	0.4438	0.0950	0.2244	0.2999	0.5131	0.6639	<.0001
Se*C*S	0.0186	0.0004	0.2783	0.0222	<.0001	0.0129	0.1284	0.7310	0.0736	0.0003	<.0001
E*Se*C	0.4197	0.0066	0.0100	0.7196	0.0069	0.0527	0.1036	0.1319	0.1733	0.3860	<.0001
E*Se*S	0.1014	0.0002	0.4338	0.8470	0.7067	0.6144	0.0394	0.3251	0.2177	0.0024	<.0001
E*Se*C*S	0.6281	0.0011	0.3989	0.3174	0.0115	0.0834	0.9394	0.7360	0.6404	0.0141	<.0001

[a-c] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table 2.3 The mean value of vitamin E content in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched egg affected by the main factors and interactions: cooking, storage, vitamin E.

Effects	n[1]	vitamin E
Cooking (Mean)		
Raw	16	96.83a
Boiled	16	75.52b
Fried	16	54.22c
SEM[2]		0.939
Storage (Mean)		
0day	24	82.33a
28days	24	68.71b
SEM		0.767
Vitamin E (Mean)		
High	24	83.10a
Low	24	67.95b
SEM		0.767
Source of Variation		**Probability**
Cooking (C)		<.0001
Storage (S)		<.0001
Vitamin E (E)		<.0001
E*C		0.0136
E*S		<.0001
C*S		0.0277
E*C*S		0.0658

Mean values of the vitamin E ug/g sample.

[a-c] Means within a column in each treatment with no common superscripts differ ($P<0.05$).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] SEM= standard error of the means

Table 2.4 The mean value of cholesterol oxidation products (COPs) content in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium. (dwb[1])

Effects	n[2]	7-KC	7α-OH	7β-OH	Total
Cooking (Mean)					
Raw	16	1.50	5.90c	1.62b	9.03b
Boiled	16	1.93	6.73b	1.96b	10.15b
Fried	16	1.86	7.74a	3.98a	13.58a
SEM[3]		0.152	0.270	0.248	0.435
Storage (Mean)					
0	24	1.67	7.46a	2.65	11.46
28	24	1.85	6.12b	2.40	10.37
SEM		0.124	0.220	0.202	0.355
Vitamin E (Mean)					
High	24	1.53b	6.19b	2.60	10.32b
Low	24	1.99a	7.38a	2.45	11.51a
SEM		0.124	0.220	0.202	0.355
Selenium (Mean)					
High	24	1.71	6.70	1.98b	10.23b
Low	24	1.82	6.88	3.07a	11.61a
SEM		0.124	0.220	0.202	0.355
Source of Variation		**----------Probability----------**			
Cooking Methods (C)		0.1282	0.0003	<.0001	<.0001
Storage (S)		0.3039	0.0002	0.3969	0.0400
Vitamin E (E)		0.0150	0.0008	0.6076	0.0268
Selenium (Se)		0.5202	0.5735	0.0008	0.0112
C*S		0.0944	0.0036	0.2545	0.0051
E*Se		<.0001	0.0087	0.3811	<.0001
E*C		0.1306	0.0011	0.0157	0.0006
Se*C		0.1892	0.2611	0.0748	0.4173
E*S		0.0642	0.3334	0.4723	0.8861
Se*S		0.0005	0.0374	0.0204	0.1810
E*C*S		0.6406	0.7031	0.5387	0.6144
Se*C*S		0.0691	0.0001	0.2080	0.0366
E*Se*C		0.0014	0.1412	0.0675	0.0040
E*Se*S		0.0064	0.0178	0.0920	0.6524
E*Se*C*S		0.3776	0.2700	0.2394	0.8619

Mean values of the COPs ug/g dry sample.

a-c Means within a column in each treatment with no common superscripts differ ($P<0.05$).

[1] dwb=dry weight basis;

[2] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[3] SEM= standard error of the means.

Table 2.5 The mean values of MDA content (TBARs) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	MDA
Cooking (Mean)		
Raw	16	0.56c
Boil	16	1.44b
Fry	16	2.02a
SEM[2]		0.077
Storage (Mean)		
0	24	1.23b
28	24	1.44a
SEM		0.062
Vitamin E (Mean)		
High	24	1.23b
Low	24	1.45a
SEM		0.062
Selenium (Mean)		
High	24	1.31
Low	24	1.37
SEM		0.062
Source of Variation		**Probability**
Cooking Methods (C)		<.0001
Storage (S)		0.0256
Vitamin E (E)		0.0239
Selenium (Se)		0.4454
C*S		0.1918
E*Se		0.0027
E*C		0.1460
Se*C		0.0156
E*S		0.7203
Se*S		0.8337
E*C*S		0.7829
Se*C*S		0.7168
E*Se*C		0.0449
E*Se*S		0.2010
E*Se*C*S		0.4456

Mean values of the TBARs, is the ug MDA/kg of sample.

[a-c] Means within a column in each treatment with no common superscripts differ ($P<0.05$).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] SEM= standard error of the means;

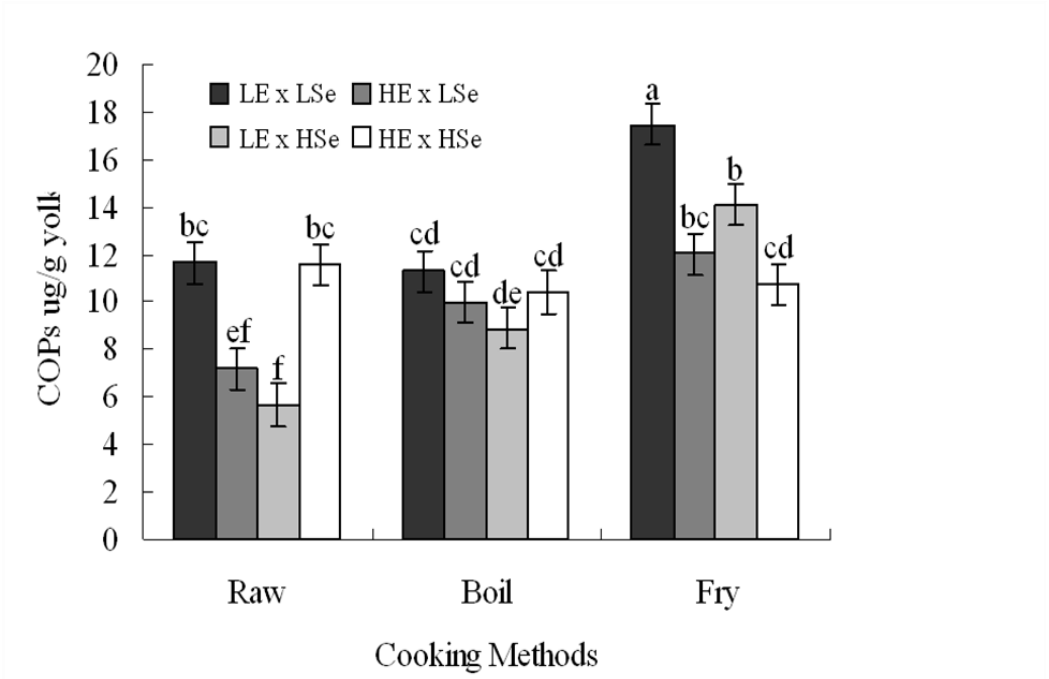

Figure 2.1 The content of cholesterol oxidation products (COPs) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs were affected by the interaction between dietary treatments and different cooking methods.
LE x LSe = low vitamin E and low selenium treatment.
HE x LSe = high vitamin E and low selenium treatment
LE x HSe = low vitamin E and high selenium treatment.
HE x HSe = high vitamin E and high selenium treatment.
[a-d] Means with no common letters differ ($P<0.05$).

