A Comparison of the Microbiological and Functional Properties of Regular and Omega-3 Chicken Eggs

By

Yang HU

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Yang Hu

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

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ABSTRACT

In recent years, there has been increasing consumer concern over the relationship of the lipid composition of egg yolk with the development of coronary heart disease. Under the demand of optimization of lipid composition in eggs, omega-3 enriched eggs, produced by hens fed with flax based diet, appeared to be one of the successful ways to solve the problem. These eggs have approximately 7 times more omega-3 fatty acids and vitamin E compared to regular eggs. Considering egg-associated salmonellosis, this study was designed to determine the growth and survival patterns of *S. typhimurium* and *E. coli* in regular and omega-3 whole eggs, egg yolk and egg albumen at 22^0 C, 8^0 C, and -20^0 C. The analysis of microbiological counts was conducted at 24 hour, 1 week, and 2 week intervals during storage at 22^0 C for 72 hours, 8^0 C for 6 weeks, and -20^0 C for 8 weeks. The results showed that the growth and/or survival of *S. typhimurium* or *E. coli* were similar among omega-3 eggs versus regular eggs. Also, the growth of *S. typhimurium* either under aerobic or anoxic conditions at 22^0 C in regular egg yolk modified with approximate 63-240 ppm of α-tocopherol was not affected. In addition, *S. typhimurium* in egg yolk heated at 56.5^0 C showed similar heat resistance regardless of the concentration of α-tocopherol (55-713 ppm) or total tocopherol (92-1238 ppm). It was confirmed by HPLC analysis of vitamin E in egg yolk that omega-3 egg yolk has approximate 5-12 times more α-tocopherol than regular egg yolk. Furthermore, it was demonstrated that there was no difference in terms of functionality of egg yolks between regular and omega-3 egg yolks. Overall, considering that the growth and survival of *S. typhimurium* was similar in regular and omega-3 egg yolk, omega-3 eggs obviously have

the advantages of providing more natural sources of omega-3 fatty acids and tocopherol to consumers.

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1.0 INTRODUCTION

In recent years, there has been increasing consumer concern over the relationship of the lipid composition of chicken eggs to the development of coronary heart disease. Coronary heart disease (CHD) is one of the leading causes of death in Western countries, being mainly related to dietary lipid combinations. The typical western diet is rich in lipid, containing about 40-50% saturated fatty acids (Shafey and Cham, 1994). Current dietary recommendations include reduction of saturated fat as well as cholesterol, increase of polyunsaturated fatty acids (PUFAs), plus a balance between the omega-6 and omega-3 PUFAs families (Lauritzen, 1994). In particular, chicken eggs contain high quality protein, vitamins, and minerals; however, their relatively high cholesterol content has been recognized as a negative dietary factor. Since restrictions on dietary cholesterol and egg consumption were proposed in 1972, the per capita consumption of eggs has declined in Western countries (Jiang and Sim, 1994; Van Elswyk, 1997). Egg consumption in the USA dropped from a peak of 405 eggs per capita in 1945 to 244 eggs per capita in recent years (Egg Nutrition Center, 1999).

Consumers continue to demand low cholesterol and low fat food products. For many years, the egg industry supported research aimed to reduce egg cholesterol. Although cholesterol content of the egg yolk is minimally altered through hen's diet (Shafey and Cham, 1994), research has been reported to indicate that the fatty acid content can be substantially modified by diet by Cruikshank in 1934 (Leskanich and Noble, 1997). However, it was not until recently that food scientists were able to alter the omega-3 polyunsaturated fatty acid (PUFA) profile of eggs by variations in the hen's

diet. Bang and Dyerburg (1972) first reported that omega-3 fatty acids had the ability to reduce the risk of CHD (Marshall et al., 1994), by observing the low mortality of Greenland Eskimos from CHD with a high dietary intake of fish. This correlation involving a high intake of omega-3 fatty acids from fish with a low incidence of CHD has now been supported by numerous clinical and epidemiological studies (Ferrier et al., 1994; Lauritzen, 1994; Sim and Jiang, 1994; Leskanich and Noble, 1997; Van Elswyk, 1997).

However, many Western populations do not consume adequate levels of omega-3 fatty acids in their daily diet. In addition, Western diets are high in omega-6 PUFAs, which are one of two essential omega PUFAs that can not be synthesized by humans. This increase is most likely due to a dramatic increase in the consumption of refined vegetable oils, margarine, spreads, shortenings, and fried foods over the last 50 years. This leads to an imbalanced ratio from 10 to 30 of omega-6:omega-3 PUFAs, compared to the optimal ratio of 6:1 (Garcia, 1998). This may result in an increase in CHD. In order to decrease the incidence of CHD, current dietary recommendations include the consumption of at least one or two 100g servings of fish per week, as this is the main source of omega-3 fatty acids (Garcia, 1998). However, realistically, the intake of fish is much lower than the recommended level. People may either not like to eat fish or may not have access to it. Therefore, the incorporation of omega-3 fatty acids in eggs may result in an alternative way to increase the intake of this nutrient, since eggs and egg products occur widely in people's diets, in items such as egg dishes, bakery products, mayonnaise, and salad dressing. In fact, compared to traditional eggs, the creation of

omega-3 fatty acids enriched eggs enhances the health standard of eggs and improves their image.

Modifications in lipid content of chicken eggs with omega-3 fatty acids can be achieved by feeding hens a diet containing fish oil or flaxseed (Van Elswyk, 1997). Currently, menhaden oil, flaxseed, and marine algae are the only available sources for the egg industry to incorporate omega-3 fatty acids in the hen's diet. Results of recent reports on fortifying the egg yolk of chicken with omega-3 fatty acids using a variety of diets were summarized in a review by Leskanich and Noble (1997). Although there were generally no negative effects on egg production and other parameters, use of dietary marine oil meal developed fishy-like flavors in the chicken eggs. This was because PUFAs in marine oil include eicosapentaenoil acid (C20:5; EPA) and docosahexaenoic acid (C22:6; DHA) that are highly susceptible to oxidation (Aymond and Van Elswyk, 1995). Oxidized fatty acids and their degeneration products are not only undesirable but are also known to cause adverse biological effects in humans (Aymond and Van Elswyk, 1995). Therefore, the feeding of flaxseed instead of marine oil has been investigated. Flaxseed is rich in α-linoleic acid (C18:3; LNA), this means fewer double bonds than EPA and DHA, thus less susceptibility to lipid oxidation. Dietary flaxseed was found to significantly increase omega-3 fatty acids in eggs while minimizing the susceptibility of PUFAs to oxidation. Omega-3 enriched eggs are currently sold through retail outlets in Australia, Canada, USA, UK, etc., and appear to have increasing consumer acceptance (Leskanich and Noble, 1997; Flaxcouncil, 1999; Manitoba Egg Producers, 2000). In particular, flaxseed grows abundantly on the Canadian prairies (Flaxcouncil, 1999), which provides an opportunity for the egg industry to produce omega-3 enriched eggs.

However, the use of flaxseed (ground or whole) does not completely prevent lipid oxidation. As a result, omege-3 fatty acids enriched eggs still require improvements with respect to organoleptic quality. For example, the addition of vitamin E to flaxseed diet could further enhance the health benefits of omega-3 eggs by preventing or reducing oxidation (Van Elswyk, 1997). In this regard, the vitamin E content in egg yolk could increase linearly with an increasing dose of vitamin E supplemented in the diet (Jiang et al., 1994; Qi and Sim, 1998). In fact, omega-3 fatty acids enriched chicken eggs contain as much as 8 and 7 times more omega-3 fatty acids and vitamin E, respectively, compared to regular eggs (Manitoba Egg Producers, 2000). Unlike commonly used synthetic antioxidants, such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA), vitamin E is a natural lipid oxidation inhibitor that has been successfully applied in the meat industry, thus increasing meat product shelf life (Cabedo et al., 1997). Recently, studies on the health benefits of vitamin E have shown that it may be anticarcinogenic, decrease coronary heart disease, and suppress inflammation. The epidemiological studies, based on the application of vitamin E in the prevention of human diseases associated with free radical mechanisms, actually suggested that it is safe to consume 50 times more vitamin E daily than the current U. S. Recommended Dietary Allowance (RDA) (Qi and Sim, 1998).

Vitamin E refers to a family of lipid-soluble antioxidants consisting of four tocopherol isomers (α, β, γ, δ) and four tocotrienol isomers (α, β, γ, δ). In terms of unique characteristics of tocopherol isomers, it is α-tocopherol that has the highest antioxidative ability and stops free radical attacks in biological systems. Interestingly, the *in vitro* antioxidant ability of vitamin E isomers is the reverse of its *in vivo* action. *In*

vitro, δ-tocopherol is the most effective antioxidant among isomers. But α-tocopherol has the highest transfer efficiency from the hen's diet into eggs. Consequently, an increase in the content of vitamin E in omega-3 eggs actually reflects the dose dependent increase of α-tocopherol in eggs. Because of its antioxidant potency in biological systems, the utilization of α-tocopherol in eggs has been studied by a number of researchers in terms of preventing PUFA oxidation. In addition to enhanced health benefits of eggs with increasing content of α-tocopherol, it was shown that α-tocopherol could maintain lipid stability of omega-3 eggs (Qi and Sim, 1998).

In addition to improved health benefits of consuming chicken eggs with enriched omega-3 fatty acids and vitamin E, the safety of eggs has received increased concern in recent years due to the presence of *Salmonella*. In 1991, the Food and Drug Administration defined shell eggs as potentially hazardous foods (Tauxe, 1991). Foods containing raw or undercooked eggs such as sunny-side-up eggs, salad dressing, mayonnaise, or scrambled eggs may be possible vehicles in *Salmonella* infections. Like other bacterial gastrointestinal illnesses, human salmonellosis may be characterized by abdominal pain, diarrhea, and fever. Children, the elderly, and immuno-comprimised people are usually more susceptible to *Salmonella* infections. In fact, severe infections may cause death. In addition, there is a concern for the presence of other pathogens, such as *Listeria monocytogenes,* in shell eggs or egg products. Although no documented outbreaks of listeriosis have been related to eggs, *L. monocytogenes* was isolated from 36-72% of raw liquid whole eggs (Muriana et al., 1996). Besides *Salmonella*, other members of the *Enterobacteriaceae*, particularly *E. coli* have been isolated from 0.5-6% of eggs through fecal contamination (Barrow, 1994)

Specifically, there has been a long history of association of human salmonellosis with chicken eggs. Before the establishment of the Egg Product Sanitation Act in USA in 1970, eggs were identified as the main vehicle to cause *Salmonella* infections besides farm animals such as pigs or poultry, which are considered to be natural reservoirs of *Salmonella* (Oosterom, 1991; Jones et al., 1995). During the period from 1970 to the late 1980's, eggs were occasionally associated with salmonellosis. Interestingly, eggs were once thought to be free of bacterial contamination (Kobayashi et al., 1997). However, since the late 1980s, new concerns have arisen due to *Salmonella enteritidis* (Humphrey, 1994a; Jones et al., 1995). Shell eggs and egg-containing foods were identified as the sources in 77% of the outbreaks caused by *S. enteritidis* (Ridzon et al., 1997). In particular, it was thought that the high nutrient composition of egg yolk might provide a suitable site for the presence and growth of *S. enteritidis*. In this respect, there was a new trend to increase consumption of pasteurized liquid egg products instead of shell eggs in order to eliminate egg-associated salmonellosis. The purpose of pasteurization was to produce a *Salmonella*-free egg product. This was extremely important for people who use raw eggs to prepare homemade ice cream, salad dressing, or mayonnaise. Up to now, pasteurization of liquid egg products was the primary means to ensure the microbiological safety of these products. The numbers of liquid egg products positive for *Salmonella* before pasteurization can range from 4.2 to 56% (Michalski et al., 1999). After pasteurization, one survey reported that 2% of pasteurized egg products were positive for *Salmonella* (Michalski et al., 1999). Generally *Salmonella* are most heat resistant in egg yolk followed by whole eggs, and lastly in albumen (Michalski et al., 1999).

It was well known that many commonly used antioxidants such as BHA and BHT may act as antimicrobial agents when incorporated into food products (Raccach, 1984). Whether vitamin E in omega-3 eggs also exerts antimicrobial activity, especially on *Salmonella*, is a question that should be answered. Currently only one study has focused on comparing the growth of spoilage and pathogenic bacteria in meat as a function of vitamin E concentration (Cabedo et al. 1998). Reports concerning the microbiological status (growth or survival of pathogens) of omega-3 and vitamin E enriched chicken eggs are not available. In addition, reports detailing the functional properties of omega-3 eggs from hens fed with flaxseed based diet, in particular egg yolk which is known to be one of the best emulsifiers in food processing, have not been published.

Specifically, the objectives of this study were to:

1. Examine the growth and survival of *S. typhimurium* and *E. coli* in regular and omega-3 eggs at different storage temperatures.
2. Compare whether regular egg yolk supplemented with a range of vitamin E (α-tocopherol) concentrations could result in different growth patterns of *S. typhimurium* either under aerobic or anoxic condition at 22^0 C.
3. Determine thermal resistance of *S. typhimurium* in regular and omega-3 egg yolk at 56.5^0 C.
4. Determine thermal resistance of *S. typhimurium* in regular egg yolk supplemented with vitamin E (α-tocopherol) at 56.5^0 C.
5. Evaluate the emulsifying properties of omega-3 and regular egg yolk.
6. Assess the presence and stability of tocopherol isomers in omega-3 and regular egg yolk during storage.

2.0 LITERATURE REVIEW

2.1 Omega-3 Fatty Acids Modified Chicken Eggs

2.1.1 Omega-3 Chicken Eggs

Since consumption of chicken eggs has declined due to consumer awareness of the high cholesterol content in eggs (Jiang and Sim, 1994), food scientists have collaborated with the egg industry to focus on modifying the lipid composition of eggs.

Lipids in eggs are almost exclusively located in the egg yolk, mainly as lipoproteins. They occupy about 31.2% of the total weight of egg yolk, and 60% of the egg yolk based on dry weight. Their composition includes 65% triacylglycerols, 28.3% phospholipids, and 5.2% cholesterol, plus other minor lipids (Pike and Peng, 1985). The major fatty acids in traditional chicken eggs are oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2), and stearic acid (C18:0), which are found most frequently in triglycerides (Sugino et al., 1997). Eicosapentaenoil acid (C20:5; EPA) and docosahexaenoic acid (C22:6; DHA) are mainly present in phospholipids as minor compounds.

High lipid and cholesterol levels in chicken eggs are negative dietary factors. Attempts to reduce the cholesterol content in eggs during the last thirty years have not succeeded. Because cholesterol is an essential nutrient required for the development of the embryo (Shafey and Cham, 1994), it remains relative resistant to alteration. On the other hand, lipid modification of the egg yolk with omega-3 fatty acids creates an alternative way to enhance the health benefits of eggs.

Current available diets used to fortify omega-3 fatty acids in chicken eggs include fish oil and flaxseed. The advantage of using flaxseed over fish oil is an increase in the concentration of omega-3 fatty acids, particularly α-linolenic acid (C18:3, LNA), and avoidance of developing a fishy flavor in eggs due to possible lipid oxidation. Secondly, flaxseed is abundant in western Canada (Jiang and Sim, 1994; Flaxcouncil, 1999), which provides a good opportunity to produce omega-3 eggs by feeding hens with a flaxseed modified diet. Flaxseed is naturally high in PUFAs and low in saturated fat. It contains as much as 41% fat, of which approximately 57% is LNA (Scheideler and Froning, 1996). The omega-6:omega-3 PUFAs ratio of 0.3 gives flaxseed the potential to provide omega-3 fatty acids in eggs with little increase in omega-6 PUFAs including linolenic acid (C18:2n-6, LA).

2.1.2 Content of Omega-3 Fatty Acids in Hen Eggs Fed Flaxseed Diet

The use of incorporating ground or whole flaxseed in hens' diets has been investigated (Aymond and Van Elswyk, 1995; Van Elswyk, 1997; Botsoglou et al., 1998). Although results often have been inconclusive partially due to variations in hen types and ages, plus lipid analysis procedures, a dose-dependent increase of LNA in the egg yolk with an increasing concentration of flaxseed in the diet was observed (Aymond and Van Elswyk, 1995).

Depending upon the dietary flaxseed concentration, LNA content in omega-3 eggs was at least 7 times higher than in the traditional eggs. For instance, Aymond and Van Elswyk (1995) reported that LNA content was 8.08 or 16.08 mg/g in egg yolk when feeding 5% or 15% ground flaxseed, respectively. It was also shown that feeding whole

flaxseed with an intact seed coat at the level of 5% or 15% provided approximately 6.62 and 12.03 mg/g of LNA in egg yolk. In comparison, control eggs from hens without a flaxseed modified diet contained only 0.95 mg/g LNA in the egg yolk. In a review by Van Elswyk (1997) which summarized the influence of dietary flaxseed on the content of egg yolk PUFAs, LNA values ranged from 8.5 to 38.0 mg/g of yolk following feeding from 5% to 30% ground flaxseed. Nevertheless, feeding whole flaxseed at levels of 5%, 10%, and 15% provided about 7.0, 13.5, and 19.3 mg/g yolk LNA, respectively. Botsoglou et al. (1998) reported the amount of LNA as 4.11% of total yolk fatty acids following feeding of 10% whole flaxseed, compared to 5.19% from 10% ground flaxseed. The reduction of LNA in the egg yolk of hens fed similar levels of whole versus ground flaxseed was attributed to the negative effect of the hard seed coats on the deposition and absorption of flaxseed in hens. Thus, grinding flaxseed prior to feed formulation increased the deposition of yolk LNA. Nevertheless, the seed coat in whole flaxseed appeared to prevent lipid oxidation during feed storage (Aymond and Van Elswyk, 1995).

In addition to LNA, yolk DHA and EPA were also produced in eggs following flaxseed feeding despite an absence of these PUFAs in flaxseed. Unlike the dose-dependence of LNA in omega-3 eggs, the yolk DHA content appeared to stay within a narrow range of 5-7 mg/g of yolk, regardless of dietary flaxseed content (Aymond and Van Elswyk, 1995; Van Elswyk, 1997). On the other hand, the yolk EPA content was either at a minimal level of 0.2 to 1.5 mg/g yolk or undetectable in omega-3 eggs. Traditional eggs produced by hens with a non-flaxseed diet contained DHA at levels of 2.09 mg/g of yolk or 1.33% of total yolk fatty acids in eggs. In addition, the EPA content

was much less than in omega-3 eggs (Aymond and Van Elswyk, 1995; Botsoglou et al., 1998). Therefore, feeding hens flaxseed increased DHA and EPA in egg yolks to a specific level.