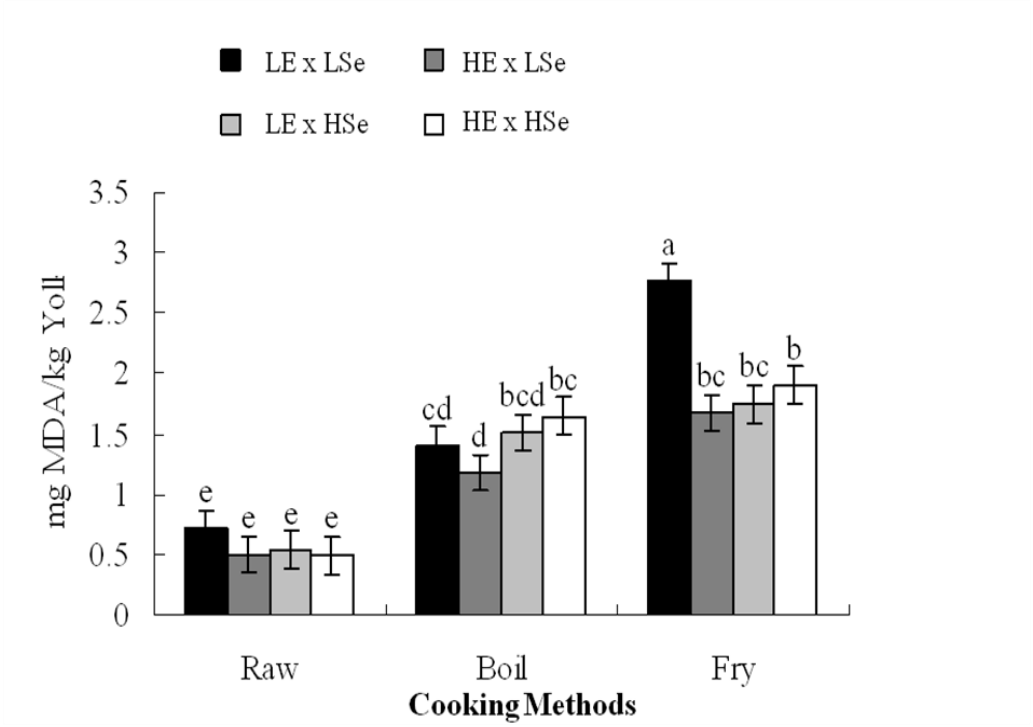

Figure 2.2 The amount of malondialdehyde (MDA) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs were affected by the interaction between dietary treatments and different cooking methods.
LE x LSe = low vitamin E and low selenium treatment.
HE x LSe = high vitamin E and low selenium treatment
LE x HSe = low vitamin E and high selenium treatment.
HE x HSe = high vitamin E and high selenium treatment.
[a-d] Means with no common letters differ ($P<0.05$).

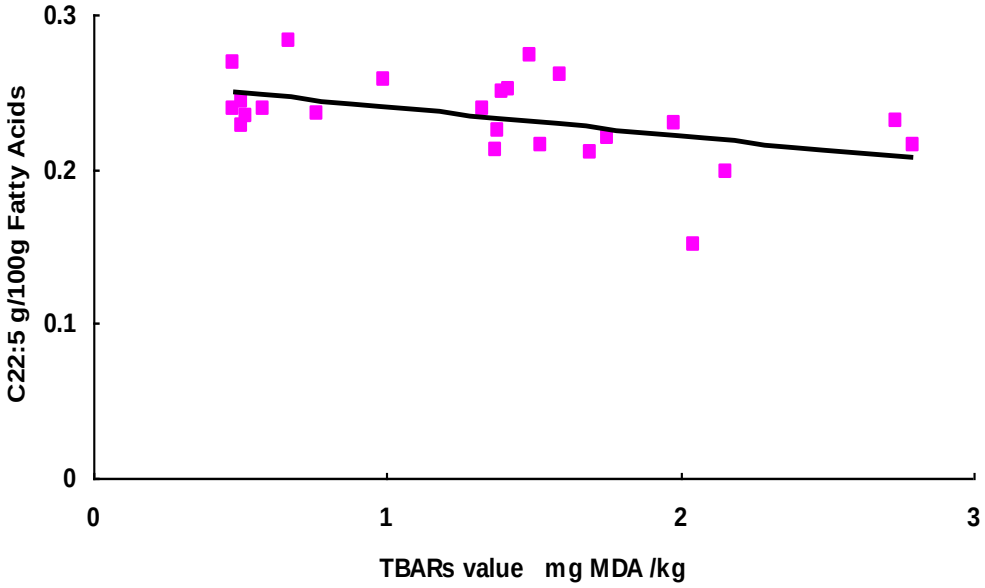

Figure 2.3 Correlation between the content of docosapentaenoic acid (DPA) and the amount of malondialdehyde (MDA) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs.
r = -0.4733, P = 0.0195

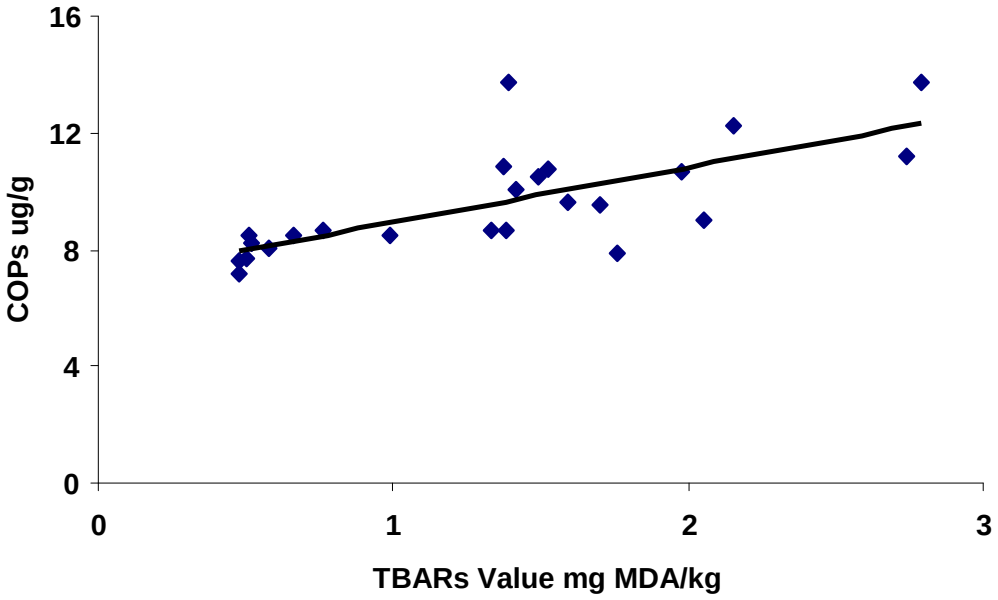

Figure 2.4 Correlation between total amount of cholesterol oxidation products (COPs) and content of malondialdehyde (MDA) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs.
r=0.7171, P<0.0001

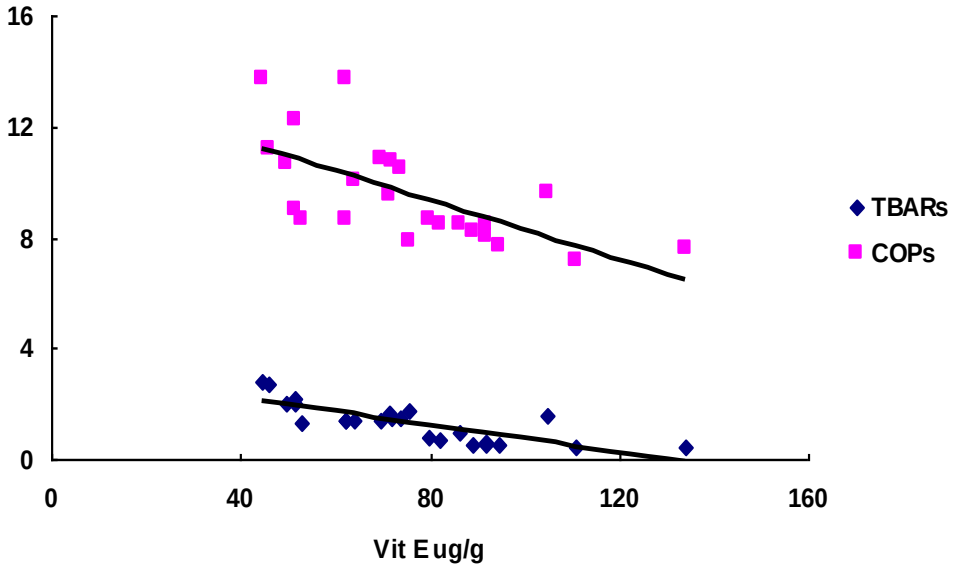

Figure 2.5 Correlation between the content of vitamin E and cholesterol oxidation products (COPs) / content of malondialdehyde (MDA) in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs.
Values of the TBARs, is mg MDA/kg of sample
Values of the COPs, is ug MDA/kg of sample (dry weight basis)
For TBARs: r = -0.7857, P <0.0001
For COPs: r = -0.6565, P = 0.0005

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CHAPTER 3 FINAL REMARKS

3.1 A New Component of Human Diet: n-3 PUFA Enriched Eggs

Enrichment with omega-3 polyunsaturated fatty acid (**n-3 PUFA**) is a strategy for adding nutritional value to eggs and egg products. Benefits of n-3 PUFA include decreased risk of cardiovascular diseases (**CVD**), and improved brain development and function. In managing CVD, for example, current findings suggest that n-3 PUFA helps lower blood triglyceride levels. Daily fat intake is one of the key dietary factors affecting onset of CVD. In addition to excess fat intake, unbalanced intake of different fatty acid classes is believed to have a wide range of health impacts. An unbalanced fatty acid ratio is a risk factor for the onset and development of cancers and coronary hearth diseases (**CHD**), especially the formation of blood clots.

There is a competition between omega-6 polyunsaturated fatty acids (**n-6 PUFA**) and n-3 PUFA for the desaturation enzymes (Δ4, Δ5 and Δ6 desaturases). Therefore, a high intake of linoleic acid (**LA**, 18:2 n-6, a major n-6 PUFA) interferes the desaturation and elongation of linolenic acid (**LNA**, 18:3 n-3, a major n-3 PUFA). Eicosapentaenoic acid (**EPA**, 20:5 n-3) and docosahexaenoic acid (**DHA**, 22:6 n-3), which is the elongation-desaturation products of LNA, will be reduced. The n-6 PUFA are an interesting group of fats because on one hand they contribute to good health, but on the other hand, too much can promote

inflammation in the body, particularly during an immune response. The n-3 PUFA promotes a different type of response, with EPA and DHA having anti-inflammation effects; therefore, it is recommended to always maintain a low n-6 to n-3 ratio. The recommended n-6 to n-3 ratio is 2:1 or less, compare to the 15:1 to 16.7:1 ratio found in the western diet. The n-6 to n-3 ratio in eggs can be easily changed by alternation of hen's diet; so, consuming the n-3 PUFA enriched eggs is one way to maintain a low n-6 to n-3 ratio in human body.

3.2 Project Summary

Previous researchers indicated that storage can affect the stability of n-3 PUFA in eggs. But there has been no research on the effect of different cooking methods and storage on the stability of n-3 PUFA on egg. It is known that the presence of antioxidants (i.e. Selenium as part of the glutathione peroxidase [**GSH-Px**] complex and vitamin E) improve the stability to unsaturated fatty acids. The present study focused on how cooking and storage affects the stability of n-3 PUFA on enriched eggs.

The results of this study are summarized as follow:

1. Total n-3 PUFA was not affected by cooking and storage, although long chain n-3 PUFA (EPA, DPA and DHA) decreased after cooking and storage.
2. The n-6 to n-3 ratio increased during cooking and storage.

3. Total n-3 PUFA composition was not affected by antioxidants but long chain PUFA (EPA, docosapentaenoic acid [**DPA**, 22:5 n-3], DHA and arachidonic acid [**AA**, 20:4 n-6]) decrease at the high level of vitamin E.
4. Both vitamin E and Sel-Plex decreased oxidation of yolk lipids and cholesterol.
5. Frying, but not boiling, increased lipid and cholesterol oxidation.
6. Four weeks of storage did not affect cholesterol oxidation products (**COPs**) content, but increased lipid oxidation.

Thus, these results suggest that storage and cooking can reduce the stability of n-3 PUFA. However, the oxidative damage in eggs is less than other foods, such as chicken meat. An egg is created by the hen to nourish a developing embryo in a closed environment. Even when egg is not fertilized, the egg contains all of the defence mechanisms required to the growing embryo. In muscle, once the blood supply is stopped at death, by the natural protection in the cell have in the alive tissue are not maintained as they are as in an egg.

3.3 Project Implications

This project demonstrated n-3 PUFA enriched eggs to be relatively stable under normal storage and cooking conditions and that these lipids could be further stabilized by antioxidants in the hen diet. Even though there was evidence for breakdown of n-3 PUFA and for co-oxidation of cholesterol into undesirable

COPs, the amounts were much lower than what would normally appear in many meat products containing n-3 PUFA. The natural defenses against oxidation already existing in the egg in combination with the ability to further stabilize it with antioxidants in the hen diet will enhance consumer confidence in the safety of this quality food product. This can in turn support sustainable growth of the Canadian egg industry. Findings from the study can also be used by the egg industry to educate consumers about the safety of omega-3 PUFA in enriched eggs under home-style cooking methods.

The cooking methods used in this study were limited to boiling and frying. Processes involving exposure to higher temperatures (such as microwaving) or to heat for longer times (industrial processing) may demonstrate more challenges for egg n-3 PUFA stability and need to be further explored.

Many n-3 PUFA enriched eggs use dietary flax as the sole source of enrichment. The form of n-3 PUFA in flax is LNA, a medium chain fatty acid that has health benefits, but not as many as that of the longer chain n-3 PUFA such as EPA, DPA and DHA. In the current study, LNA represents about 2/3 of the total n-3 PUFA in enriched eggs and long chain n-3 PUFA make up the remaining 1/3 of total n-3 PUFA. Compared to the source diet, where there was no detectable long-chain n-3 PUFA, the final egg product contains considerable more of the desirable n-3 PUFA forms. Much of this may result from conversion of the LNA to long chain n-3 PUFA, which would deliver more of the expected health benefits.