Considering that omega-3 fatty acids in flaxseed include solely LNA, the conversion of α-LNA to DHA and EPA was discussed by many studies (Aymond and Van Elswyk, 1995; Van Elswyk, 1997; Botsoglou et al., 1998). Alpha-LNA, also called the parent compound of the omega-3 fatty acids family, can serve as a precursor for the production of DHA and EPA *in vivo* by a series of desaturation and chain elongation reactions. Desaturation adds a double bond by removal of hydrogen which is catalyzed by delta-desaturase. The elongation reactions add two carbon atoms. In specific, α-LNA is desaturated to stearidonic acid (C18:4n-3) by delta-6-desaturase. This is followed by elongation to C20:3n-6 by an elongase. Then EPA (C20:5n-3) is created by desaturation of C20:3n-6. Finally, EPA is elongated and desaturated to DHA (C22:6n-3) by delta-4-desaturase. A similar desaturation-elongation cycle occurs in the conversion of linoleic acid (C18:2n-6) to arachidonic acid (C20:4n-6) (Cherian and Sim, 1994; Aymond and Van Elswyk, 1995).

The conversion of α-LNA (C18:3n-3) and linoleic acid (C18:2n-6) to their respective metabolites requires the same desaturation enzyme, which leads to competition between these two essential fatty acids. In particular, excessive linoleic acid could interfere with the conversion of α-LNA, thus reducing the biosynthesis of EPA and DHA (Aymond and Van Elswyk, 1995). Fortunately, an omega-6:omega-3 PUFAs ratio of 0.3 in flaxseed ensures a lowering of the ratio from 7.53 in regular egg yolk to approximately 2-3 in omega-3 egg yolk for various flaxseed diets (Botsoglou et al., 1998). Therefore,

flaxseed feeding increases the conversion of α-LNA to DHA and EPA for yolk deposition. However, the conversion is somewhat slow and inefficient in the laying hen as well as in the egg yolk mainly due to the predominance of omega-6 fatty acids both in the diet and the eggs.

The minimal deposition of EPA in omega-3 eggs from either a flaxseed or a marine source diet still needs further investigation. EPA and DHA are mainly found from marine sources, with EPA being predominant in marine oil relative to DHA. Interestingly, the DHA content in eggs from hens with a marine oil diet is much higher than EPA. According to Aymond and Van Elswyk (1995), eggs from a 1.5% menhaden oil diet contain 6.15 mg DHA/g of yolk compared to EPA at a level of 1.12 mg/g yolk. They also indicated that a 15% dietary ground flaxseed could produce a similar amount of DHA as with 1.5% menhaden oil and a higher LNA content. However, 15% ground flaxseed in the diet resulted in noticeably lower egg production (Aymond and Van Elswyk, 1995). Therefore, it is important that the level of flaxseed in laying hen diets be limited to minimize adverse effects in the eggs including economic viability and organoleptic properties. Also, dietary flaxseed content needs to remain stable during the laying period in order to maintain the presence of yolk omega-3 fatty acids in the eggs.

Omega-3 enriched eggs in retail outlets in Canada are produced from layers with 10-20% dietary flaxseed (Manitoba Egg Producers, 2000). The distribution of PUFAs in omega-3 egg yolks from 10-20% flaxseed versus control eggs is given in Table 1 (Ferrier et al., 1995). As shown in Table 1, the total levels of omega-3 fatty acids in omega-3 eggs produced from a 20% flaxseed diet were about 6 times higher than those in control eggs. LNA increased to 10.7% from 0.5% in control eggs. In addition, DHA increased

Table 1. Composition of PUFAs in egg yolk for control and omega-3 enriched eggs.

	% (w/w) of total yolk lipid		
	Omega-3 egg		Control egg
Fatty acid	10% flaxseed	20% flaxseed	
Linoleic acid (18:2n-6)	15.2	16.3	16.8
Linolenic acid (18:3n-3, LNA)	5.5	10.7	0.5
Arachidonic acid (20:4n-6)	1.1	0.9	2.2
Eicosapentaenoic acid (20:5n-3, EPA)	0.2	0.2	0.1
Docosatetraenoic acid (22:4n-6)	0.3	0.2	0.4
Docosapentaenoic acid (22:5n-3, DPA)	0.3	0.4	0.2
Docosahexaenoic acid (22:6n-3, DHA)	1.7	1.8	1.0
Total omega-3 PUFAs	7.7	13.1	1.8
Total omega-6 PUFAs	16.6	17.4	19.4
Ratio of omega-6:omega-3	2.2	1.3	10.8

Source: Ferrier et al. (1995)

approximately 2 fold, whereas EPA increased minimally compared to control eggs. The ratio of omega-6:omega-3 PUFAs dropped dramatically from 10.8 in control eggs to 1.3 in omega-3 enriched eggs for 20% flaxseed diet.

2.1.3 Health Benefits of Omega-3 Eggs

Omega-3 enriched chicken eggs provide a practical alternative to fish as they increase the intake of omega-3 fatty acids in the human diet. Insufficient levels of omega-3 fatty acids in the human diet can result in an increased risk of CHD. For this reason, an intake level of 200 mg/day total omega-3 fatty acids including LNA, EPA and DHA is recommended (Leskanich and Noble, 1997). As indicated previously, one omega-3 enriched egg could provide total omega-3 fatty acids equal to or higher than the recommended intake level (Leskanich and Noble, 1997).

The importance of omega-3 fatty acids to human health was realized during the past decade (Leskanich and Noble, 1997). Nevertheless, most of the clinical and epidemiologic studies were conducted with marine foods. It was demonstrated that omega-3 fatty acids, especially EPA and DHA, not only have beneficial effects on the vascular system, but also reduce blood triglycerides and blood pressure, all of which help to decrease the risk of CHD (Van Elswyk, 1997). In addition to their effects on CHD, EPA and DHA have been shown to suppress inflammatory reactions. Moreover, DHA is essential for the growth and function of the human brain and retina. Deficiency of DHA leads to loss of visual acuity in infants.

Since eggs and egg products are widely consumed, it is important to demonstrate the health benefits of omega-3 enriched eggs along with their introduction to the market.

In fact, consumption of omega-3 enriched eggs has been reported to lower blood pressure and plasma triglycerides in human, which have been associated with reduced risk of CHD (Oh et al., 1991; Ferrier et al., 1992; Ferrier et al., 1995). Oh et al. (1991) found that blood pressure was significantly reduced in human subjects following the consumption of four omega-3 enriched eggs (produced using 10% fish oil) per day for four weeks and significantly increased with four control eggs per day. In this study, plasma triglyceride levels were also reduced but the decrease was not always significant (Oh et al. 1991). This could be due to an insufficient amount of omega-3 fatty acids obtained from consuming four omega-3 eggs a day for a short term to cause consistent reduction of triglycerides, compared to direct consumption of fish oil in other published studies (Oh et al., 1991).

Another study involving omega-3 eggs from hens fed with 10% or 20% ground flaxseed was conducted (Ferrier et al., 1995). Although the levels of plasma triglyceride were inconsistent following consumption of omega-3 eggs in comparison with their preliminary study (Ferrier et al., 1992), there was a significant rise of DHA in platelet phospholipids of human subjects consuming omega-3 enriched eggs in both studies. Accumulation of DHA as well as LNA, which could be further converted to EPA and DHA, appeared to reduce platelet aggregation, thus reducing the risk of CHD.

In addition, Cherian and Sim (1994) reported that the incorporation of extractable omega-3 egg yolk lipids could serve as a source of omega-3 fatty acids, particularly DHA, in infant formula preparation. It is well established that DHA is essential for optimal development of the nervous system and retina of infants, and is rapidly accumulated in brain lipid and retina during the last trimester of pregnancy and the first

year of life (Cherian and Sim, 1994; Flaxcouncil, 1999). Therefore, it is possible that insufficient DHA in premature infants undergoing rapid brain development could lead to impaired visual function and learning difficulties (Cherian and Sim, 1994; Flaxcouncil, 1999). Sources of omega-3 fatty acids (DHA) for infants include breast milk and formula. Breast milk usually provides an optimum source of nutrients for infants. However, breast milk is totally dependent on the mother's diet which is often low in omega-3 fatty acids. On the other hand, currently available infant formulas contain no DHA, since the fats in the formulas are most likely from vegetable oils that do not contain long chain PUFAs (Cherian and Sim, 1994).

Cherian and Sim (1994) investigated the effect of omega-3 enriched eggs on the content of omega-3 fatty acids in breast milk in nursing women. The researchers reported that DHA significantly increased along with an increase of LNA when nursing women consumed two omega-3 eggs in addition to their usual diets that were seafood free.

2.1.4 Sensory Quality and Functional Properties of Omega-3 Eggs

Health benefits of omega-3 eggs may be significant to the egg industry as well as the health conscious public. However, consumer acceptance of a new food item also depends upon the overall quality of the item. In the case of omega-3 eggs, incorporation of omega-3 fatty acids in the lipid component of eggs could alter their sensory quality resulting in lower consumer acceptability. In particular, increasing the degree of polyunsaturation would accelerate oxidative deterioration in the egg yolk, leading to off-flavors in eggs (Jiang et al., 1992). Oxidized fatty acids and their degeneration products

are not only undesirable but are also known to cause adverse biological effects in human (Qi and Sim, 1998).

Chemical measurement of lipid oxidation in eggs could be based on the thiobarbituric acid reactive substances (TBARS) test, which involves monitoring the reaction of malondialdehyde (MA), a secondary degeneration product of lipid oxidation, and thiobarbituric acid (TBA). Yolk TBARS of fresh eggs produced by hens fed 1.5% menhaden oil was significantly higher in comparison with that of the control diet (Van Elswyk, 1997). Theoretically, feeding of layers with flaxseed instead of marine oil would decrease off-flavor resulting from lipid oxidation. However, Aymond and Van Elswyk (1995) reported that yolk TBARS of fresh eggs were not different, regardless of dietary omega-3 fatty acids source. Fresh eggs from layers fed whole or ground flaxseed at the same level exhibited almost similar TBARS values. Studies have suggested that the deposition of TBARS in fresh egg yolk was either from oxidized lipid in the diet or the gastrointestinal tract (Van Elswyk, 1997; Qi and Sim, 1998).

Off-flavors in omega-3 eggs have been reported in several studies (Jiang et al., 1992; Caston et al., 1994). In one study, Jiang et al. (1992) reported a fishy-flavor in eggs from hens fed a 15% flaxseed diet. In addition, Caston et al. (1994) reported that taste panelists preferred the flavor of control eggs to that of omega-3 eggs. However, the panelists were not always consistent in their perception of the fishy-flavor. This could have been due to the method of preparation and source of sample eggs plus training of panelists (Caston et al., 1994).

Further studies are required to determine the actual cause of off-flavor in omega-3 eggs from layers fed flaxseed. Although borderline level of TBARS for detection of off-

flavors in eggs have not been established, the deposition of TBARS in the egg yolk nevertheless results in off-flavors, and trace amounts of oxidized lipid could produce fishy-flavors in eggs (Jiang and Sim, 1994). Fortunately, incorporation of a natural antioxidant such as vitamin E in the laying hen diet could eliminate dietary and egg yolk lipid oxidation, which would maintain and enhance health benefits of omega-3 eggs. For instance, TBARS values of egg yolk from hens fed diets supplemented with tocopherol at 0, 200, 400, 800 mg per kg diet significantly decreased from 41.32 to 18.57 nmol MA/g yolk (Qi and Sim, 1998). Moreover, the role of vitamin E against cancer, CHD, and suppression of inflammation enhanced the health benefits of omega-3 eggs by deposition of vitamin E in the egg yolk (Ahmad, 1996).

In addition to nutritional value and sensory quality, another feature involving the evaluation of the overall quality of omega-3 chicken eggs is the functional properties of the eggs. Egg yolk is recognized as one of the best emulsifying agents and it is used extensively in food preparations such as mayonnaise, salad dressing, and bakery products. The emulsifying properties of egg yolks have been attributed to the presence of lipoprotein in the egg yolk, especially low density lipoprotein (LDL). LDL contains 12.5% protein and 86-89% lipids, which, in turn, include 27% phospholipids, 69% triacylglycerol, and 4% total cholesterol (Mine, 1998a). Among the constituents, proteins are more important contributors to emulsion properties than phospholipids (Mine, 1998b). Nevertheless, the excellent emulsifying properties of LDL are also due to the amount of lipids bound to LDL (Hatta et al., 1997; Juneja and Kim, 1997). Mine (1998a) suggested that lipids including cholesterol and phospholipids could interact with protein

components at oil-in-water interfaces in order for them to play an important role in stable emulsion formation.

Up to now, the effect of various external conditions such as pH, oil volume and salts on the emulsifying properties of egg yolk has been well established (Mine, 1998b). However, a few studies reported the relationship between emulsion properties of egg yolk and the interaction of individual egg yolk components. In particular, small changes in egg yolk lipid composition resulting from diets supplemented with different types of fat could affect functional properties. For example, Jordan et al. (1962) indicated that eggs from hens fed a 10% corn oil diet could produce sponge cake with greater volume than that of the control diet. However, there was no significant difference in terms of the emulsion capacity of egg yolks when comparing a 10% corn oil or beef fallow diet. In a study by Pankey and Stadelman (1969), supplementation of 10% corn oil, soybean oil, and olive oil in the hens' diet resulted in a significant increase of linoleic acid in the triglyceride fraction; however, this did not result in significant differences in emulsification capacity or sponge cake volume.

In the case of omega-3 eggs, the deposition of LNA occurred in the triacyglycerol fraction and longer PUFAs such as EPA, DPA, and DHA were exclusively incorporated in the phospholipid fraction (Leskanick and Noble, 1997). Van Elswyk et al. (1992) also reported no significant differences in emulsion capacity and sponge cake volume between omega-3 eggs from hens fed 3% menhaden oil and control eggs, even though the DHA content in omega-3 eggs was much higher than that of control eggs.

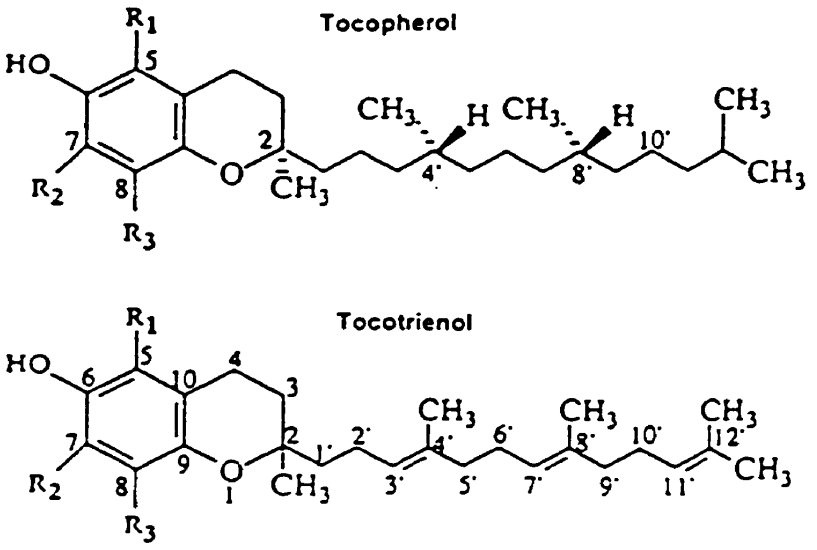
2.2 Vitamin E and Its Application

2.2.1 Vitamin E Family and Their Structures

Vitamin E, an essential nutrient that functions as an antioxidant (Ahmad, 1996; Kelly, 1997), is actually a generic term for fat-soluble 6-hydroxychroman compounds that exhibit the biological activity of α-tocopherol. Vitamin E occurs in nature as 8 different forms including α-, β-, γ- and δ- tocopherol and their corresponding unsaturated tocotrienols, which have 3 double bonds in the tail at 3', 7' and 11' positions (Fig. 1) (Eitenmiller, 1997). The tocopherols basically consist of a "ring" portion called a chromanol "head" and a "tail" portion called a "phytyl" group which contains a saturated 16-carbon isoprenoid (Burton, 1990). Differences between the various vitamin E isomers include the number and position of methyl groups on the chroman ring. Alpha-tocopherol has 3 methyl groups, one each on carbon numbered 5, 7 and 8 (Fig. 1). Beta- and γ-tocopherol have two methyl groups in position 5 and 8, plus 7 and 8, respectively. Delta -tocopherol has only one methyl group at carbon numberred 8 (Fig. 1). Among these, d-α-tocopherol (RRR-α-tocopherol) has the highest biological activity (Eitenmiller, 1997).

Chemically synthesized vitamin E introduced in the 1940's for supplementing diet and feeds (Piironen et al., 1991), is also called all-rac- α-tocopherol or dl-α-tocopherol. It is a mixture of eight stereoisomers including the natural d-α-tocopherol and seven other α-tocopherol isomers in equal amounts (Piironen et al., 1991). However the free active form of vitamin E is easily destroyed by oxygen; destruction is also accelerated by

Figure 1. Structure of Vitamin E



Position of methyl groups	Tocopherols	Tocotrienols
5, 7, 8 – Trimethyl	α	α
5, 8 - Dimethyl	β	β
7, 8 - Dimethyl	γ	γ
8 - Monomethyl	δ	δ

Source: Eitenmille (1997)

light, heat, etc. (Bauernfeind, 1977; Eitenmiller, 1997). Therefore, esterification of tocopherols with organic acids such as acetic acid has been used to provide a more stable form of vitamin E. In fact, synthetic tocopheryl acetate is the most common form of vitamin E used to supplement feeds in poultry and cattle (Piironen et al., 1991; Jensen et al., 1998). Nevertheless, the ester form of vitamin E does not serve as an antioxidant until it is converted back to the free active form in the digestive tract (Jensen et al., 1998).

The unesterified forms of tocopherols are prevalent in a variety of plants such as nuts, seeds, oil, fruits, vegetables, and grasses (Bauernfeind, 1977). Among them, vegetable oils are the richest source of vitamin E in the human diet. For instance, fats and oils such as soybean oil or corn oil supply 20% of the vitamin E in the USA diet (Eitenmiller, 1997). Nevertheless, corn oil or soybean oil contain 6 to 10 times more γ-tocopherol than α-tocopherol (Eitenmiller, 1997). However, regardless of the types of vitamin E isomers in human and animals diets, it is α-tocopherol that is predominant in the human body and in most animal products including meat, eggs and dairy products (Bauernfeind, 1977; Chen et al., 1998). Unlike other tocopherols or tocotrienols, α-tocopherol has the lowest antioxidative activity *in vitro* whereas the highest biological activity *in vivo* due to the presence of a specific α-tocopherol binding protein (Cherian et al., 1996b; Qi and Sim, 1998).