The reported conversion efficiency of LNA to long chain PUFA is less than 10% in healthy human subjects. The hen appears to have a higher efficiency ratio. This could be due to a combination of conversion to the longer forms through elongation and desaturation in combination with a reduced efficiency for moving LNA from the feed to the egg. Dietary n-3 PUFA concentration was higher than the resulting n-3 PUFA concentration in the egg, demonstrating that the fatty acid composition of the egg, while pliable, is not as easy to change as carcass fat composition. The egg is created to support the normal development of the embryo to a chick, a process that requires fairly specific resources. However, it is encouraging for the consumer that the egg contains such a high amount of n-3 PUFA in the long-chain form. These amounts could be further enhanced by using mixed n-3 PUFA sources in the hen diet, such as flax in combination with menhaden oil.

In Canada, there is no distinction between LNA and long chain PUFA in the labeling of n-3 PUFA enriched products. This can be problematic when the vast majority of the n-3 PUFA is not in the desirable long-chain forms. For example, the recommended intake of long chain n-3 PUFA is 0.5 to 1 g per day for adult but one n-3 PUFA enriched egg normally contains ~200 mg LNA and ~100 mg long chain n-3 PUFA. Countries such as Australia and the UK require specific labeling of DHA content rather than total n-3 PUFA. This would allow producers to more clearly demonstrate the potential health benefits of their products to the consumer. Clearly eggs are at an advantage in this market due to their relatively high ratio of long chain n-3 PUFA to medium chain n-3 PUFA.

In conclusion, cooking and storage can cause some loss in long chain n-3 PUFA and increase oxidation. However, it is possible to make the n-3 PUFA enriched eggs more stable by adding the dietary antioxidants or a combination of dietary antioxidants.

APPENDIX

A.1 Lipid profile in yolk on dry weight basis. (Table A.1)

A.2 Lipid profile of triglyceride in yolk on dry weight basis and fatty acids composition of triglyceride in yolk. (Table A.2a and Table A.2b)

A.3 Lipid profile of phospholipids in yolk on dry weight basis and fatty acids composition of phospholipids in yolk. (Table A.3a and Table A.3b)

A.4 Lipid profile in fried whole eggs on dry weight basis. (Table A.4)

A.5 Cholesterol oxidation product (COPs) content in fried whole eggs on dry weight basis. (Table A.5)

A.6 Egg Traits. (Table A.6)

A.7 The fatty acid composition in feed. (Table A.7)

A.8 The vitamin E content in feed. (Table A.8)

A.9 The fatty acid composition in original commercial table eggs. (Table A.9)

A.10 The total omega-3 polyunsaturated fatty acids content in enriched eggs affected by the interaction of cooking, vitamin E and selenium (Figure A.1).

A.11 The linolenic acid content of enriched eggs affected by the interaction of cooking, vitamin E and selenium (Figure A.2).

Table A.1 The lipid profile in yolk sample (mg/g dry yolk sample) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	29.41c	11.67c	3.42c	0.48b	0.50b	4.64c	86.14c	67.26b	33.39c	17.29c	1.93b
Boiled	16	36.45a	14.58a	4.00a	0.53a	0.59a	5.56a	102.9a	79.51a	41.14a	21.26a	1.94b
Fried	16	33.97b	13.34b	3.77b	0.49b	0.492b	5.04b	95.98b	76.66a	38.39b	19.35b	1.99a
SEM[4]		0.448	0.223	0.032	0.011	0.006	0.052	2.037	1.198	0.472	0.261	0.005
Storage (Mean)												
0day	24	32.52b	12.87b	3.77	0.51a	0.55a	5.25a	92.96	73.47	36.94b	19.19	1.93b
28days	24	34.04a	13.53a	3.69	0.48b	0.50b	4.90b	97.03	75.47	38.34a	19.41	1.98a
SEM		0.366	0.182	0.026	0.009	0.005	0.042	1.663	0.978	0.385	0.213	0.004
Vitamin E (Mean)												
High	24	33.35	13.22	3.64b	0.49	0.51b	5.02	94.66	74.31	37.60	19.24	1.96
Low	24	33.20	13.18	3.82a	0.51	0.54a	5.14	95.33	74.64	37.67	19.36	1.95
SEM		0.366	0.182	0.026	0.009	0.005	0.042	1.663	0.978	0.385	0.213	0.004
Selenium (Mean)												
High	24	33.21	13.17	3.69b	0.49	0.51b	5.06	95.08	75.00	37.51	19.24	1.96
Low	24	33.34	13.22	3.77a	0.51	0.54a	5.09	94.91	73.86	37.77	19.37	1.95
SEM		0.366	0.182	0.026	0.009	0.005	0.042	1.663	0.978	0.385	0.213	0.004
Source of Variation		**Probability**										
Cooking (C)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1648
Storage (S)		0.7915	0.9568	0.6138	0.3041	0.1669	0.4053	0.7188	0.3315	0.8169	0.9490	0.0848
C*S		0.0559	0.0225	0.8737	0.1563	0.8045	0.8366	0.1548	0.7559	0.0564	0.0263	0.0008
Vitamin E (E)		0.2030	0.2355	0.3968	0.0943	0.0609	0.0148	0.2662	0.3088	0.1772	0.2094	0.9158
Selenium (Se)		0.2903	0.3472	0.5027	0.3871	0.9684	0.8988	0.1182	0.0199	0.2818	0.3751	0.2254

E*Se	0.9208	0.1702	0.0580	0.0503	0.8486	0.6962	0.4786	0.0157	0.9061	0.1975	<.0001
E*C	0.3567	0.2997	0.9765	0.2029	0.3071	0.6334	0.3510	0.0007	0.3269	0.3263	<.0001
Se*C	0.5324	0.6521	0.0034	0.3517	0.2083	0.1395	0.4491	0.0269	0.5112	0.5945	0.6273
E*S	0.0533	0.0412	0.0716	0.2400	0.1410	0.2035	0.0997	0.1645	0.0452	0.0479	0.1723
Se*S	0.4223	0.4053	0.2058	0.0527	0.4307	0.7676	0.4389	0.2022	0.4375	0.3780	0.0831
E*C*S	0.8543	0.7472	0.3102	0.4223	0.5485	0.6723	0.9849	0.9532	0.8312	0.7655	0.0012
Se*C*S	0.0868	0.1317	0.6892	0.1036	0.0011	0.0732	0.2566	0.7122	0.0815	0.1461	0.0218
E*Se*C	0.1425	0.0605	0.8612	0.2571	0.0132	0.0794	0.0793	0.3813	0.1392	0.0594	<.0001
E*Se*S	0.2014	0.4409	0.5614	0.0083	0.2633	0.5463	0.0285	0.4491	0.2160	0.4155	0.0004
E*Se*C*S	0.0121	0.0045	0.0826	0.2707	0.0011	0.0008	0.0125	0.0573	0.0097	0.0042	<.0001