2.2.2 Lipid Peroxidation and Antioxidant Activity of Vitamin E

The main role of vitamin E appears to be as an antioxidant by protecting PUFAs and other lipids from peroxidation in cellular membranes and food systems (McCay, 1985; Burton and Traber, 1990; Mukai et al., 1993a, 1993b; Kitts, 1997). In food

systems, specifically meat and meat products, lipid peroxidation is one of the primary processes of quality deterioration, leading to adverse changes in flavor, color, texture, and nutritive value plus the possible production of toxic lipid oxidation compounds (Kitts, 1997).

Lipid peroxidation (or autoxidation) is significant in living systems and food products and involves a chain reaction consisting of three stages: initiation, propagation and termination (McCay and King, 1980; Fung et al., 1985; Burton and Traber, 1990; Ahmad, 1996). Most importantly, long term damage of the body is associated with free radicals (Ahmad, 1996). Free radicals are formed naturally in the body as byproducts of metabolic processes (Ahmad, 1996; Kitts, 1997) or outside the body by environmental pollutants including nitrogen dioxide and ozone of polluted air, cigarette smoke and ultra violet radiation (Ahmad, 1996). Unchecked by an antioxidant, the highly unstable free radicals attack cell constituents such as DNA, particularly those containing PUFAs. As a result, a chain reaction occurs that generates free radicals in profusion, leading up to degenerative diseases (Ahmad, 1996). For instance, free radicals play a role in the onset of cancer, coronary heart disease, plus arthritis and perhaps even more diseases (Fiala et al., 1985; Ahmad, 1996; Kitts, 1997).

Therefore, primary antioxidants are required to prevent the chain reaction of lipid peroxidation by reacting with initial free radicals to form stable or nonradical products (Burton and Traber, 1990; Ahmad, 1996). Members of this type are called inhibitor or radical scavengers, including mainly phenols such as natural or synthetic tocopherols, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) (Burton and Traber, 1990; Ahmad, 1996).

It was demonstrated that α-tocopherol is the most effective antioxidant available *in vivo* (McCay, 1985; Burton and Traber, 1990). Moreover, the ideal antioxidant should be at least fat-soluble, so that it can prevent lipid peroxidation in cell membranes. In comparison to BHA and BHT that are partially soluble in fats and oils (Raccach, 1984), vitamin E is the only major lipid-soluble, chain terminating antioxidant that has been identified in biological systems (Burton and Traber, 1990). Although the antioxidative ability of phenols are entirely related to the hydroxyl groups on the phenols (or chromanol) ring, the phytyl tail of vitamin E is responsible for the hydrophobic activity of vitamin E so that it can be transported within membranes (McCay, 1985; Burton and Traber, 1990).

The advantages of vitamin E as an antioxidant also include its nontoxic effect. By comparison, BHA and BHT, two commonly synthetic antioxidants used in food systems, appear to promote tumor growth in long-term animal and human studies when used in excess of their required level (Qi and Sim, 1998). In addition, a number of recent studies emphasized the nutritional significance of vitamin E in health and disease (McCay, 1985; Ahmad, 1996; Kelly, 1997; Kitts, 1997). In this respect, it is generally recommended that a daily intake of vitamin E (20-50 mg) be included in the diet, because it can not be synthesized by our body (Bauernfeind, 1977; Kelly, 1997; Smith, 2000).

In recent years, there has been a trend to incorporate vitamin E in cattle and chicken diets, which results in the accumulation of α-tocopherol in meat and egg products. Thus, α-tocopherol not only plays a role as an antioxidant in the prevention of lipid peroxidation in foods but also provides a dietary supplementation for humans.

2.2.3 Incorporation of α-Tocopherol into Omega-3 Eggs

Sensory evaluation and chemical measurement of byproducts of peroxides derived from PUFAs result in the development of off-flavors and lower consumer acceptability. For this reason, vitamin E has been incorporated into layer diets for the production of omega-3 eggs.

The tocopherol content in egg yolk relies mainly on its source and the dietary level of vitamin E. In a study by Qi and Sim (1998), a natural tocopherol mixture was used in the layer diet, in which α-, γ-, and δ-tocopherols accounted for about 10, 60, and 30% of total tocopherol mixture, respectively. When total tocopherol mixture increased from 0 to 800 mg/kg in the diet, α-tocopherol still accounted for 90.96% to 57.60% of total tocopherol in eggs. In addition, there were only small amounts of γ- and δ-tocopherols transferred into egg yolks. This reflected the discrimination of transfer in favor of α-tocopherol into eggs in comparison to γ- and δ-tocopherols that exist in greater quantities than α-tocopherol in the diet. A similar pattern was also observed by Cherian et al. (1996a, 1996b). Moreover, the incorporation of α-tocopherol in eggs triggered a significant difference in TBARS values of egg yolks (Qi and Sim, 1998). For instance, TBARS dropped to 18.57 nmol MA/g of egg yolk as a result of a tocopherol mixture (800 mg/kg diet) in contrast to 41.32 of TBARS value within the control diet without tocopherol supplementation (Qi and Sim, 1998). Despite this, sensory analysis did not show significant differences among treatments, although the flavor and taste values were most favorable with the highest level of tocopherol mixture in the diet (800mg/kg).

However, incorporation of a natural tocopherol mixture is not ideal in commercial egg production, since it can easily react with free radicals prior to feeding. Instead, the more air-stable all-rac-α-tocopheryl acetate, is commonly used to supplement feeds (Piironen et al., 1991). The addition of 16.0 mg/kg of dietary total α-tocopherol resulted in a transfer of about 72.2 mg/kg total α-tocopherol into the egg yolk (Piionen et al., 1991). The amount of egg yolk α-tocopherol increased linearly as the α-tocopheryl acetate content increased in the diet. For instance, egg yolk α-tocopherol content increased to 477 ppm when 400 mg dl-α-tocopheryl acetate per kg diet was applied compared to 144 ppm in the control without vitamin E supplementation (Jiang et al., 1994).

The concentration of α-tocopherol in eggs must be controlled because α-tocopherol acts as an antioxidant at low concentrations but as a proxidant at high concentrations (Mukai et al., 1993a, 1993b; Huang et al., 1995; Kitts, 1997; Chen et al., 1998). While most of the proxidant activity of α-tocopherol was conducted in liquid model systems such as purified soybean oil, Chen et al. (1998) reported that the concentration of dietary α-tocopherol resulting in the egg yolk proxidant activity was 75 ppm of yolk or above. Leeson et al. (1998) suggested that the lower acceptability of eggs from layers fed 100 mg vitamin E/kg diet was possibly due to proxidation.

2.2.4 Antimicrobial Effects of Phenolic Antioxidants

The antimicrobial activity of phenols such as BHA, BHT and TBHQ either in media or in model food systems has been well established for many years (Raccach,

1984; Fung et al., 1985). The dual functions of synthetic antioxidants allow them to be applied to meat, poultry, seafood and other food products to inhibit the growth of bacteria, mold and yeast as well as to prevent lipid oxidation. In general, phenols are allowed in food products up to 200 ppm (or 0.02%) based on the fat or oil content (Raccach, 1984; Fung et al., 1985). For instance, BHA (100 ppm) in combination with potassium sorbate inhibited the growth of *S. typhimurium* in cooked turkey meat during 8 days of refrigerated storage (Fung et al., 1985). The combination of BHA and TBHQ at a total concentration of 200 ppm resulted in from 56 to 71% aflatoxin inhibition by *Aspergillus parasiticus* (Fung et al., 1985).

The antimicrobial activity of phenols is affected by a number of factors including microbial species and strains, type and concentration of phenols, and food components (Raccach, 1984). Raccach (1984) reported that phenols are less effective against gram negative than gram positive bacteria. Food components such as protein and fats were also reported to depress the antimicrobial effect of phenolic antioxidants (Raccach, 1984; Fung et al., 1985). In some cases, the antimicrobial activity was totally diminished due to the presence of lipid in foods. The lipophilic feature of phenols allows them to be located in the lipid portion of food thus making them unavailable as antimicorbials (Davidson and Branden, 1981; Raccach 1984; Fung et al., 1985). Because the antimicrobial activity of phenolic antioxidants depends on the presence of a free hydroxyl group on the molecule, it is possible to lose the antimicrobial activity by hydrogen donation from the hydroxyl groups of phenols during free radical termination (Raccach, 1984). Therefore, higher amount of phenols in lipid-based foods are required.

The incorporation of vitamin E into food products provides an excellent alternative to retard lipid oxidation (Qi and Sim, 1998). However, little work has been carried out to verify the effect of vitamin E on bacterial growth. One study did determine that when dietary α-tocopheryl acetate was fed to animals at a level of 1000 or 2000 IU, it resulted in α-tocopherol levels of 6.12 and 7.77 μg/g meat, respectively. In comparison, the control group contained 2.50 μg α-tocopherol /g meat (Cabedo et al., 1998). Regardless of the α-tocopherol content, similar numbers of aerobic bacteria, coliforms, or *Listeria monocytogenes* were present in ground beef patties displayed at 4^0 C or 12^0 C.

2.3 *Salmonella* in Chicken Eggs and Egg Products

2.3.1 Prevalence of *Salmonella* in Shell Eggs

Egg safety has received increased concern in recent years due to the presence of *Salmonella sps.* in eggs and egg-containing foods. Since the late 1980's, there has been an increase in egg-associated *Salmonella enteritidis* infections (Humphrey, 1994a; Jones et al., 1995). Specifically, shell eggs and egg-containing foods were identified in 77% of the outbreaks caused by *S. enteritidis* (Ridzon et al., 1997). In addition, a survey indicted that antibiotic resistant *S. typhimurium* were involved in 40% of *Salmonella* outbreaks (Tauxe, 1991).

Although *Salmonella* has been isolated from intact grade A eggs (Humphrey, 1994a, 1994b; Schoeni et al., 1995), its prevalence is quite low. Commonly isolated *Salmonella* species include *S. enteritidis*, *S. typhimirium* and *S. heidelberg* (Schoeni et al., 1995). In terms of recent problems involving *S. enteritidis* and shell eggs, the incidence

of *S. enteritidis* positive eggs is one or two per 10,000 (Anderson, 1997) with one to ten *S. enteritidis* cells per egg (Hammack et al., 1993). However, salmonellae in shell eggs can increase during prolonged storage at room temperature (Humphrey, 1994a).

Eggshells can become contaminated with salmonellae either as a result of infection in the oviduct after formation of the shell or fecal carriage (Humphrey, 1994a). In Spain 4 of 372 eggs (1.1%) from *Salmonella*-positive flocks were shell-positive for *S. enteritidis* (Humphrey, 1994a). However, the rate of shell contamination decreased to 0.5% with eggs from flocks which were not associated with salmonellosis (Humphrey, 1994a). Moreover, the prevalence of eggshell contamination with *Salmonella* was intermittent. In two independent studies from the UK (Mawer et al., 1989; Humphrey et al., 1989) with the same flocks, none of the 360 eggs from *Salmonella*-positive flocks were found to be shell contaminated with *S. enteritidis*. Nevertheless, *S. enteritidis* were isolated from egg contents (Mawer et al., 1989). In contrast, Humphrey et al. (1989) reported that *S. enteritidis* phage type 4 (PT4) was isolated from eggshells at the rate of 7.4% with the same flocks.

The prevalence of *S. enteritidis* in egg contents from naturally infected flocks was 1.0% or lower (Humphrey, 1994b). Overall, the prevalence of *S. enteritidis* in egg contents varies due to a number of factors including sampling and test procedures, plus the sites within the egg that were tested. The most isolated site of *S. enteritidis* in contaminated egg contents was the albumen or the outside of the vitelline membrane (Humphrey, 1994b). The number of salmonellae isolated from fresh egg contents was low. On the other hand, egg yolks are quite high in nutrients, making them suitable for microbial growth. Hammack et al. (1993) reported that salmonellae grew rapidly in

artificially contaminated egg yolks, especially at room temperature. Therefore, it was suggested by Humphrey (1994b) that the egg yolk was not a common site of *Salmonella* contamination, even though migration and growth of the microorganism in egg yolk was demonstrated (Humphrey, 1994a).

In the event of contamination of the albumen or associated membrane, salmonellae compete with the antimicrobial factors and may survive the initial 3-4 weeks. At some point, in conjunction with the degradation of the vitelline membrane, salmonellae can migrate into the yolk, growing rapidly especially at room temperature.

2.3.2 Transmission Routes of *Salmonella* in Shell Eggs

There are two possible routes by which shell eggs become contaminated with *Salmonella*; these include transovarian and trans-shell routes.

Transovarian contamination of egg contents occurs at oviposition before the shell is formed; this is also known as vertical transmission. *Salmonella spp.* can be isolated from many sites of the hen, such as the peritoneum, ovules, oviduct, and ovary (Chen et al., 1996). Being thought to be the natural reservoir of *Salmonella*, most hens show no signs of illness while they are infected with *Salmonella*. During egg formation, *Salmonella* can be transferred directly from the oviduct into the egg contents. For example, *S. enteritidis* has been isolated from both the ovary and the oviduct (Humphrey, 1994a; Humphrey, 1994b; Chen et al., 1996); therefore, it is able to contaminate egg contents during egg formation.

Shell eggs can also become infected with *Salmonella* by cross-contamination or horizontal transmission. In this case, *Salmonella* can originate from fecal carriage or the

environment. For example, Jones et al. (1995) reported that *Salmonella* were isolated from 72% of samples collected from a laying house environment which included egg belts, egg collectors, ventilation fans, and flush water. Oosterom (1991) also reported that the incidence of *Salmonella* in the Canadian egg operations was about 53%. Since *Salmonella* are widely spread in the environment, it is quite difficult to eliminate contamination of eggshells with *Salmonella* after the eggs are laid. *Salmonella* on eggshells can die rapidly (Humphrey, 1994b). However, their survival is affected by the storage temperature and the ability to penetrate the shell.

Once *Salmonella* penetrate the shell, they initially appear to be located in the albumen where they compete with the natural antimicrobial factors present, mainly lysozyme, which hydrolyzes gram-positive bacteria, and ovotransferrin, which chelates iron (Board et al., 1994). Schoeni et al. (1995) suggested that these antimicrobials might be limited to prevent *Salmonella* growth. For instance, lysozyme does not inhibit the growth of *Salmonella* and other gram-negative organisms (Schoeni et al., 1995). Three dominant species including *S. enteritidis*, *S. typhimurium*, and *S. heidelberg* were used in the study. It was found that the number of salmonellae in the albumen remained stable or slightly increased even at a temperature as low as 4^0 C. Weakening of the vitelline membrane during storage mitigates *Salmonella* penetration into the egg yolk.

In this respect, Liong et al. (1997) conducted a study of *S. enteritidis* penetration of the shell membranes using confocal scanning laser microscopy (CSLM). This study not only agreed with a study by Nascimento et al. (1992) that the interfiber distances between the paired membranes allow for penetration of *S. enteritidis*, but also demonstrated microbial penetration through the membranes. In another study, Chen et al.

(1996) used a luminescent strain of *S. enteritidis*, containing bacterial luciferase genes, to successfully monitor penetration in eggs.

2.3.3 Thermal Resistance of *Salmonella* in Liquid Egg Product

In recent years, there has been increased interest in the sale of a variety of processed egg products such as pasteurized liquid egg and frozen dried egg products due to the demand of safer egg products by consumers and food processing plants. It was estimated that the use of shell eggs for egg products increased from 13.8% (8.7 billion eggs) in 1980 to 24.6% (15 billion) in 1992 (Schuman and Sheldon, 1997; Michalski et al., 1999). The advantages of using refrigerated liquid egg products as well as frozen dried egg products include convenience, storage-space savings as well as microbiological safety (Schuman and Sheldon, 1997).

Liquid egg products which include liquid whole egg, egg yolk (fortified with sugar, salt, or albumen) and albumen are more susceptible to microbial contamination, since all physical barriers have been removed. In fact, these products serve as media for microbial growth. Moreover, because the source of raw liquid eggs might include cracked shell eggs, that could be easily contaminated by *Salmonella* or eggs involved in egg-related salmonellosis (Muriana et al., 1996), there is a greater chance of *Salmonella* in raw liquid eggs. For example, it was shown that 451 of 937 samples of unpasteurized liquid eggs in USDA Animal and Plant Health Inspection Service (APHIS) regions in 1995 were *Salmonella*-positive, among which 179 (19%) were *S. enteritidis* positive (Hogue et al., 1997). Another study indicated that 15% of raw liquid whole eggs were *S. enteritidis* positive (Michalski et al., 1999). Therefore, the potential for *Salmonella*

contamination in raw liquid eggs requires it to be pasteurized in order to ensure microbiological safety.

The time and temperature combination of pasteurization requirements vary among countries, and ranges from 61^0 C for 1 min to 65^0 C for 5 min depending upon the type of egg products involved (Stadelman, 1994). For instance, pasteurization of albumen requires a lower temperature of about 57^0 C in comparison to whole egg and egg yolk due to the possibility of protein denaturation at higher temperatures (Stadelman, 1994; Michalski et al., 1999). Because *Salmonella* is more heat resistant in egg yolk than in whole egg, the yolk must be pasteurized at a higher temperature or for a longer time at the same temperature. In particular, following the recommendation by USDA to provide a wide safety margin, all egg products are pasteurized (plate heat exchanger) for 3.5min at 60^0 C (whole egg), 56.7^0 C (albumen) and 61.1^0 C (egg yolk) (Stadelman, 1994; Michalski et al., 1999). Canadian Processed Egg Regulations (1991) requires the pasteurization of albumen without chemical additives for 3.5 min at 54^0 C, whole egg and egg yolk for 3.5 min at 61-63^0 C, depending on the added agents.

Even though raw liquid eggs contain low numbers of *Salmonella*, they can increase dramatically through temperature abuse during storage, transportation and breaking (Shah et al., 1991). Therefore, the number of salmonellae present in the egg could influence the margin of safety achieved by pasteurization.

The adequacy of pasteurization has been addressed in various studies involving different heat sources (e.g., water bath, flow heat exchanger) and vessels (test tubes versus capillary tubes), making comparisons of data difficult (Schuman and Sheldon, 1997). For example, both Schuman and Sheldon (1997) and Michalski et al. (1999)

tested thermal inactivation of *S. enteritidis* in liquid egg products using capillary tubes instead of capped test tubes. They found that the decimal reduction (D) value (the time taken to kill 90% of the population) was lower in capillary tubes than in test tubes. This may be attributed to increased heat transfer in capillary tubes. In addition, thermal inactivation of *Salmonella* in egg yolk using capped test tubes often resulted in tailing of survivor curves that affected calculation of D values (Schuman and Sheldon, 1997; Chantarapanont et al., 2000). Regarding the evaluation of USDA egg product pasteurization protocols, Michalski et al (1999) showed that a greater than 7- to 8- $\log_{10}$ reduction of *S. enteritidis* inoculated in liquid egg products could be accomplished by pasteurization using a pilot-scale continuous flow heat exchanger. In contrast, pasteurization of liquid egg yolk supplemented with 5-10% salt or sucrose, using USDA minimum protocols only resulted in a 4.2-$\log_{10}$ reduction using an inoculum of 10^7 to 10^8 *S. enteritidis* (Michalski et al., 1999).

Based on various studies, it is concluded that the thermal resistance of *Salmonella* is affected by the nature of the products pH and a_w, as well as the nature (time, temperature) of the thermal treatment.

3.0 MATERIALS AND METHODS

3.1 Cultures and Maintenance

Salmonella typhimurium (S266) was obtained from Alexander Gill in the Department of Food Science (University of Manitoba, Winnipeg, MB), and generic *Escherichia coli* (Bio Type I) was provided by Garry Palmateer in the Ministry of the Environment (London, ON). Both *S. typhimurium* and *E. coli* exhibited nalidixic acid resistance (50 μg/ml). Each organism was grown in 50 ml of trypticase soy broth (TSB; BBL, Becton Dickinson and Company, Cockeysville, MD) for 18-24 h at room temperature (20-22^0 C). Resulting populations were verified by serial dilution and enumeration using tryptic soy agar (TSA; BBL). An inocolum level of approximately 10^2-10^3 colony forming units (CFU/g) egg contents was used throughout the study. The organisms were maintained on TSA (BBL) slants at 4^0 C following growth at 35^0 C for 24 h. On a monthly basis, the organisms were transferred to freshly prepared slants.

3.2 Eggs

Regular and omega-3 shell eggs were obtained from a local retail outlet. Any cracked eggs and eggs exhibiting fecal contamination were discarded. Shell eggs were surface sanitized (200 ppm chlorine) and aseptically broken out into sterile stomacher bags (17.8 x 30.5 cm, Fisher Scientific Ltd., Nepean, ON). Egg yolk and albumen were obtained by aseptically separating whole eggs using a sanitized (200 ppm chlorine) plastic egg-yolk separator. Contents were collected in sterile stomacher bags. Egg contents including whole egg, egg yolk or albumen were subsequently mixed for

approximately 60 s using a stomacher (model 6021; Seward Laboratory, London, England). Samples (10±0.01 g) were transferred to a series of sterile, plastic vials (50 ml capacity; Fisher Scientific Ltd., Nepean, ON). Prior to experimentation, egg samples were assessed for the presence of nalidixic acid resistant organisms by spread plating 0.1g on TSA (BBL) containing 50 ppm nalidixic acid. A nalidixic acid solution was prepared by mixing free nalidixic acid (Sigma, St. Louis, MO) with 0.05 N NaOH and sterilized using filters (0.2μm SFCA filter; Fisher Scientific Ltd., Nepean, ON).

3.3 Aerobic Plate Count: Regular and Omega-3 Whole Eggs, Egg Yolk and Albumen

A series of sterile, plastic vials (Fisher Scientific Ltd., Nepean, ON) containing 10±0.01 g whole egg, egg yolk or albumen were subsequently incubated at 22^0 C (abusive temperature) or 8^0 C (slightly abusive temperature). Aerobic plate counts were determined at 0, 24, 48, 72 h for samples stored at 22^0 C or on a biweekly basis (0, 2, 4, 6 wk) for samples stored at 8^0 C. Egg samples were serially diluted in saline (0.85% NaCl) and surface spread plated (0.1 g) in duplicate using standard plate count agar (SPC; BBL, Becton Dickinson and Company, Cockeysville, MD). Plates were incubated at 32^0 C for 48 h. Evaluation for all egg products was conducted in duplicate; results were expressed as $\log_{10}$ CFU/g.

3.4 Growth and/or Survival of *S .typhimurium* and *E. coli* in Regular and Omega-3 Whole Eggs, Egg Yolk and Albumen

Portions (10±0.01 g) of egg samples (regular and omega-3 whole eggs, egg yolk, and albumen), contained in sterile vials (Fisher Scientific Ltd., Nepean, ON), were

subsequently inoculated with either *S. typhimurium* or *E. coli* (0.1 ml) resulting in a final concentration of approximately 10^2-10^3 CFU/g egg contents and incubated at 22^0 C, 8^0 C or -20^0 C. Samples stored at 22^0 C were assessed on a daily basis, samples stored at 8^0 C and -20^0 C were assessed on a weekly basis and biweekly basis, respectively. Growth or survival for each bacterium was determined by surface plating serial dilutions of samples on TSA containing 50 ppm nalidixic acid and incubating at 35^0 C for 24 h. If organisms were not detected at the lowest dilution, the samples were enriched in TSB containing 50 ppm nalidixic acid at 35^0 C for 24 h with agitation and subsequently replated on TSA containing 50 ppm nalidixic acid and incubated at 35^0 C for 24 h. Colonies growing on TSA with 50 ppm nalidixic acid were counted as presumptive *S. typhimurium* or *E. coli*. Colonies were confirmed as *S. typhimurium* by streaking about 5-10 colonies from the highest dilution plate onto brilliant green agar with sulfadiazine (BGSA; BBL) containing 50 ppm nalidixic acid. *E. coli* colonies were confirmed using MacConkey sorbitol agar (SMAC; Difco Laboratories, Detroit, MI). Evaluation for all egg products was conducted in duplicate and the results were expressed as $\log_{10}$ CFU/g.

3.5 Growth of *S. typhimurium* in Regular Egg Yolk Fortified with Different Levels of Vitamin E under Aerobic or Anoxic Conditions at 22^0 C

Regular egg yolks were obtained as described previously (Section 3.2). Each 10±0.01 g egg yolk was fortified with 0.3±0.01 g of vitamin E solutions and egg yolk fortified with the same amount of corn oil (arbitrarily chosen) as control group. The vitamin E solutions were prepared by adding 0.26±0.01 g, 0.56±0.01 g, and 1.12±0.01 g vitamin E (Tenox® GT-2 food grade antioxidant in soybean oil, 700mg/g total tocopherol

concentration; Eastman Chemical Company, Kingsport, TN) into 11.3±0.01 g Mazola® corn oil (Best Foods Canada Inc., Etobicoke, ON), which resulted in 3 levels of vitamin E solution. Vitamin E enhanced egg yolks (10±0.01 g) contained in sterile vials (Fisher Scientific Ltd., Nepean, ON) were subsequently inoculated with 0.1 ml of a 24 h culture of *S. typhimurium* resulting in a final concentration of about 10^2-10^3 CFU/g egg yolk. Following incubation at 22^0 C for 24-48 h under aerobic conditions, growth was determined on a daily basis by surface plating serial dilutions of samples (0.1 g) on TSA containing 50 ppm nalidixic acid; plates were incubated at 35^0 C for 24 h. A similar protocol was conducted under anoxic conditions. In this case, sterile petri dishes (Fisher Scientific Ltd., Nepean, ON) containing egg yolks (30±0.01 g) with 0.9±0.01 g of vitamin E solutions were incubated in anaerobic jars at 22^0 C for 24-48 h. GasPak Anaerobic System Envelopes (BBL; Becton Dickinson Microbiology Systems, Cockeysville, MD) were used to provide anoxic conditions. The actual concentration of vitamin E isomers fortified in egg yolk was analyzed by HPLC (Section 3.8).

3.6 Thermal Inactivation of *S. typhimurium* at 56.5^0 C in Regular and Omega-3 Egg Yolks

Thermal inactivation of *S. typhimurium* in egg yolk was assessed using the procedure of Humphrey et al. (1990) with some modification. Egg yolks (1±0.01 g) contained in 15 x 100 mm round bottom screw-capped glass vials (VWR Scientific Products, San Francisco, CA) were inoculated with 0.1ml *S. typhimurium* (final population of approximately 10^6 CFU/g egg yolk per vial). Vials were subsequently placed in a thermostatically controlled water bath (Blue M Electric Company, Blue

Island, IL) and allowed to equilibrate for approximately 3.5 min to reach the required temperature (56.5^0 C). Following equilibration, the vials were removed at 1 min intervals for a total of 5 min and cooled rapidly in an ice water bath. The temperature was monitored by using a thermocouple (Omega Engineering, Inc., Stamford, CO) which was placed in the geometric center of the vial contents (non-inoculated sample). Survivors were determined using an Automated Spiral Plater (Autoplate® 4000; Spiral Biotech Inc, Bethesda, MD) and enumerated on TSA containing 50 ppm nalidixic acid following incubation at 35^0 C for 24 h.

3.7 Thermal Inactivation of *S. typhimurium* at 56.5^0 C in Regular Egg Yolk Fortified with Vitamin E

As previously described (Section 3.6), regular egg yolks (1±0.01 g) fortified with 0.03±0.01 g vitamin E solutions were used. The vitamin E solutions were prepared by adding 0.26±0.01 g, 0.56±0.01 g, 0.82±0.01 g, and 1.12±0.01 g vitamin E (Tenox® GT-2, 700mg/g total tocopherol concentration; Eastman Chemical Company, Kingsport, TN)) into 11.3±0.01 g Mazola® corn oil (Best Foods Canada Inc., Etobicoke, ON), which resulted in 4 levels of vitamin E solution. The control group was prepared as regular egg yolk (1±0.01 g) added with 0.03±0.01 g Mazola® corn oil (Best Foods Canada Inc.). The concentration of different tocopherol isomers was analyzed by HPLC (Section 3.8).

3.8 Analysis of Tocopherols in Egg Yolk by HPLC

Tocopherols are exclusively contained in the lipid portion of egg yolk. Therefore all egg yolk samples inoculated with *S. typhimurium* involved in the previous sections 3.4-3.7 (except the trial under anoxic condition) were analyzed for various tocopherol isomers. In this regards, the egg yolk samples were frozen at -20^0 C prior to lipid extraction. Thawed egg yolks (2.00±0.01g) were weighed and total lipids were extracted from these samples with chloroform/methanol (2:1 v/v) according to the procedure of Bligh and Dyer (1959). The final lipid was weighed and dissolved in 3ml hexane prior to HPLC analysis. Lipid samples were prepared in 1.5ml gas chromatography vial with a concentration of 150mg/ml in hexane and analyzed with HPLC (normal phase) using a fluorescence detector and a Shimadzu SIL-10A autoinjector (Shimadzu Corp., Kyoto, Japan) according to AOCS Official Method Ce 8-89 (1990). Separation was achieved on a Prodigy silica column (250mm × 3.2mm, Phenomenex®, Torrance, CA) using a mobile phase of *tert*-butyl ether/hexane (5:95 v/v) at a 0.8 ml/min flow rate with a running time of 22 min/sample. Tocopherols were detected with a Hewlett Packard HP 1046A programmable fluorescence detector (λ_{ex} = 290nm; λ_{em} = 330nm) and quantified using separate calibration curves for α-, β-, γ- and δ-tocopherol. The lowest detection level of tocopherol isomer was 10^{-9} g.

3.9 Comparison of Functional Properties of Regular and Omega-3 Egg Yolk

The functional properties, emulsifying capacity and mayonnaise stability of regular and omega-3 egg yolk were assessed according to the method of Varadarajulu and Cunningham (1972).

3.10 Statistical Analysis

Statistical calculations were processed by using SAS statistical analysis software program, version 6.12. An analysis of variance was conducted by the general linear models (GLM) procedure of SAS. Means determined to be significantly different ($P<0.05$) by GLM were separated using Student-Newman-Keuls (SNK) test.

4.0 RESULTS

4.1 Aerobic Plate Counts in Regular and Omega-3 Eggs at 22^0 C and 8^0 C

Time course aerobic plate counts in both regular and omega-3 whole eggs, egg yolk and albumen at 22^0 C or 8^0 C are shown in Figures 2-4, respectively. The entire protocol was performed in order to establish a background count.

Aerobic plate counts increased with time in both regular and omega-3 whole eggs when stored at 22^0 C (Fig. 2a), although values appeared significantly ($P<0.05$) higher in regular whole eggs throughout the 72 h storage period (Fig. 2a). At 8^0 C, counts also increased with time in regular whole eggs especially after 4 weeks; by 6 weeks counts reached approximately 10^6 CFU/g. In contrast, counts in omega-3 whole eggs at 8^0 C increased from 10^2 to approximately 10^4 CFU/g by week 2, thereafter, dropped to 10^3 CFU/g at 6 weeks. Thus, the population in regular whole eggs appeared about 3 $\log_{10}$ higher ($P<0.05$) compared to omega-3 eggs at 6 weeks when stored at 8^0 C (Fig. 2b).

Storage at 22^0 C for 48 h resulted in an increase in aerobic counts from 10^2 to 10^8 CFU/g in both regular and omega-3 egg yolks (Fig. 3a). When stored at 8^0 C, aerobic counts remained higher in regular egg yolks until 4 weeks of storage. Thereafter, aerobic counts in omega-3 egg yolks increased from 10^3 (week 4) to 10^6 CFU/g (week 6), resulting in approximately a 2 $\log_{10}$ higher populations than in regular egg yolk ($P<0.05$) (Fig. 3b). As shown in Figure 4 (a, b), aerobic counts decreased with storage time from 10^2 to 10^1 or below 10 CFU/g in regular and omega-3 egg albumen when stored at 22^0 C or 8^0 C, respectively. At 22^0 C, population levels were significantly higher in omega-3 albumen (Fig. 4a). However, the reverse trend was observed at 8^0 C. In this case, aerobic

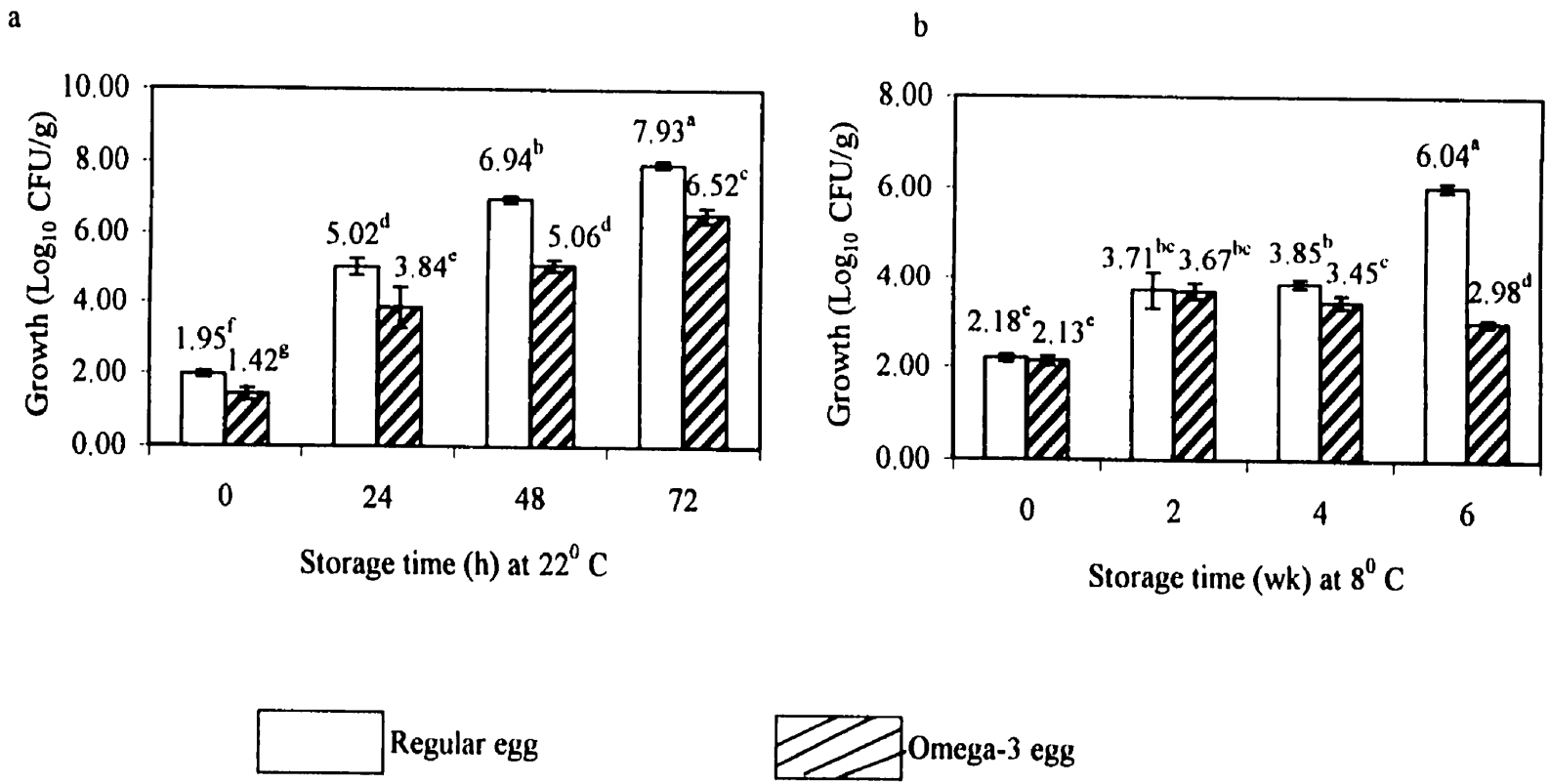


Figure 2a, b. Aerobic plate counts in regular and omega-3 whole eggs. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 1). Values with different superscripts are significantly different ($P<0.05$).