[a-c] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table A.2a The lipid profile of triglyceride (mg/g dry yolk sample) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	30.32a	15.65a	0.304a	0.206a	0.19a	0.38a	112.00a	55.67a	30.99a	16.67a	0.27
Boiled	16	29.99a	15.44a	0.3a	0.221a	0.181a	0.38a	107.00a	56.96a	30.66a	16.43a	0.27
Fried	16	21.51b	11.25b	0.168b	0.129b	0.131b	0.26b	74.61b	38.71b	21.94b	11.89b	0.27
SEM[4]		0.845	0.463	0.015	0.011	0.006	0.013	3.036	2.276	0.853	0.496	0.001
Storage (Mean)												
0day	24	27.14	14.13	0.252	0.17	0.17	0.35	97.25	49.14	27.75	15.01	0.27
28days	24	27.40	14.10	0.262	0.19	0.16	0.34	98.53	51.75	27.98	14.98	0.27
SEM		0.69	0.378	0.012	0.009	0.005	0.01	2.479	1.858	0.697	0.405	8.50E-04
Vitamin E (Mean)												
High	24	26.64	13.79	0.249	0.17	0.16	0.32b	95.89	51.81	27.18	14.63	0.27
Low	24	27.91	14.44	0.265	0.19	0.17	0.36a	99.88	49.08	28.55	15.36	0.27
SEM		0.69	0.378	0.012	0.009	0.005	0.01	2.479	1.858	0.697	0.405	8.50E-04
Selenium (Mean)												
High	24	27.80	14.37	0.251	0.19	0.16	0.35	100.7	53.72a	28.41	15.25	0.27
Low	24	26.75	13.86	0.263	0.18	0.16	0.34	95.05	47.17b	27.32	14.74	0.27
SEM		0.69	0.378	0.012	0.009	0.005	0.01	2.479	1.858	0.697	0.405	8.50E-04
Source of Variation		**Probability**										
Cooking (C)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1648
Storage (S)		0.7915	0.9568	0.6138	0.3041	0.1669	0.4053	0.7188	0.3315	0.8169	0.9490	0.0848
C*S		0.0559	0.0225	0.8737	0.1563	0.8045	0.8366	0.1548	0.7559	0.0564	0.0263	0.0008
Vitamin E (E)		0.2030	0.2355	0.3968	0.0943	0.0609	0.0148	0.2662	0.3088	0.1772	0.2094	0.9158
Selenium (Se)		0.2903	0.3472	0.5027	0.3871	0.9684	0.8988	0.1182	0.0199	0.2818	0.3751	0.2254

E*Se	0.9208	0.1702	0.0580	0.0503	0.8486	0.6962	0.4786	0.0157	0.9061	0.1975	<.0001
E*C	0.3567	0.2997	0.9765	0.2029	0.3071	0.6334	0.3510	0.0007	0.3269	0.3263	<.0001
Se*C	0.5324	0.6521	0.0034	0.3517	0.2083	0.1395	0.4491	0.0269	0.5112	0.5945	0.6273
E*S	0.0533	0.0412	0.0716	0.2400	0.1410	0.2035	0.0997	0.1645	0.0452	0.0479	0.1723
Se*S	0.4223	0.4053	0.2058	0.0527	0.4307	0.7676	0.4389	0.2022	0.4375	0.3780	0.0831
E*C*S	0.8543	0.7472	0.3102	0.4223	0.5485	0.6723	0.9849	0.9532	0.8312	0.7655	0.0012
Se*C*S	0.0868	0.1317	0.6892	0.1036	0.0011	0.0732	0.2566	0.7122	0.0815	0.1461	0.0218
E*Se*C	0.1425	0.0605	0.8612	0.2571	0.0132	0.0794	0.0793	0.3813	0.1392	0.0594	<.0001
E*Se*S	0.2014	0.4409	0.5614	0.0083	0.2633	0.5463	0.0285	0.4491	0.2160	0.4155	0.0004
E*Se*C*S	0.0121	0.0045	0.0826	0.2707	0.0011	0.0008	0.0125	0.0573	0.0097	0.0042	<.0001

[a-b] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table A.2b The fatty acids composition of triglyceride (g/100g total fatty acids) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	14.11	7.28b	0.14a	0.10a	0.09	0.18	52.15	25.62	14.42	7.75	1.86
Boiled	16	14.21	7.31b	0.14a	0.10a	0.09	0.18	50.76	26.85	14.53	7.78	1.87
Fried	16	14.63	7.63a	0.12b	0.09b	0.09	0.18	50.72	26.24	14.92	8.07	1.85
SEM[4]		0.188	0.105	0.009	0.005	0.002	0.004	0.672	0.965	0.193	0.112	0.007
Storage (Mean)												
0day	24	14.37	7.48	0.13	0.09	0.09a	0.18	51.28	26.02	14.69	7.94	1.86
28days	24	14.26	7.33	0.14	0.10	0.08b	0.18	51.15	26.45	14.55	7.79	1.87
SEM		0.153	0.086	0.007	0.004	0.001	0.003	0.549	0.788	0.157	0.092	0.006
Vitamin E (Mean)												
High	24	14.13	7.31	0.13	0.09	0.08b	0.17b	50.58	27.19	14.41	7.75	1.86
Low	24	14.50	7.50	0.14	0.10	0.09a	0.19a	51.84	25.28	14.83	7.98	1.86
SEM		0.153	0.086	0.007	0.004	0.001	0.003	0.549	0.788	0.157	0.092	0.006
Selenium (Mean)												
High	24	14.04b	7.26b	0.12b	0.09	0.08b	0.17b	50.89	27.02	14.34b	7.70b	1.87
Low	24	14.59a	7.55a	0.15a	0.10	0.09a	0.19a	51.54	25.46	14.90a	8.03a	1.86
SEM		0.153	0.086	0.007	0.004	0.001	0.003	0.549	0.788	0.157	0.092	0.006
Source of Variation		**Probability**										
Cooking (C)		0.1386	0.0484	0.0559	0.1270	0.3987	0.6720	0.2513	0.6665	0.1768	0.1076	0.1648
Storage (S)		0.6031	0.2288	0.7827	0.3970	0.0013	0.0727	0.8666	0.7073	0.5423	0.2339	0.0848
C*S		0.0526	0.0055	0.7248	0.2083	0.1890	0.5379	0.4260	0.2121	0.0576	0.0083	0.0008
Vitamin E (E)		0.1037	0.1385	0.5222	0.1194	0.0043	0.0016	0.1171	0.1002	0.0723	0.0997	0.9158
Selenium (Se)		0.0193	0.0229	0.0308	0.5543	0.0003	0.0018	0.4086	0.1742	0.0196	0.0154	0.2254

E*Se	0.1398	0.1219	0.0283	0.0793	0.2976	0.1324	0.0017	0.0160	0.1582	0.1655	<.0001
E*C	0.0465	0.0051	0.5773	0.8293	0.0191	0.1503	0.0115	0.0134	0.0522	0.0054	<.0001
Se*C	0.0326	0.0774	0.0018	0.3112	0.0006	0.0020	0.0044	0.0155	0.0300	0.0483	0.6273
E*S	0.0077	0.0025	0.1446	0.0860	0.0684	0.2855	0.0544	0.0242	0.0052	0.0036	0.1723
Se*S	0.1935	0.1473	0.2150	0.0124	0.3499	0.9039	0.2112	0.1917	0.2238	0.1142	0.0831
E*C*S	0.3874	0.0866	0.2744	0.1752	0.0210	0.2209	0.9130	0.7564	0.2963	0.1036	0.0012
Se*C*S	0.1533	0.1025	0.7779	0.0052	<.0001	0.1675	0.9241	0.6826	0.1432	0.1411	0.0218
E*Se*C	0.5773	0.2289	0.7815	0.7527	0.0002	0.0657	0.1469	0.2153	0.5121	0.2084	<.0001
E*Se*S	0.3740	0.0384	0.7626	0.0563	0.4460	0.0698	0.1715	0.6114	0.2921	0.0461	0.0004
E*Se*C*S	0.1883	0.0635	0.6393	0.8775	0.0047	0.0184	0.0991	0.1324	0.2405	0.0596	<.0001