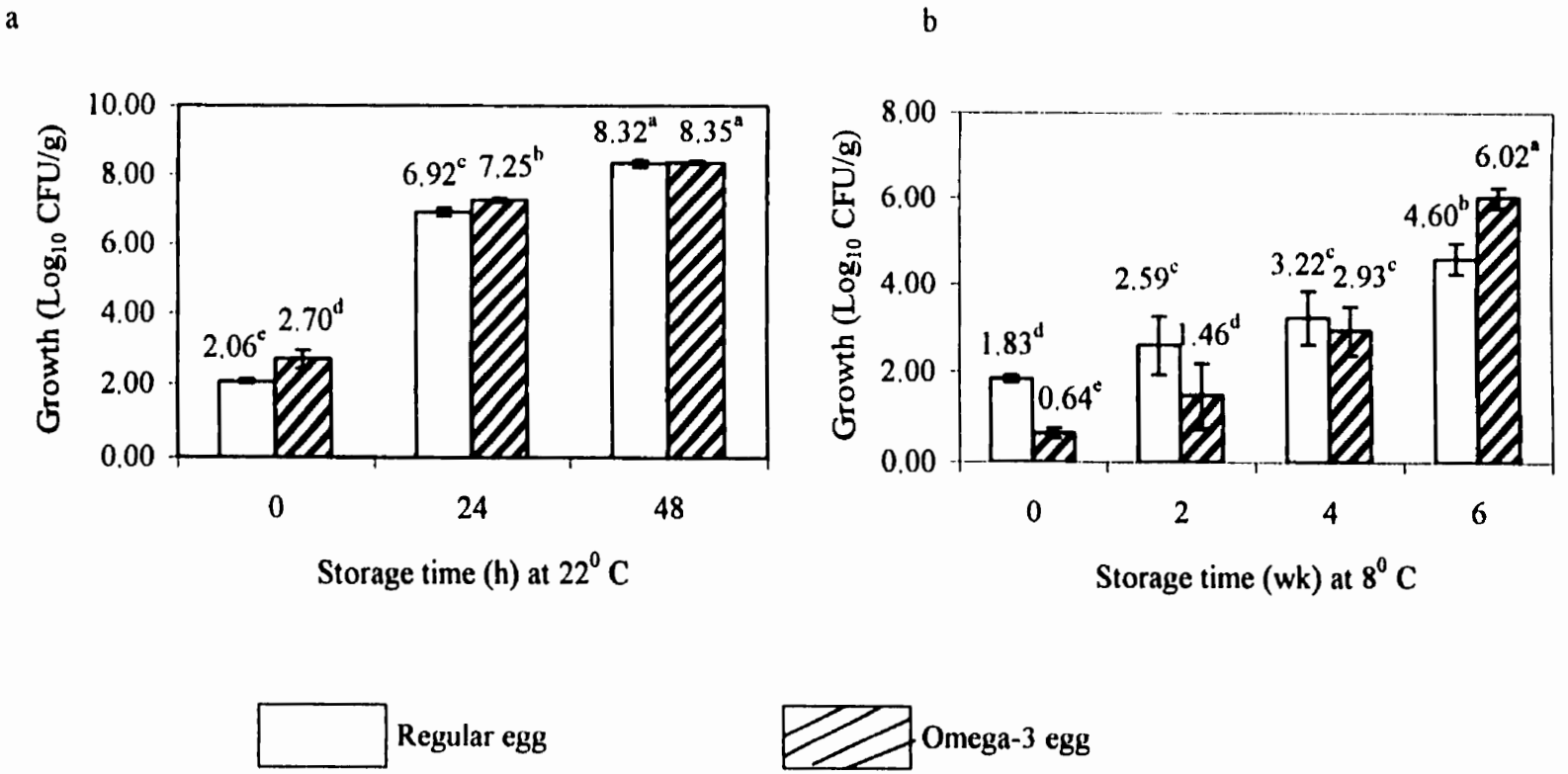


Figure 3a, b. Aerobic plate counts in regular and omega-3 egg yolk. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 2). Values with different superscripts are significantly different ($P<0.05$).

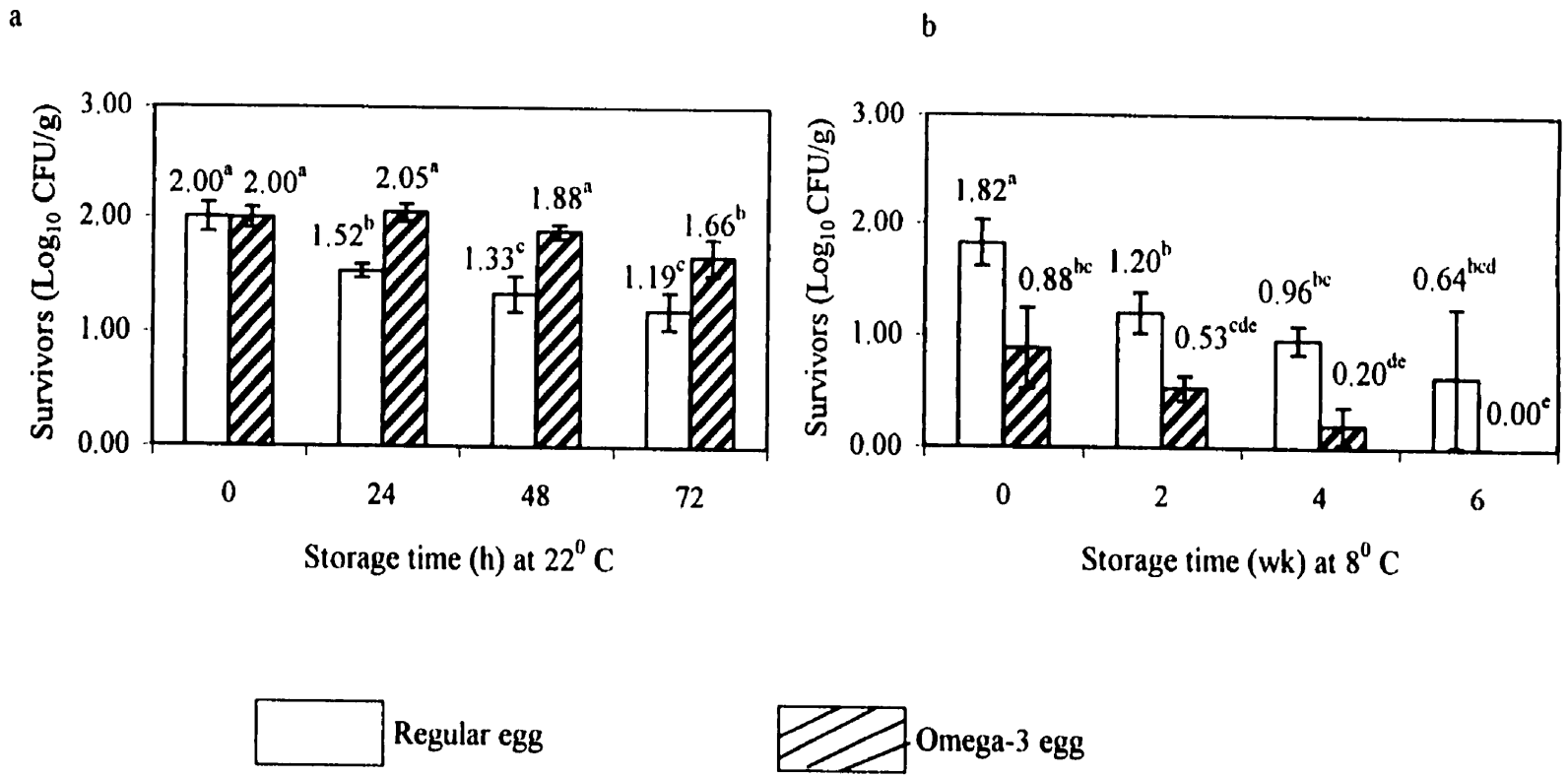


Figure 4a, b. Aerobic plate counts in regular and omega-3 egg albumen. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 3). Values with different superscripts are significantly different ($P<0.05$).

plate counts appeared significantly higher in regular egg albumen throughout the 6 week storage period ($P<0.05$) (Fig. 4b).

4.2 Growth and/or Survival of *S. typhimurium* and *E. coli* in Regular and Omega-3 Whole Egg and Albumen at 22^0 C, 8^0 C, and -20^0 C

No nalidixic acid resistant organisms (other than the inocula) were detected in the egg samples that were used in this study. Also, colonies assessed using SMAC or BGSA appeared as either *Salmonella* or *E. coli*, respectively.

As shown in Figures 5 and 6, the growth and/or survival patterns of both *S. typhimurium* and *E. coli* in regular whole eggs were similar to that of omega-3 eggs. For instance, *Salmonella* populations grew from an initial inoculum level of 10^2 to 10^8 CFU/g after 2 days of incubation at 22^0 C in both types of whole eggs (Fig. 5a). Regardless of the egg type, *Salmonella* counts remained stable or increased slightly above the initial level of 10^3 CFU/g during the first 4 weeks of incubation at 8^0 C. Thereafter, the populations significantly ($P<0.05$) increased from 10^3 to 10^6 CFU/g in both regular and omega-3 whole eggs (Fig. 5b). In comparison from week 2 to week 8, *Salmonella* counts appeared slightly higher in omega-3 whole eggs than in regular eggs ($P<0.05$) when stored at -20^0 C; populations remained above 10^1 CFU/g after 8 weeks in both types of whole egg products (Fig. 5c).

Growth and/or survival profiles of *E. coli* appeared similar regardless of whole egg type (Fig. 6). Storage at 22^0 C resulted in a population increasing to 10^8 CFU/g after 2 days for both regular and omega-3 whole eggs (Fig. 6a). Unlike the growth of *S. typhimurium* in whole eggs at 8^0 C, the corresponding growth of *E. coli* was more rapid

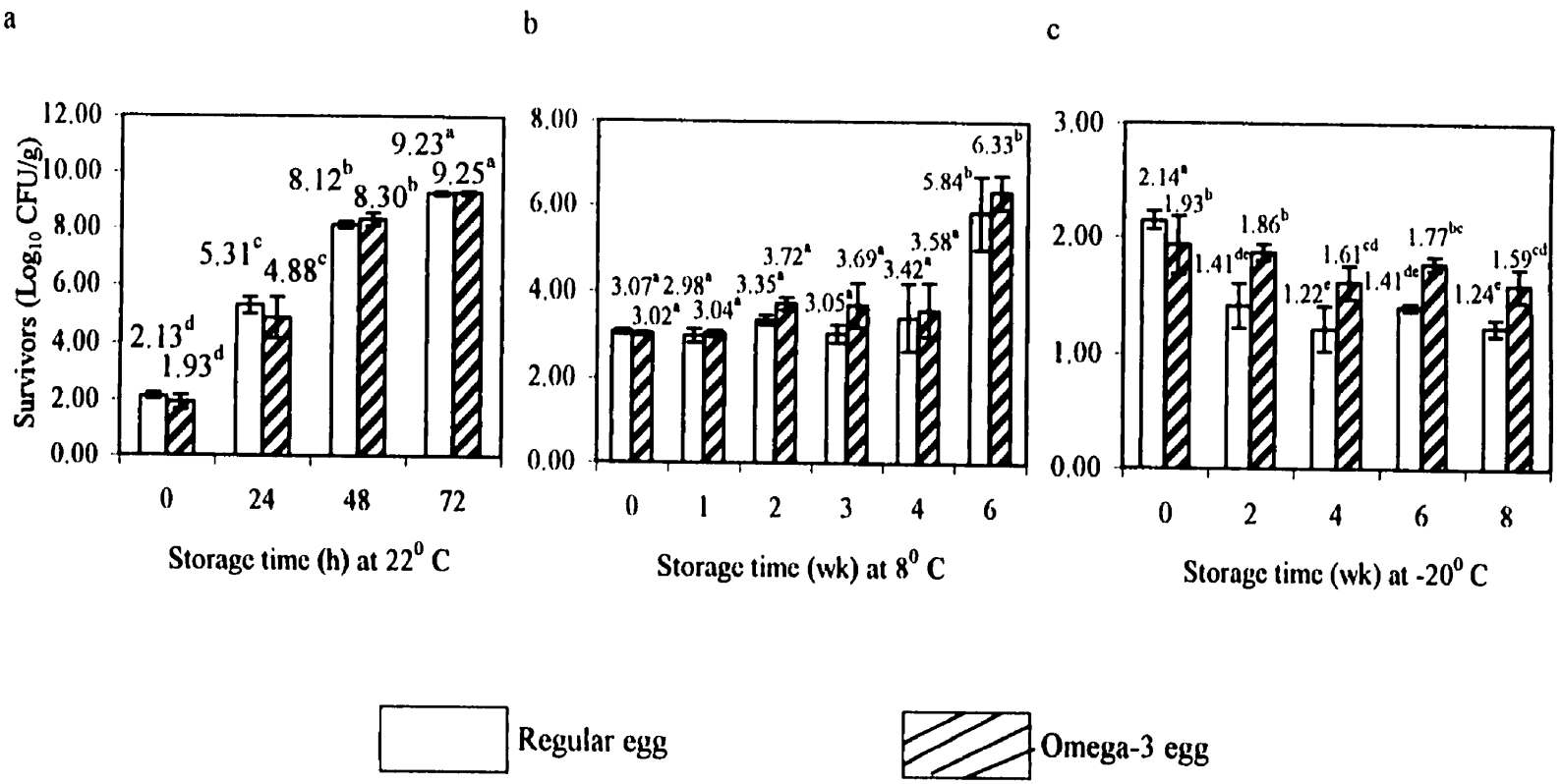


Figure 5a, b, c. Growth and/or survival of *S. typhimurium* in regular and omega-3 whole eggs. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 4). Values with different superscripts are significantly different ($P<0.05$).

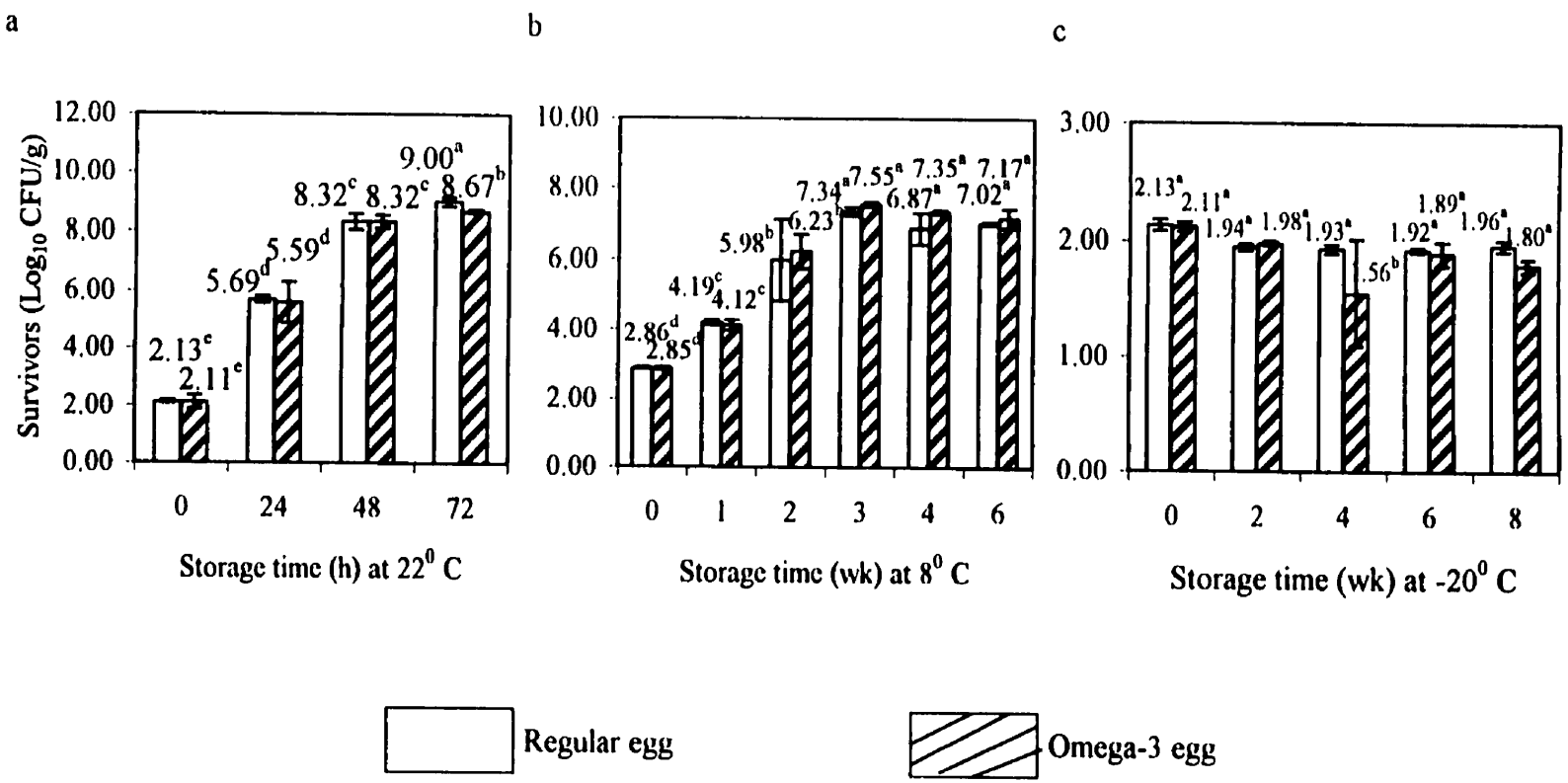


Figure 6a, b, c. Growth and/or survival of *E. coli* in regular and omega-3 whole eggs. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 5). Values with different superscripts are significantly different ($P<0.05$).

in both types of whole eggs (Fig. 6b). Population levels greater than 3 $\log_{10}$ were reached at 3 weeks. Moreover, *E. coli* counts remained relatively constant in both types of whole eggs when stored at -20^0 C (Fig. 6c).

Initial inoculum levels for *Salmonella* and *E. coli* in albumen at zero time appeared slightly lower than those used previously (Fig. 7, 8). Although *E. coli* could be detected by enrichment procedures after 3 days storage at 22^0 C or 2 weeks at 8^0 C, none were detected at -20^0 C by week 2 (Appendix 6). Compared to *E. coli*, *S. typhimurium* showed enhanced survival in albumen, although numbers still decreased gradually when stored at 8^0 C and -20^0 C (Fig. 8b, c). Nevertheless, *Salmonella* counts were significantly higher in omega-3 egg albumen especially from 2 to 8 weeks of storage at -20^0 C; differences in the range of 0.12 to 0.43 $\log_{10}$ between omega-3 and regular egg albumen were observed (Fig. 8c). Additionally, *Salmonella* counts remained relatively constant in regular egg albumen for a 3 day period, while the numbers increased about 0.5 $\log_{10}$ by day 3 in omega-3 egg albumen (22^0 C). This value was significantly ($P<0.05$) different from regular egg albumen (Fig. 8a). However, at 8^0 C survival patterns of salmonellae in albumen were similar in both regular and omega-3 egg types (Fig. 8b).

4.3 Growth and/or Survival of *S. typhimurium* and *E. coli* in Regular and Omega-3 Egg Yolk at 22^0 C, 8^0 C, or -20^0 C

Similar growth patterns for *S. typhimurium* were observed in regular and omega-3 egg yolk incubated at 22^0 C despite an eight-fold increase in α-tocopherol in the latter

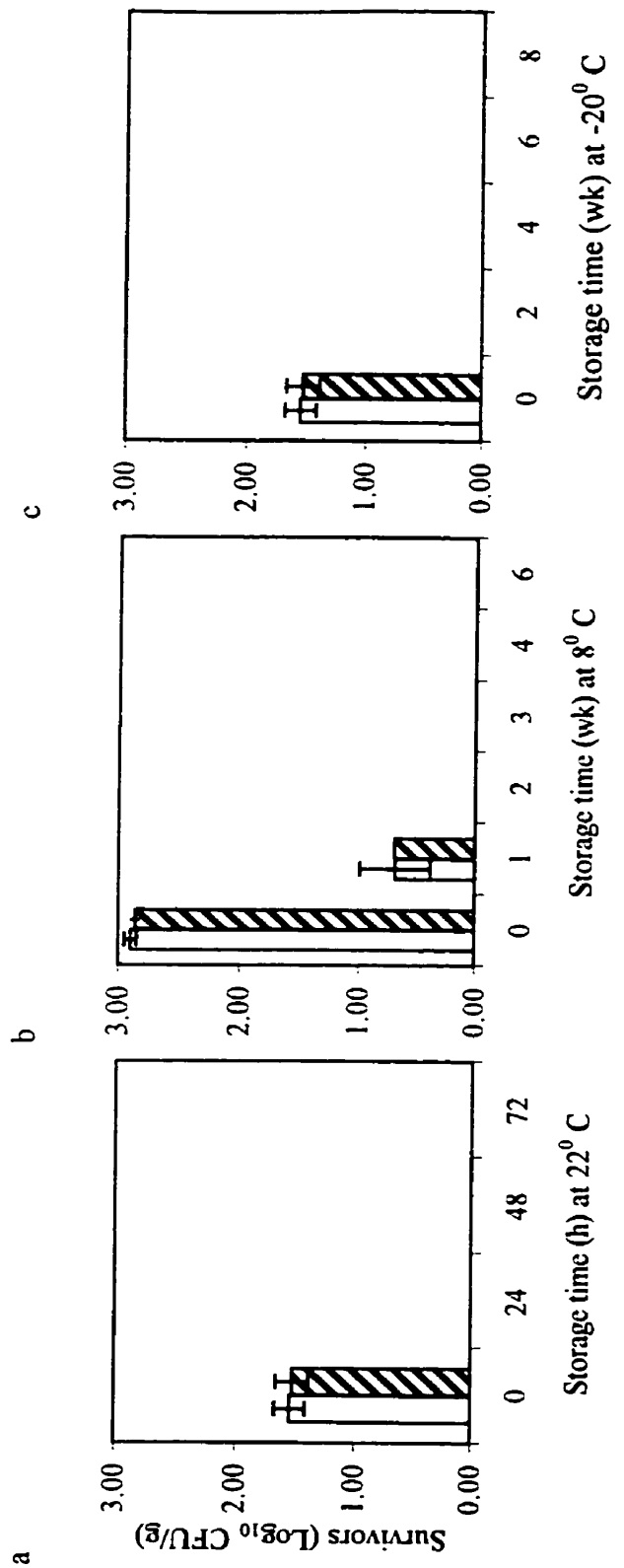


Figure 7a, b, c. Survival of *E. coli* in regular and omega-3 egg albumen. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 6).

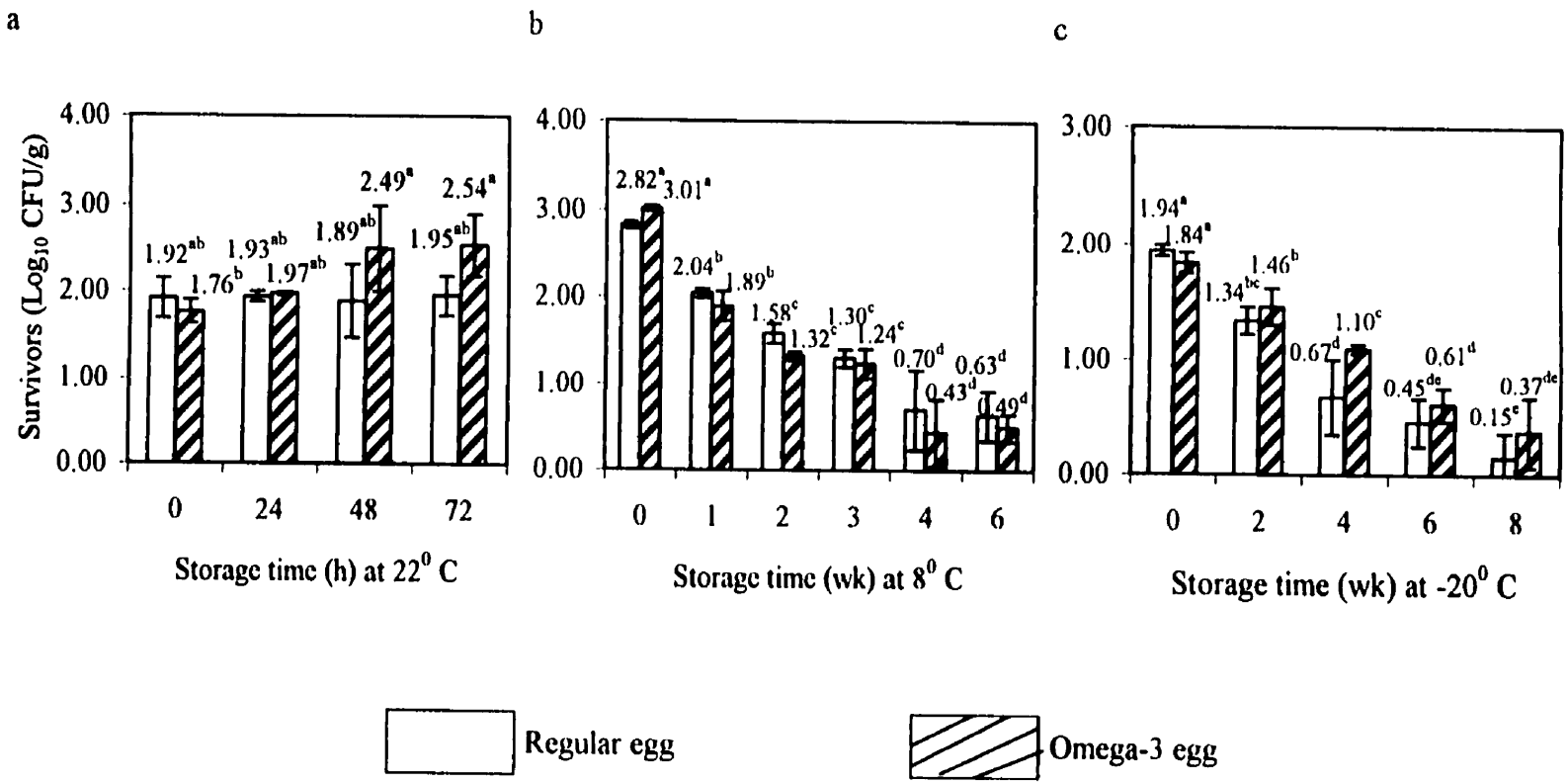


Figure 8a, b, c. Growth and/or survival of *S. typhimurium* in regular and omega-3 egg albumen. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 7). Values with different superscipts are significantly different ($P<0.05$).

product (Fig. 9, Table 2). *Salmonella* levels reached more than 10^9 CFU/g after 2 days storage in both types of egg yolk (Fig. 9). In regard to tocopherol components, the two main isomers, α and γ, as well as trace amounts of β- and δ-tocopherol were detected in both types of egg yolks (Table 2). Among them, α- and γ-tocopherol were approximately 8 and 2 times higher in omega-3yolks than in regular egg yolk, respectively (Table 2). In addition, α-tocopherol accounted for about 80% of the total tocopherols in regular egg yolk, compared to 95% in omega-3 egg yolk (Table 2).

Survival profiles of *S. typhimurium* in egg yolk at -20^0 C are illustrated in Figure 10. Regardless of egg yolk type, *Salmonella* populations decreased from an initial inoculation level of 3 to 1.7 $\log_{10}$ CFU/g after 8 weeks incubation. No significant differences in terms of *Salmonella* survival were observed between regular and omega-3 egg yolks except at week 2. At this time regular egg yolk contained 0.3 $\log_{10}$ more *S. typhimurium* compared to omega-3 egg yolk ($P < 0.05$) . As shown in Table 3, omega-3 egg yolk contained approximately 7 and 2 times more α- and γ-tocopherol than regular egg yolk, respectively. Beta-tocopherol contents were in the range of 0.76-1.08 ppm in both types of egg yolk. However, total tocopherol decreased 50 ppm in omega-3 egg yolk throughout 8 weeks storage at -20^0 C (Table 3); this was mainly attributed to the decrease of α-tocopherol during storage which was not observed in regular egg yolk (Table 3).

When stored at 8^0 C, salmonellae levels increased with time in both types of egg yolk, although regular egg yolk contained significantly higher levels at week 2, 3, and 4 ($P<0.05$) (Fig. 11). However, by week 6 population levels reached approximately 4.6

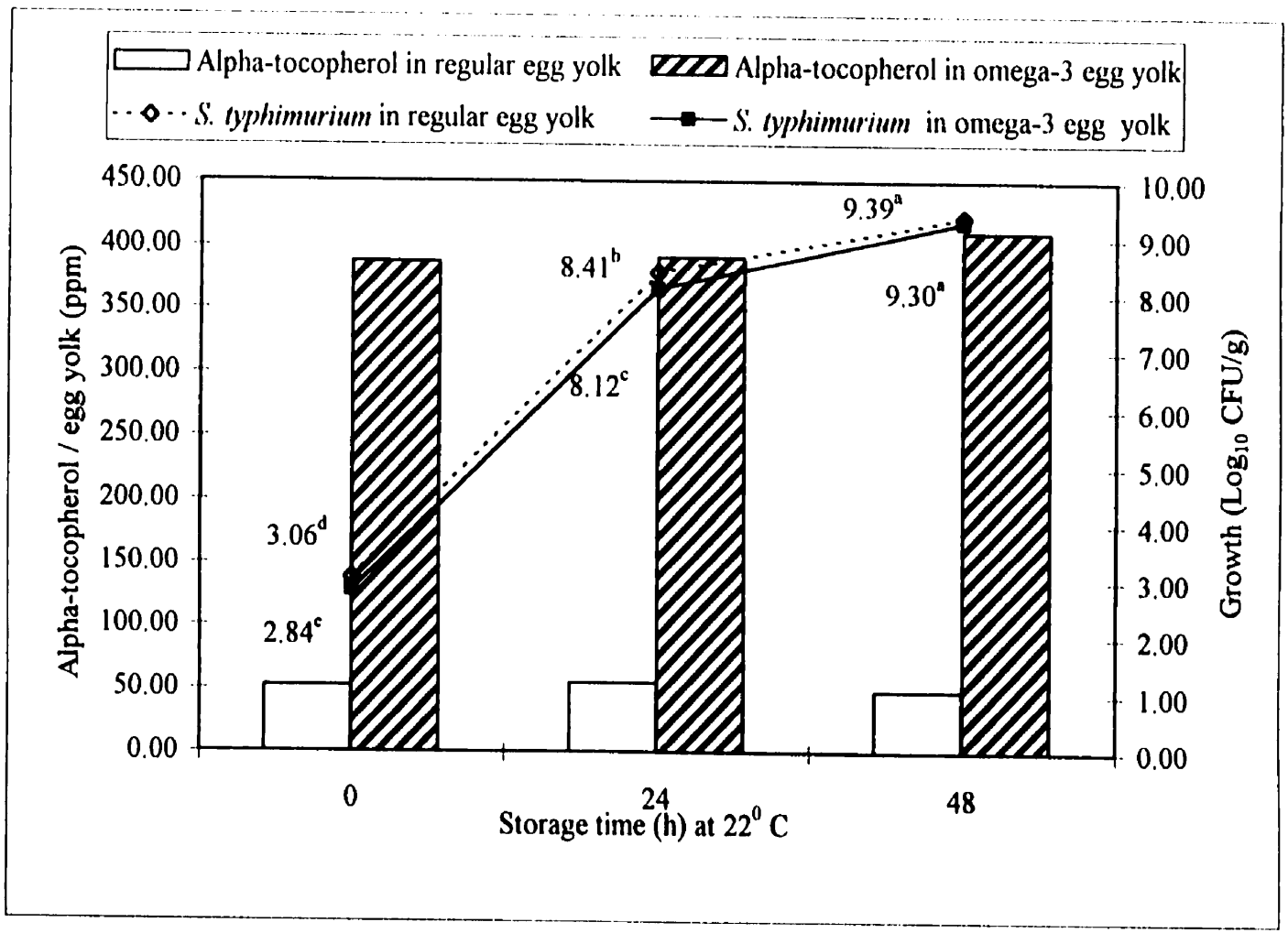


Figure 9. Growth of *S. typhimurium* and α-tocopherol content in regular and omega-3 egg yolk at 22° C. Each bar and point represents the mean of 2 and 4 replicates, respectively (Appendix 8). Values with different superscripts are significantly different ($P<0.05$).

Table 2. Tocopherol content in regular and omega-3 egg yolk inoculated with *S. typhimurium* and stored at 22^0 C.

Egg sample	Storage time (h)	Tocopherol (ppm)[1]				
		Alpha	Beta	Gamma	Delta	Total
Regular	0	52.71	0.76	11.63	ND[2]	65.10
	24	55.22	0.88	12.25	ND	68.35
	48	48.07	0.84	11.02	ND	59.93
Omega-3	0	386.94	0.82	20.78	ND	408.54
	24	390.47	0.97	21.26	ND	412.70
	48	410.66	1.14	21.87	0.93	433.67

[1] Value represents the mean of 2 replicates.

[2] Not Detectable.

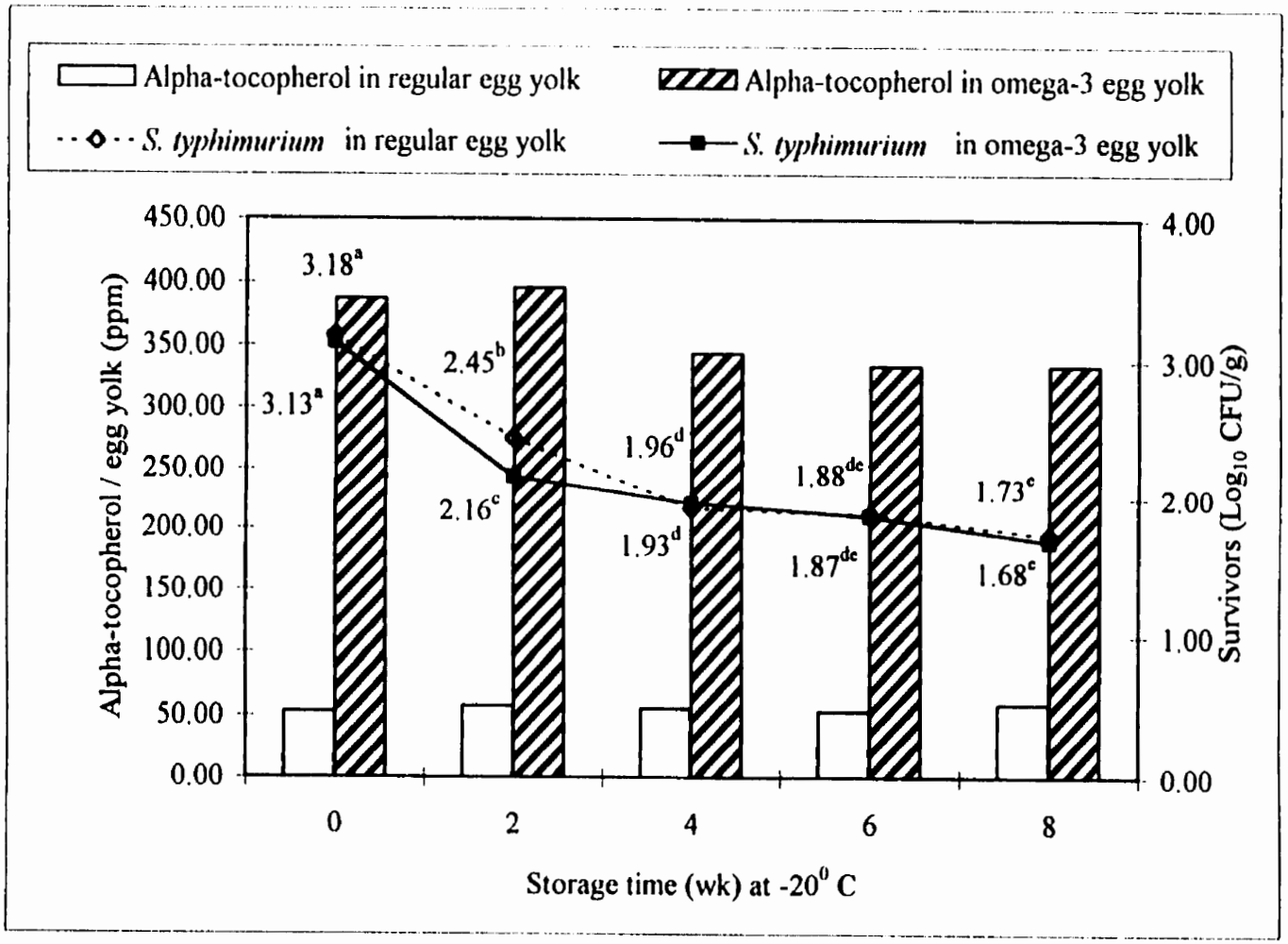


Figure 10. Survival of *S. typhimurium* and α-tocopherol content in regular and omega-3 egg yolk at -20^0 C. Each bar and point represents the mean of 2 and 4 replicates, respectively (Appendix 9). Values with different superscripts are significantly different (P<0.05).

Table 3. Tocopherol content in regular and omega-3 egg yolk inoculated with *S. typhimurium* and stored at -20^0 C.

Egg sample	Storage time (week)	Tocopherol (ppm)[1]				
		Alpha	Beta	Gamma	Delta	Total
Regular	0	52.71	0.76	11.63	ND[2]	65.10
	2	57.38	0.88	12.89	1.23	71.15
	4	55.66	0.88	13.78	ND	70.32
	6	53.18	0.91	12.15	ND	66.24
	8	59.05	0.95	11.86	ND	71.86
Omega-3	0	386.94	0.82	20.78	ND	408.54
	2	395.53	1.01	21.70	ND	418.24
	4	343.88	0.97	19.62	ND	364.47
	6	333.97	1.03	18.81	ND	353.81
	8	333.65	1.08	19.02	ND	353.75

[1] Value represents the mean of 2 replicates.

[2] Not Detectable.