[a-b] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table A.3a The lipid profile of phospholipids (mg/g dry yolk sample) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	7.92a	0.76a	2.30a	0.33a	0.25a	3.13a	16.12a	25.03a	10.57a	4.46a	0.34c
Boiled	16	8.65a	0.84a	2.35a	0.32a	0.27a	3.19a	17.49a	27.17a	11.38a	4.62a	0.35b
Fried	16	6.14b	0.59b	1.62b	0.22b	0.19b	2.19b	11.66b	19.01b	8.03b	3.19b	0.36a
SEM[4]		0.313	0.03	0.089	0.011	0.01	0.126	0.754	0.997	0.414	0.175	0.002
Storage (Mean)												
0day	24	7.52	0.73	2.08	0.29	0.24	2.84	15.14	23.47	9.92	4.10	0.35b
28days	24	7.63	0.72	2.11	0.29	0.23	2.83	15.04	24.00	10.07	4.08	0.36a
SEM		0.256	0.025	0.072	0.009	0.009	0.103	0.616	0.814	0.338	0.143	0.001
Vitamin E (Mean)												
High	24	7.28	0.69b	1.97b	0.28	0.23	2.72	14.10b	22.65	9.57	3.92	0.35
Low	24	7.86	0.77a	2.21a	0.30	0.24	2.95	16.07a	24.83	10.41	4.26	0.35
SEM		0.256	0.025	0.072	0.009	0.009	0.103	0.616	0.814	0.338	0.143	0.001
Selenium (Mean)												
High	24	7.28	0.69b	1.97b	0.28	0.23	2.72	14.10b	22.65	9.57	3.92	0.35
Low	24	7.86	0.77a	2.21a	0.30	0.24	2.95	16.07a	24.83	10.41	4.26	0.35
SEM		0.256	0.025	0.072	0.009	0.009	0.103	0.616	0.814	0.338	0.143	0.001
Source of Variation		**Probability**										
Cooking (C)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Storage (S)		0.7586	0.7795	0.7645	0.8740	0.8604	0.9210	0.9095	0.9044	0.6527	0.7609	0.0002
C*S		0.6185	0.7320	0.7630	0.6040	0.3899	0.8711	0.4628	0.8549	0.7261	0.6542	<.0001
Vitamin E (E)		0.1183	0.0447	0.0329	0.1599	0.2896	0.1199	0.0328	0.1072	0.0704	0.0906	0.3308
Selenium (Se)		0.0985	0.1085	0.0654	0.1699	0.0584	0.0372	0.0515	0.0489	0.1208	0.0924	0.0156

E*Se	0.4270	0.1245	0.6611	0.0796	0.8218	0.7400	0.9368	0.5296	0.5980	0.4730	0.3410
E*C	0.4777	0.5230	0.4583	0.4794	0.1346	0.2924	0.4879	0.3284	0.4307	0.4784	0.0325
Se*C	0.6031	0.9076	0.2466	0.3062	0.1693	0.3670	0.9659	0.4354	0.5926	0.5066	0.0386
E*S	0.2751	0.2155	0.3284	0.1763	0.0493	0.4492	0.9537	0.3377	0.4326	0.2868	0.5460
Se*S	0.0787	0.0782	0.1485	0.0908	0.0133	0.0698	0.0415	0.0636	0.0740	0.0889	0.9181
E*C*S	0.7302	0.6357	0.6753	0.6495	0.3804	0.8422	0.7180	0.7914	0.8381	0.7336	0.9347
Se*C*S	0.0304	0.0138	0.0380	0.1496	0.0090	0.0080	0.1902	0.0102	0.0373	0.0324	<.0001
E*Se*C	0.6357	0.3790	0.8694	0.1506	0.9513	0.6757	0.2663	0.6118	0.6713	0.6845	0.0045
E*Se*S	0.0347	0.0908	0.0202	0.0782	0.1635	0.0487	0.1692	0.0580	0.0360	0.0307	0.0047
E*Se*C*S	0.0314	0.0125	0.0278	0.0092	0.0160	0.0206	0.0211	0.0172	0.0254	0.0314	0.0105

[a-c] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table A.3b The fatty acids composition of phospholipids (g/100g total fatty acids) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs affected by the main factors: cooking, storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Cooking (Mean)												
Raw	16	14.09b	1.35b	4.09a	0.59a	0.45	5.54a	28.64	44.50	18.81	7.92a	2.38c
Boiled	16	14.25ab	1.38ab	3.86b	0.53b	0.44	5.26b	28.78	44.74	18.74	7.60b	2.47b
Fried	16	14.73a	1.42a	3.89b	0.53b	0.44	5.24b	27.38	45.58	19.25	7.64ab	2.53a
SEM[4]		0.195	0.019	0.054	0.009	0.009	0.079	0.929	0.576	0.255	0.11	0.012
Storage (Mean)												
0day	24	14.27	1.39	3.92	0.55	0.45	5.38	28.73	44.55	18.82	7.77	2.43b
28days	24	14.44	1.37	3.98	0.55	0.44	5.31	27.81	45.33	19.05	7.67	2.49a
SEM		0.159	0.016	0.044	0.008	0.007	0.064	0.759	0.471	0.208	0.09	0.01
Vitamin E (Mean)												
High	24	14.57	1.38	3.95	0.56a	0.45	5.42	27.62	45.27	19.15	7.82	2.45
Low	24	14.14	1.38	3.95	0.54b	0.43	5.27	28.92	44.61	18.71	7.62	2.46
SEM		0.159	0.016	0.044	0.008	0.007	0.064	0.759	0.471	0.208	0.09	0.01
Selenium (Mean)												
High	24	14.24	1.37	3.94	0.54b	0.44	5.38	28.86	44.48	18.79	7.73	2.44b
Low	24	14.47	1.39	3.97	0.56a	0.44	5.32	27.67	45.40	19.08	7.71	2.48a
SEM		0.159	0.016	0.044	0.008	0.007	0.064	0.759	0.471	0.208	0.09	0.01
Source of Variation		**Probability**										
Cooking (C)		0.0716	0.0366	0.0130	0.0004	0.6183	0.0214	0.5111	0.3964	0.3144	0.0997	<.0001
Storage (S)		0.4757	0.2765	0.3166	0.4827	0.5080	0.4650	0.3992	0.2512	0.4440	0.4790	0.0002
C*S		0.1702	0.5648	0.0122	0.1762	0.6363	0.2773	0.3641	0.4077	0.1138	0.5128	<.0001
Vitamin E (E)		0.0701	0.7337	0.9274	0.0412	0.0582	0.1080	0.2389	0.3317	0.1507	0.1218	0.3308
Selenium (Se)		0.3196	0.3235	0.6183	0.0474	0.9885	0.5362	0.2801	0.1815	0.3369	0.9277	0.0156