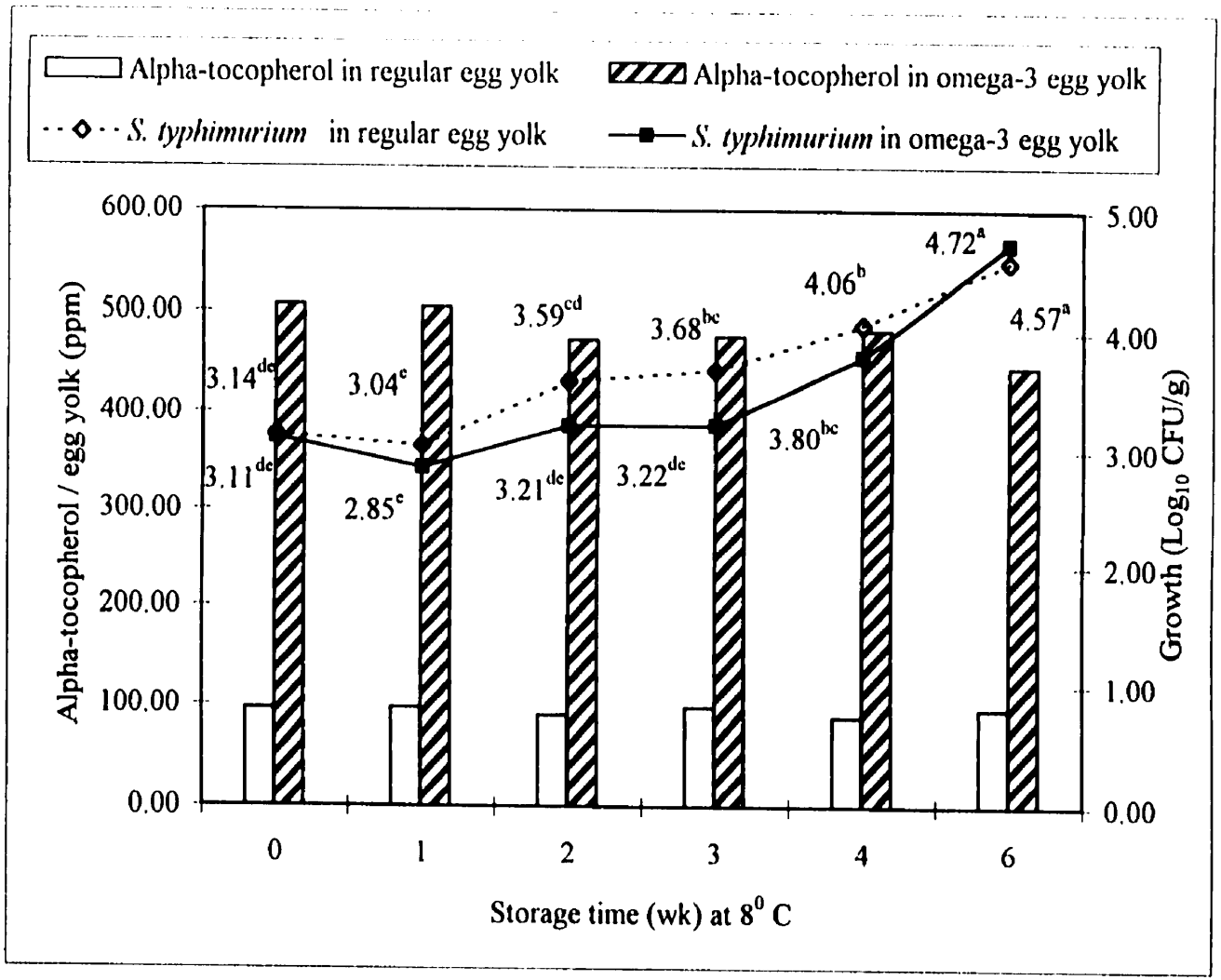


Figure 11. Growth of *S. typhimurium* and α-tocopherol content in regular and omega-3 egg yolk at 8^0 C. Each bar and point represents the mean of 2 and 4 replicates, respectively (Appendix 10). Values with different superscripts are significantly different ($P<0.05$).

$\log_{10}$ CFU/g in both products. On the other hand, tocopherol isomer values were different compared to values at 22^0 C or -20^0 C. In this respect, omega-3 egg yolk contained approximately 5 times more α- and total tocopherols than regular egg yolk, while γ-tocopherol content was similar in both products (Table 4). Similar to previous results (storage at -20^0 C), α- and total tocopherols were observed to decrease in omega-3 egg yolk when stored at 8^0 C (Table 4). Alpha-tocopherol dropped from an initial levels of 506 to 445 ppm by week 6; this resulted in a decrease in total-tocopherol levels from 520 to 458 ppm at the termination of the trial (Table 4). This pattern was not observed in regular egg yolk (Table 4).

As shown in Figure 12a, b, and c, similar growth and/or survival patterns of *E. coli* were observed in both regular and omega-3 egg yolk regardless of storage temperature. No differences in the level of *E. coli* growth were observed between regular and omega-3 egg yolks during storage at 22^0 C for 2 days (Fig. 12a). However, significant ($P<0.05$) differences in *E. coli* levels were observed at week 1 during storage at 8^0 C, and at week 2 and 6 at -20^0 C (Fig. 12b, c).

4.4 Growth of *S. typhimurium* in Regular Egg Yolk Fortified with Vitamin E at 22^0 C under Aerobic or Anoxic Conditions

In order to further demonstrate the effect of vitamin E (tocopherol) on the microbiological properties of egg yolks, regular egg yolks were fortified with vitamin E and subsequently inoculated with *S. typhimurium*. Total tocopherols in the control group consisted of 85% α-tocopherol (Table 5). Gamma-tocopherol accounted for about 50% of

Table 4. Tocopherol content in regular and omega-3 egg yolk inoculated with *S. typhimurium* and stored at 8^0 C.

Egg sample	Storage time (week)	Tocopherol (ppm)[1]				
		Alpha	Beta	Gamma	Delta	Total
Regular	0	96.01	1.85	10.80	ND[2]	108.66
	1	97.00	1.57	14.36	ND	112.93
	2	89.70	1.32	8.92	ND	99.94
	3	97.64	1.53	9.18	ND	108.35
	4	88.75	1.47	8.76	ND	98.98
	6	95.94	1.87	11.20	ND	109.01
Omega-3	0	506.42	ND[2]	13.21	ND	519.63
	1	503.52	ND	11.82	ND	515.34
	2	472.37	0.29	12.32	ND	484.98
	3	476.25	ND	14.17	ND	490.42
	4	481.54	0.23	13.35	ND	495.12
	6	445.28	0.63	11.96	ND	457.87

[1] Value epresents the mean of 2 replicates.

[2] Not Detectable.

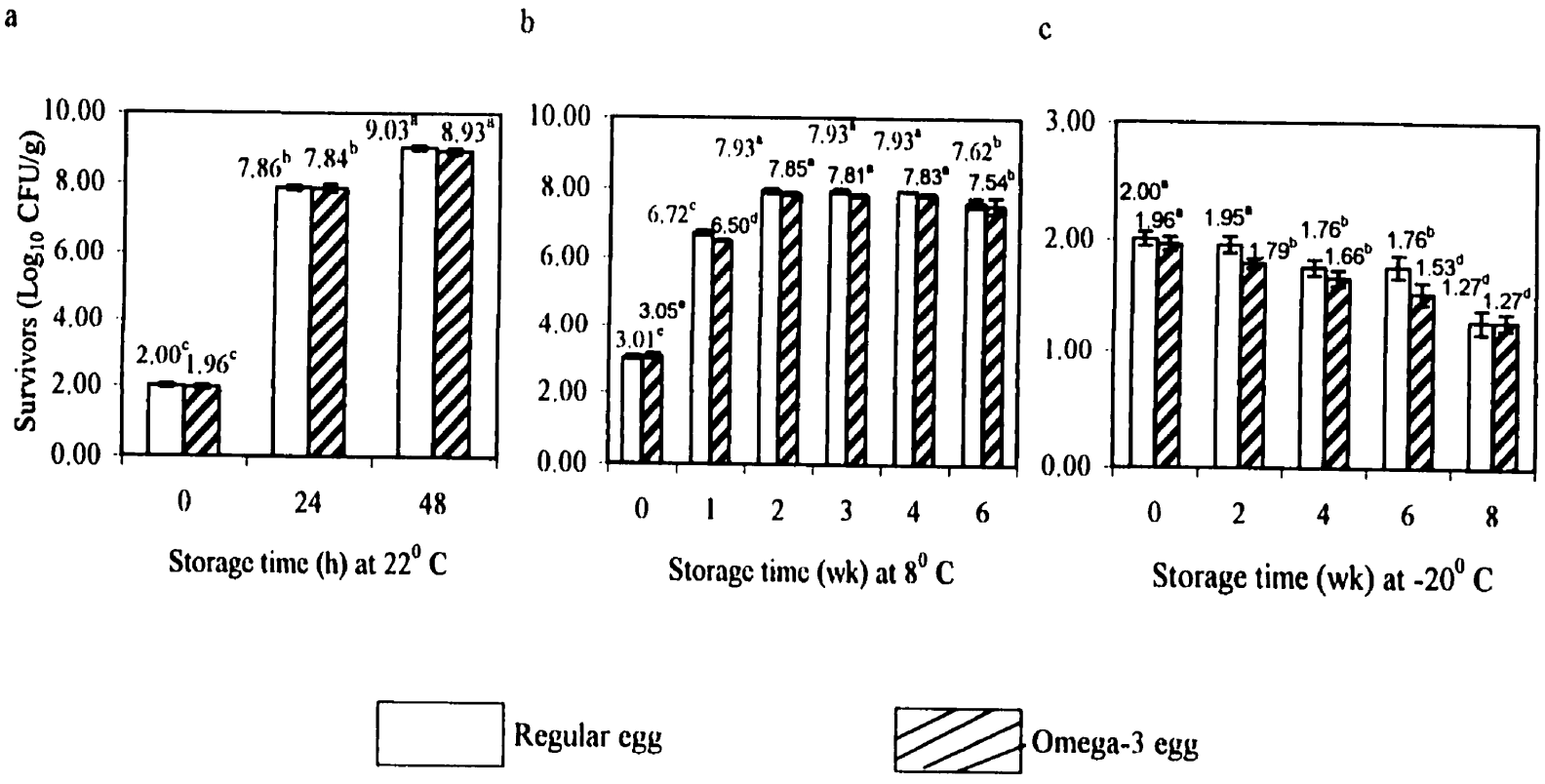


Figure 12a, b, c. Growth and/or survival of *E. coli* in regular and omega-3 egg yolk. Each bar and error bar represents the mean and standard deviation of 4 replicates, respectively (Appendix 11). Value with different superscripts are significant different ($P<0.05$)

Table 5. Tocopherol content in regular egg yolk fortified with vitamin E and inoculated with *S. typhimurium* and stored at 22^0 C under aerobic conditions.

Egg sample	Storage time (h)	Tocopherol (ppm)[1]				
		Alpha	Beta	Gamma	Delta	Total
control	0	62.91	2.28	8.73	ND[2]	73.92
	24	51.39	1.35	11.84	2.81	67.39
RY1	0	107.78	5.83	281.78	115.03	510.41
	24	97.70	4.98	255.38	104.59	462.65
RY2	0	119.99	7.78	392.46	162.86	683.09
	24	125.22	7.57	405.49	168.57	706.85
RY3	0	239.85	14.51	796.60	332.22	1383.17
	24	143.13	8.74	483.65	200.64	836.15

[1] Value represents the mean of 2 replicates.
[2] Not Detectable.

total tocopherols in samples RY1, RY2, and RY3 (Table 5). As shown in Table 5, α-tocopherol levels increased from 63 ppm in the control to 240 ppm in RY3. In contrast, γ-tocopherol levels increased from 9 to about 797 ppm in the control and RY3 samples, respectively. This resulted in a change in total tocopherol from 74 to 1437 ppm in RY3 samples (Table 5). Moreover, all individual isomer levels as well as total tocopherol decreased after 24 h of storage in RY1 and RY3 samples (Table 5).

Growth patterns of *S. typhimurium* at 22^0 C under aerobic and anoxic conditions were not significantly ($P<0.05$) different with respect to tocopherol contents in egg yolk. The population increased to 10^9 CFU in 24 h under aerobic conditions (Fig 13a); similar levels were observed by 48 h under anoxic conditions (Fig 13b).

4.5 Thermal Inactivation of *S. typhimurium* in Regular and Omega-3 Egg Yolk and in Regular Egg Yolk Fortified with Vitamin E

Thermal inactivation of *S. typhimurium* in regular and omega-3 egg yolk at 56.5^0C is shown in Figure 14. The thermal resistance patterns of *Salmonella* in both types of egg yolk appeared similar. The D value calculated as the reciprocal of the slope of the thermal inactivation curve for *S. typhimurium* in regular egg yolk (1.66 ± 0.38) (Table 6) was not significantly ($P<0.05$) different from that of omega-3 egg yolk (1.61 ± 0.42). However, the correlation coefficients (r^2), 0.70 and 0.74 for regular and omega-3 egg yolk, respectively, indicated that the inactivation curve was not linear. This may have been due to tailing effects which appeared at the 4^{th} and 5^{th} min of heating. As shown in Table 7, omega-3 egg yolks contained about 13 and 2 times more α-and γ-tocopherol,

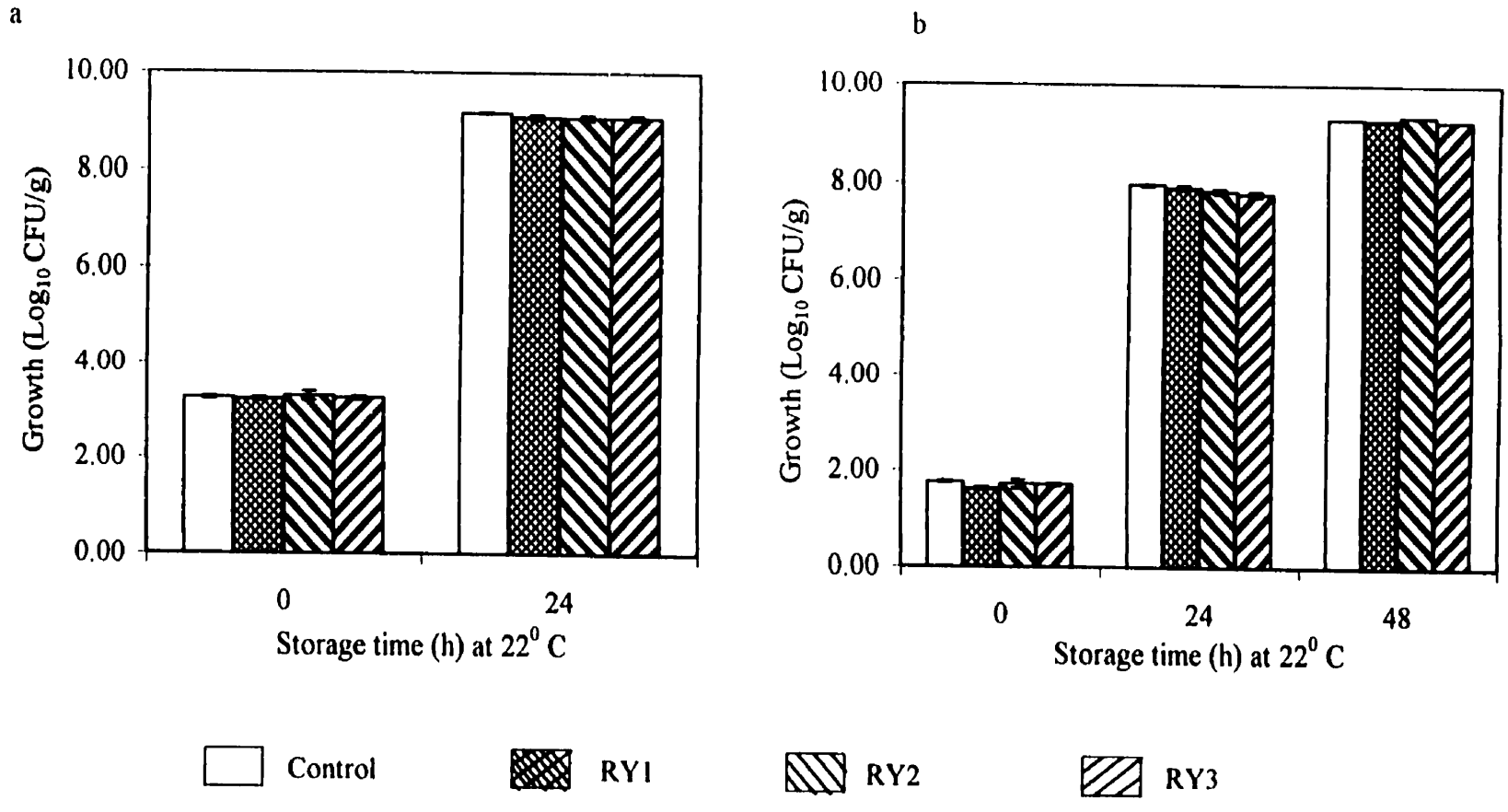


Figure 13a, b. Growth of *S. typhimurium* in regular egg yolk fortified with vitamin E at 22^0 C under aerobic (a) and anoxic (b) conditions. Each bar represents the mean of 4 replicates (Appendix 12).