E*Se	0.0880	0.0007	0.5392	0.0018	0.6602	0.9724	0.2924	0.4024	0.1385	0.3071	0.3410
E*C	0.3979	0.9548	0.0542	0.1912	0.2400	0.2867	0.2966	0.2713	0.2770	0.4718	0.0325
Se*C	0.3511	0.7432	0.0050	0.0505	0.0062	0.0438	0.2334	0.3366	0.1588	0.0816	0.0386
E*S	0.0786	0.0358	0.1587	0.3105	0.0055	0.3964	0.2499	0.3995	0.0931	0.1908	0.5460
Se*S	0.3311	0.2658	0.0348	0.2637	0.2975	0.2755	0.3206	0.3809	0.2237	0.3236	0.9181
E*C*S	0.1691	0.0300	0.7584	0.8615	0.3466	0.3930	0.3776	0.4409	0.2725	0.3486	0.9347
Se*C*S	0.3372	0.0053	0.5649	0.0977	0.0111	0.0012	0.3748	0.6287	0.4034	0.0037	<.0001
E*Se*C	0.3691	0.4548	0.0347	0.0122	0.1343	0.3506	0.3070	0.3187	0.2499	0.2748	0.0045
E*Se*S	0.2606	0.6085	0.1551	0.3246	0.5662	0.7578	0.3662	0.3440	0.2418	0.9984	0.0047
E*Se*C*S	0.1090	0.5740	0.5247	0.8612	0.0386	0.9463	0.3564	0.3680	0.2214	0.8331	0.0105

[a-b] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)

[4] SEM=standard error of the means

Table A.4 The lipid profile (mg/g dry whole egg sample) of omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs (fried whole eggs) affected by the main factors: storage, vitamin E and selenium.

Effects	n[1]	Fatty acid profile										
		C18:2 n-6	C18:3 n-3	C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	MUFA[2]	SFA[3]	Total n-6	Total n-3	n-6: n-3
Storage (Mean)												
0day	24	21.90	8.85	2.44	0.33	0.36	3.40	63.31	48.70	24.46	12.94	1.89
28days	24	22.32	9.18	2.34	0.32	0.36	3.28	65.92	51.29	24.77	13.14	1.89
SEM[4]		0.643	0.281	0.066	0.009	0.011	0.102	1.996	1.478	0.703	0.389	0.006
Vitamin E (Mean)												
High	24	21.90	8.97	2.36	0.32	0.36	3.34	63.66	49.31	24.38	12.99	1.88b
Low	24	22.32	9.06	2.42	0.33	0.35	3.34	65.56	50.68	24.86	13.08	1.90a
SEM		0.643	0.281	0.066	0.009	0.011	0.102	1.996	1.478	0.703	0.389	0.006
Selenium (Mean)												
High	24	22.14	9.19	2.36	0.34	0.37	3.30	65.46	49.96	24.60	13.19	1.86b
Low	24	22.09	8.84	2.42	0.32	0.34	3.38	63.76	50.02	24.63	12.88	1.91a
SEM		0.643	0.281	0.066	0.009	0.011	0.102	1.996	1.478	0.703	0.389	0.006
Source of Variation		**Probability**										
Vitamin E (E)		0.6564	0.8218	0.5095	0.4436	0.4046	0.9583	0.5194	0.5284	0.6419	0.8640	0.0344
Selenium (Se)		0.9553	0.4019	0.4995	0.1895	0.0877	0.5795	0.5640	0.9777	0.9785	0.5805	0.0008
Storage (S)		0.6548	0.4314	0.2860	0.6075	0.8169	0.4375	0.3812	0.2517	0.7621	0.7256	0.5926
E*Se		0.3485	0.6851	0.2939	0.1429	0.9942	0.4341	0.3547	0.1630	0.3379	0.5914	0.0067
E*S		0.6726	0.6567	0.3615	0.2912	0.6988	0.7664	0.6495	0.6665	0.6339	0.6788	0.7205
Se*S		0.7258	0.6652	0.6784	0.8974	0.8400	0.7812	0.2677	0.3120	0.7084	0.8167	0.2366
E*Se*S		0.4811	0.4606	0.3648	0.5067	0.2245	0.8932	0.5455	0.7453	0.4624	0.6288	0.0753

[a-b] Means within a column in each treatment with no common superscripts differ (P<0.05).

[1] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[2] MUFA=monounsaturated fatty acid. (16:1 + 18:1 n-7 + 18:1 n-9)

[3] SFA=saturated fatty acid. (14:0 + 16:0 + 18:0)
[4] SEM=standard error of the means

Table A.5 The mean value of cholesterol oxidation products (COPs) content in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs (fried whole eggs) affected by the main factors: storage, vitamin E and selenium. (dwb[1])

Effects	n[2]	7-KC	7a-OH	7b-OH	Total
Vitamin E (Mean)					
Low	8	1.15	5.06	2.39a	8.60a
High	8	0.78	4.81	1.82b	7.24b
SEM[3]		0.141	0.151	0.078	0.158
Selenium (Mean)					
Low	8	1.34a	5.08	2.52a	8.77a
High	8	0.60b	4.79	1.69b	7.07b
SEM		0.141	0.151	0.078	0.158
Storage (Mean)					
0day	8	1.03	5.09	1.59b	7.54b
28days	8	0.90	4.78	2.62a	8.30a
SEM		0.141	0.151	0.078	0.158
Source of Variation		**----------Probability----------**			
Vitamin E (E)		0.1012	0.2771	0.0009	0.0003
Selenium (Se)		0.0056	0.2100	<.0001	<.0001
Storage (S)		0.5290	0.1888	<.0001	0.0095
E*Se		0.0311	0.8495	0.0388	0.0020
E*S		0.1645	0.7327	0.8765	0.4190
Se*S		0.1197	0.9561	0.2216	0.1246
E*Se*S		0.8392	0.9827	0.1367	0.2054

Mean values of the COPs ug/g dry sample.

[a-b] Means within a column in each treatment with no common superscripts differ ($P<0.05$).

[1] dwb=dry weight basis;

[2] n= sample number, each sample calculation of 8 eggs, (pool of 8 eggs) = (sample)

[3] SEM= standard error of the means;

Table A.6 Vitamin E and selenium effect on albumen height, shell thickness, shell weight, specific gravity, yolk weight and egg weight in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched eggs.

Treatments name	**LE x LSe**	**HE x LSe**	**LE x HSe**	**HE x HSe**	SEM
Albumen Height (mm)	8.600b	8.900ab	8.900ab	9.200a	0.200
Shell Thickness (mm)	0.355	0.356	0.357	0.359	0.003
Shell Weight (g)	5.830	5.904	5.828	5.896	0.053
Specific Gravity (g/cm^3)	1.086ab	1.085b	1.085b	1.087a	0.001
Yolk Weight (g)	16.90b	17.60a	17.30ab	17.40a	0.200
Egg Weight (g)	61.70	61.80	60.70	61.00	0.500

[a-b] Means within a column in each treatment with no common superscripts differ ($P<0.05$).
SEM = standard error of the means.
LE x LSe = low vitamin E and low selenium treatment.
HE x LSe = high vitamin E and low selenium treatment
LE x HSe = low vitamin E and high selenium treatment.
HE x HSe = high vitamin E and high selenium treatment.

Table A.7 Vitamin E content in omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched laying hen feed.

Treatments name	LE x LSe	HE x LSe	LE x HSe	HE x HSe
Vitamin E Content	7.95b	20.65ab	13.29ab	24.91a
SEM	2.488	2.488	2.488	2.488

Mean values of the Fatty acid composition mg/g sample.

[a-b] Means within a column in each treatment with no common superscripts differ ($P<0.05$)

SEM = standard error of the means.

LE x LSe = low vitamin E and low selenium treatment.

HE x LSe = high vitamin E and low selenium treatment

LE x HSe = low vitamin E and high selenium treatment.

HE x HSe = high vitamin E and high selenium treatment.

Table A.8 The fatty acid composition of the omega-3 polyunsaturated fatty acids (n-3 PUFA) enriched laying hen base diet.

Fatty Acids profile in feed	—— mg/g ——
16:0	5.71
16:1	0.12
18:0	1.65
18:1 n-7	1.39
18:1 n-9	22.53
18:2 n-6	18.48
18:3 n-3	25.95
18:3 n-6	0.10
20:0	0.22
20:2 n-6	0.05
20:3 n-6	0.05
20:4 n-6	ND
20:5 n-3	ND
22:0	0.15
24:01:00	0.04
22:1 n-9	ND
22:4 n-6	ND
22:5 n-3	ND
22:6 n-3	ND
SFA[2]	7.99
MUFA[3]	24.85
PUFA[4]	45.15
Total n-3	25.95
Total n-6	18.62

Mean values of the fatty acid composition mg/g feed.
SEM = standard error of the mean
[1] ND = Not detected.
[2] SFA: Saturated fatty acids, 14:0 + 16:0 + 18:0 + 20:0 + 22:0
[3] MUFA: Monounsaturated fatty acids, 16:1 + 18:1 n-7 + 18:1 n-9 + 22:1 n-9 + 24:1
[4] PUFA: Polyunsaturated fatty acids, 18:2 n-6 + 18:3 n-3 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-3 + 22:6 n-3.

Table A.9 The fatty acid composition in original commercial table eggs[a].

Fatty acid composition	
8:0 Caprylic	0.05
10:0 Capric	0.05
12:0 Lauric	0.05
14:0 Myristic	0.41
16:0 Palmitic	26.91
18:0 Stearic	9.48
20:0 Arachidic	0.48
14:1 Myristoleic	0.12
16:1 Palmitoleic	3.60
18:1 Oleic	41.97
20:1 Eicosenoic	0.34
22:1 Erucic	0.05
18:2 Linoleic	13.88
18:3 Linolenic	0.41
20:4 Arachidonic	1.72
20:5 Eicosapentaenoic	0.05
22:6 Docosahexaenoic	0.44
Saturated--total	37.48
Monounsaturated--total	46.06
Polyunsaturated--total	16.49

Mean values of the fatty acid composition g/100g of total fatty acids.
[a]Adapt from American Egg Board (2008)

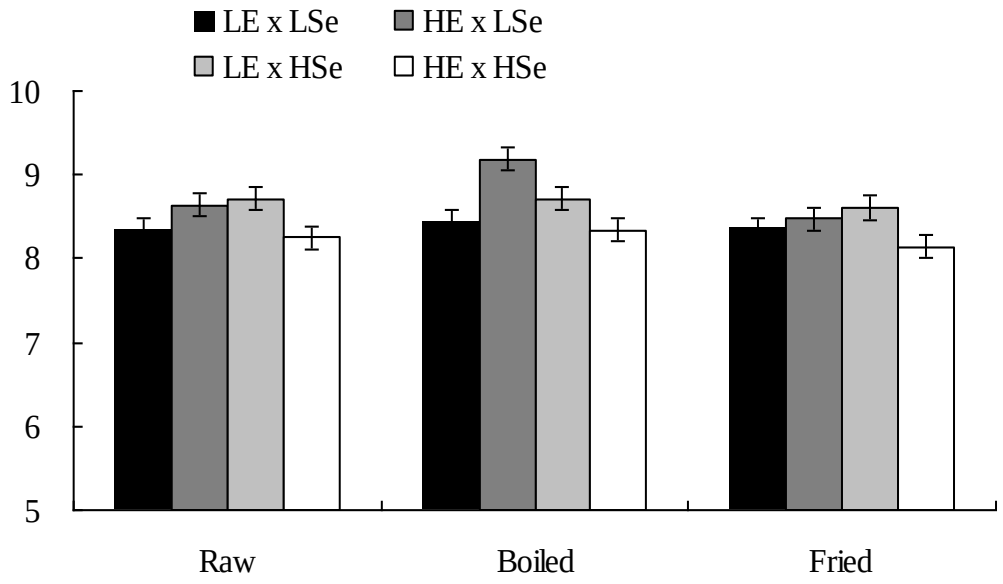

Figure A.1 The total omega-3 polyunsaturated fatty acids (n-3 PUFA) content (g/100g total fatty acids) of enriched eggs affected by the interaction of cooking, vitamin E and selenium.
LE x LSe = low vitamin E and low selenium treatment.
HE x LSe = high vitamin E and low selenium treatment
LE x HSe = low vitamin E and high selenium treatment.
HE x HSe = high vitamin E and high selenium treatment.
Treatments were not significantly different (P = 0.3860)

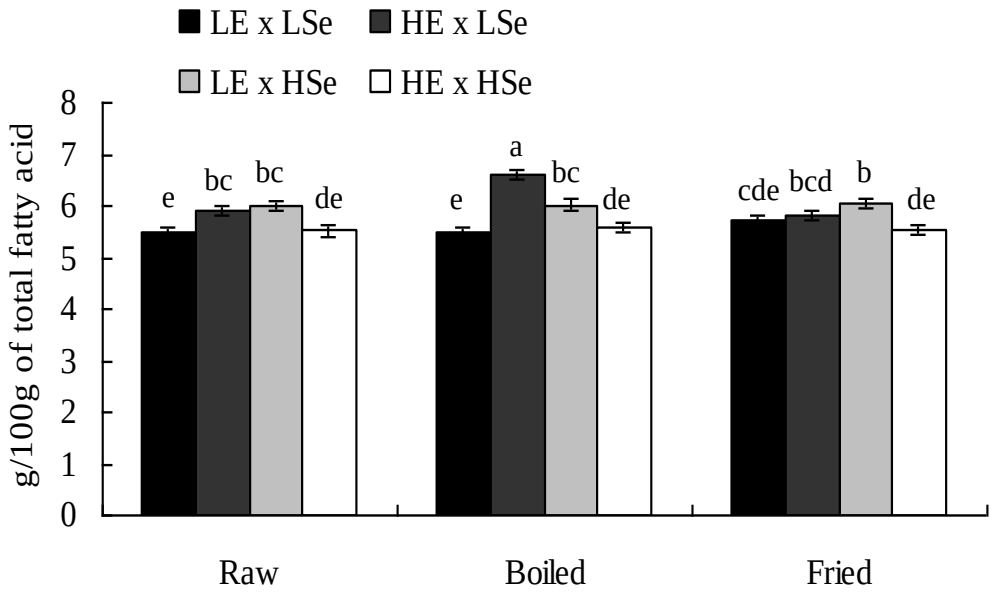

Figure A.2 The linolenic acid (LNA) content (g/100g total fatty acids) of enriched eggs affected by the interaction of cooking, vitamin E and selenium.
LE x LSe = low vitamin E and low selenium treatment.
HE x LSe = high vitamin E and low selenium treatment
LE x HSe = low vitamin E and high selenium treatment.
HE x HSe = high vitamin E and high selenium treatment.
Treatments were not significantly different (P = 0.3860)