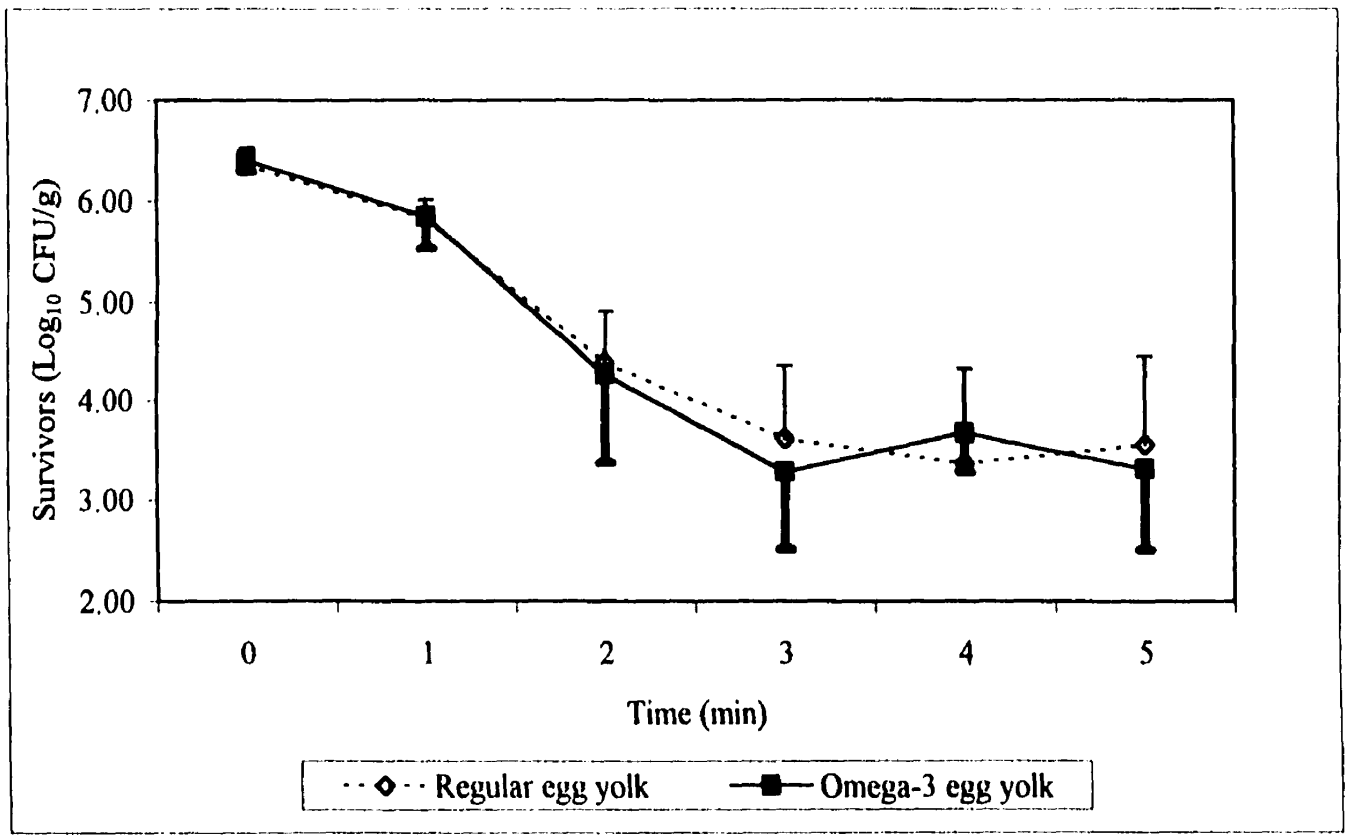


Figure 14. Thermal inactivation of *S. typhimurium* in regular and omega-3 egg yolk at 56.5^0 C. Each point and error bar represents the mean and standard deviation of 12 replicates, respectively (Appendix 13).

Table 6. Regression analysis of D-value[1] data for thermal inactivation of *S. typhimurium* in egg yolk at 56.5^0 C.

Sample	Regular egg yolk	Omega-3 egg yolk	Regular egg yolk fortified with vitamin E				
			RY0	RY1	RY2	RY3	RY4
D-value[3]	1.66^{a}	1.61^{a}	1.03^{bc}	1.14^{b}	0.88^{c}	1.01^{bc}	0.98^{bc}
SD[2]	0.38	0.42	0.13	0.22	0.16	0.22	0.17
R-square[3]	0.70	0.74	0.90	0.92	0.92	0.92	0.89
SD[2]	0.13	0.18	0.09	0.09	0.05	0.07	0.16

[1] Means with identical superscripts within a row are not significantly different (P>0.05).

[2] SD- standard deviation.

[3] Value represents the mean of 12 replicates.

Table 7. Tocopherol content in regular and omega-3 egg yolk, plus regular egg yolk fortified with vitamin E prior to heat treatment at 56.5^0 C.

Egg sample		Tocopherol (ppm)[1]				
		Alpha	Beta	Gamma	Delta	Total
Regular egg yolk fortified with vitamin E	RY0 (control)	53.72	1.94	31.10	8.37	95.13
	RY1	96.23	5.62	231.24	91.05	424.14
	RY2	136.41	8.74	447.50	182.75	775.40
	RY3	150.67	10.08	534.96	220.01	915.72
	RY4	190.25	13.53	729.90	303.82	1237.50
Regular egg yolk		55.38	1.83	24.71	10.01	91.93
Omega-3 egg yolk		713.47	1.35	58.42	6.08	779.32

[1] Value represents the mean of 2 replicates.

respectively, than regular egg yolks. Additionally, the total tocopherol level was 8.5 times greater than in regular egg yolks.

The thermal inactivation of *S. typhimurium* in regular egg yolk fortified with varying levels of vitamin E was also conducted in order to compare D values. Alpha-, γ- and δ-tocopherols in regular egg yolk fortified with vitamin E solutions increased at different rates. For instance, α-tocopherol increased from 54 ppm in the control (RY0) to 151 ppm in RY4 samples. RY4 samples contained about 304 ppm δ-tocopherol compared to 8 ppm in the control (RY0). Gamma-tocopherol also increased from 31 ppm in the control to 730 ppm in RY4 samples (Table 7). Thus, the total amount of γ- and δ-tocopherols accounted for more than 50% of total tocopherol in egg yolk with the control (RY0) as an exception.

Due to the large variations in survivors shown after 3min in the preliminary study (Appendix 14, 15), it was decided that a 2.5min thermal treatment (Fig. 15) be used to calculate the D values (Table 6). D values varied from 0.88 to 1.14. Overall, however, it appeared that increasing the total level of vitamin E (total tocopherol) had minimal effect on the D values. The correlation coefficient (r^2) improved to about 0.90, indicating the inactivation curve was linear in the first 2.5min of heat treatment (Table 6).

4.6 Emulsion and Mayonnaise Stability in Regular and Omega-3 Egg yolk

As shown in Table 8, emulsion and mayonnaise stability of omega-3 egg yolk did not significantly ($P<0.05$) differ from that of regular egg yolk.

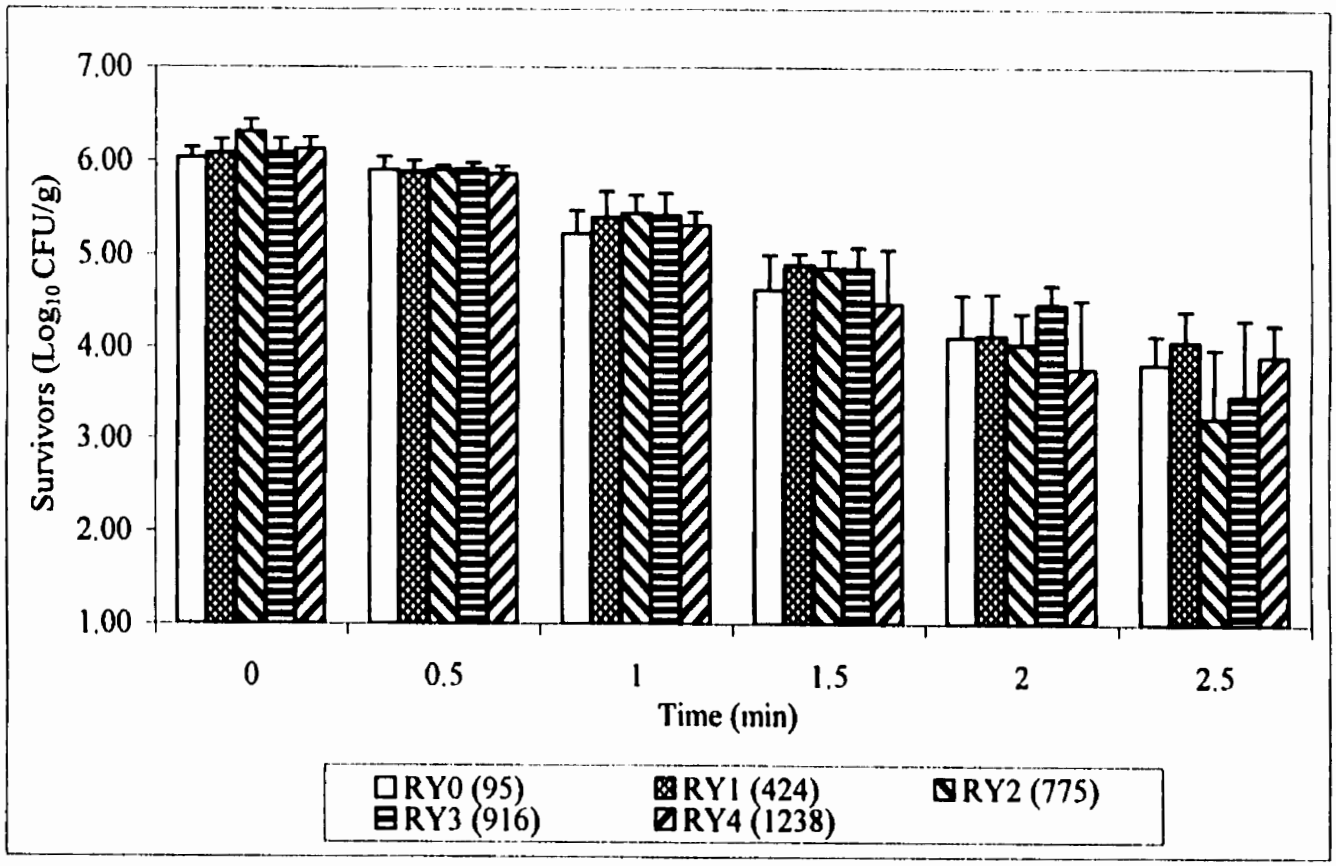


Figure 15. Thermal inactivation of *S. typhimurium* in regular egg yolk fortified with vitamin E at 56.5^0 C. Each bar and error bar represents the mean and standard deviation of 12 replicates, respectively (Appendix 16).

Table 8. Emulsion and mayonnaise stability in regular and omega-3 egg yolk

Functional properties [1]			Regular	Omega-3
Emulsion stability [2]	Emulsion separation (ml)	After 60 min	0.46 ± 0.09[a]	0.38 ± 0.01[a]
		After 120 min	1.40 ± 0.16[b]	1.48 ● 0.06[b]
Mayonnaise stability (%)[2]			0.30 ● 0.02[c]	0.30 ± 0.03[c]

[1] Functional properties following by the same superscript were not significantly different (P>0.05).

[2] Value represents the mean ± SD of 4 replicates.

5.0 DISCUSSION

Based on the results of this study, it would appeared that growth and/or survival of *S. typhimurium* in omega-3 and regular whole egg and egg yolk were similar under all conditions evaluated. This was also true for *E. coli* which has been isolated in 0.5-6% eggs (Barrow, 1994).

For both regular and omega-3 egg yolk and whole egg *S. typhimurium* numbers increased rapidly to 10^9 CFU/g by 48 h or 72 h, respectively, at 22^0 C in spite of approximately 7 times more total tocopherols in omega-3 egg yolk than in regular egg yolk. These results are in agreement with previous studies which indicated similar growth profiles for *Salmonella* in whole eggs and egg yolk at room temperature (Saeed and Koons, 1993; Schoeni et al. 1995). Saeed and Koons (1993) reported that *S. enteritidis* grew to 10^6 CFU/ml within 24 h and reached 10^9 CFU/ml within 72 h in homogenized whole eggs at 23^0 C. Furthermore, Schoeni et al. (1995) demonstrated that storage at 25^0 C for 3 days resulted in an increase from an initial inoculation level of 10^2 or 10^4 CFU/g to 10^8-10^9 CFU/g in egg yolk for three *Salmonella* strains - *S. enteritidis*, *S. typhimurium*, and *S. heidelberg*. In addition, Kobayashi et al. (1997) pointed out that *Salmonella* (*S. typhimurium, S. cerro, and S. infantis*) reached 10^8-10^9 CFU/g in liquid whole eggs and egg yolk after storing for as little as 24 h at 25^0 C.

When whole eggs were stored at 8^0 C, no significant differences in *Salmonella* numbers were observed between both types of eggs. Major differences in *Salmonella* populations between regular and omega-3 egg yolk were also not observed during storage at 8^0 C. Overall, salmonellae growth at 8^0 C during 4 weeks of incubation was poor in

both regular and omega-3 whole eggs and egg yolk. In contrast, Schoeni et al. (1995) reported that both *S. enteritidis* and *S. typhimurium* grew in egg yolk at 4^0 C. At a higher temperature, Curtis (2000) indicated that *S. enteritidis* numbers increased approximately 1 $\log_{10}$ in whole egg when stored at 10^0 C. Overall, at -20^0 C, there were no significant differences in *Salmonella* population levels between regular and omega-3 whole egg and egg yolk. The survival pattern of salmonellae at frozen temperature was also supported by Kobayashi et al. (1997) and Marshall (1998). Cotterill and Glauert (1972) indicated that *S. oranienburg* survived frozen storage through 28 weeks at -25^0 C.

Regardless of egg type, *E. coli* also showed similar growth and/or survival profiles in whole egg or egg yolk in most cases. Although few studies on generic *E. coli* in eggs have been performed, the growth and/or survival patterns of this microorganism are in agreement with previous studies involving *E coli* O157:H7. For instance, Yang and Chou (2000) reported that *E. coli* O157:H7 numbers increased to 10^9 CFU/g after 24 h of incubation at 22^0 C in egg products. When stored at -20^0 C, *E. coli* remained or slightly decreased in both regular and omega-3 whole egg and egg yolk. Ansay et al. (1999) also reported that *E. coli* O157:H7 numbers decreased approximately 1 $\log_{10}$ during frozen storage in ground beef. However, in this study, an increase of *E coli* numbers from 10^3 to 10^7 CFU/g in eggs was observed after incubating for 6 weeks at 8^0 C. This was contrary to those of Yang and Chou (2000) who reported no change in *E coli* O157:H7 numbers in egg products after 36 h at 5^0 C. The discrepancy may be attributed to the difference in the strain of *E coli* and duration of incubation.

S. typhimurium multiplied slowly in albumen during incubation at room temperature and was detected for up to 6 or 8 weeks at 8^0 C and -20^0 C, respectively.

Kobayashi et al. (1997) also reported only a 1 $\log_{10}$ increase for salmonellae in albumen at room temperature. Further, similar growth and/or survival patterns of *Salmonella* in albumen were observed by Schoeni et al. (1995) at 4^0 C, 10^0 C, and 25^0 C. However, the survival profiles for salmonellae at low temperature in this study were contrary to those of Lock and Board (1992). They reported that *S. typhimurium* did not persist at 6 weeks of storage at 4^0 C. It has been suggested that strains differences might cause variable responses among salmonellae during time–temperature experiments (Lock and Board, 1992). In the case of *E. coli*, levels rapidly decreased in albumen, regardless of storage temperature and egg type. Poor growth and/or survival of both bacteria may be attributed to the presence of antimicrobial factors in albumen such as lysozyme and ovotransferrin (Baron et al., 1997) and/or the alkali–sensitive nature of *E coli*. This is important since albumen pH increases to ca. 9.3 after storage in comparison to pH 7.2 in fresh laid egg albumen (Board et al., 1994). Although the alkali-sensitivity of *E coli* strain used in this study was not specifically characterized, it seems reasonable to suggest that rapid diminution of *E coli* in egg albumen may be attributed to alkali–sensitive *E coli*. Rowbury et al. (1996) indicated that the alkali tolerance of *E. coli* was partially strain dependent; in fact some *E coli* strains were more alkali–tolerant than others.

No differences regarding bacterial growth and/or survival were observed in response to tocopherol concentrations in whole egg and egg yolk. Concentrations of tocopherol were either too low or have no antimicrobial activity in eggs. For example, the lipid solubility of synthetic phenols was suggested to diminish the antimicrobial activity of phenols such as BHA in the presence of lipid-based foods (Raccach, 1984, Fung et al., 1985). In particular, the phytyl "tail" in the structure of tocopherol provides its totally

lipophilic activity (McCay, 1985; Burton and Trader, 1990). Thus, the impact of the absolute lipid solubility of tocopherol on antimicrobial activity needs further investigation.

Furthermore, studies have been demonstrated that γ- and δ-tocopherols have higher antioxidative activity *in vitro* in comparison to α-tocopherol which is a superb antioxidant in biological systems (Burton and Traber, 1990; Qi and Sim, 1998). In this study, fortification of vitamin E (diluted with corn oil) in regular egg yolks resulted in a moderate increase of α-tocopherol from 60 to 240 ppm and increased γ-tocopherol from 8 to about 800 ppm. The latter effect is presumably due to the fact that corn oil is known to contain much higher amounts of γ- than α-tocopherol (Eitenmiller, 1997). However, regardless of tocopherol type, concentration and presence/absence of oxygen in the growth environment, there was no difference in salmonellae growth. Under anoxic conditions, it would be assumed the presence of free tocopherols possibly act as antimicrobials.

The D value of *S. typhimurium* in regular egg yolk was not significantly different from that of omega-3 egg yolk. D values of salmonellae in regular egg yolk fortified with different tocopherol concentrations ranged from 0.88 min to 1.14 min. Using a similar procedure, Humphrey et al. (1990) determined that the D value for *S. typhimurium* in egg yolk at 55^0 C was 8 min. In addition, Palumbo et al. (1995) used glass vials to determine D values for *S. typhimurium* in egg yolk at 60^0 C which were calculated to be 0.67 min. The use of a capillary tube method to determine heat resistance most likely result in lower D values compared to a test tube method. For instance, Schuman and Sheldon (1997) demonstrated that the D value for *S. enteritidis* in

egg yolk at 60^0 C was 0.28 min, which was lower than 0.62-0.73 min using glass vials (Palumbo et al., 1995). Therefore, direct comparisons between studies are sometimes impossible due to differences in heating protocol (test tubes versus capillary tubes) and the bacterial strains.

The addition of antimicrobials to eggs also affect the heat resistance of bacteria. For instance, lowering the pH by adding lactic acid has been demonstrated to enhance thermal inactivation of salmonellae in eggs (Doyle and Mazzotta, 2000). Also, the D value for *S typhimurium* in whole egg with 500 ppm benzoic acid at 54^0 C was 6,5 min compared to 17 min (ICMFS, 1980).

In terms of functional properties of egg yolk, the emulsion capacity of omega-3 egg yolk was also not significantly different from that of regular egg yolk. These results are in agreement with previous studies (Jordan et al., 1962; Pankey and Stadelman, 1969; Van Elswyl et al., 1992), which reported the effect of dietary fats on the functional properties of egg yolk. Pankey and Stadelman (1969) reported that there was no effect of various dietary oils (10%) including corn, soybean, olive, or safflower oil on the emulsification capacity of egg yolk. Although Jordan et al. (1960) suggested that change of lipid type in the egg yolk could affect the functional properties, the examination of the emulsion capacity and mayonnaise stability of omega-3 egg yolk compared to regular egg yolk in this study did not support their theory.

Direct comparison among studies in terms of tocopherol content in egg yolk are often not possible due to differences in the dietary concentration and types of tocopherols, types of layers as well as different analysis techniques involved in the determination of tocopherol content. There were two aspects that were clearly consistent

with previous studies. First, omega-3 egg yolk was demonstrated to contain 5-12 times more α-tocopherol than regular egg yolk. Although it was not known if synthetic or natural dietary tocopherols were used in the production of omega-3 eggs, it was known that α-tocopherol has the greatest transfer rate in biological systems which results in the highest amount of α-tocopherol in egg yolk compared to other tocopherol isomers.

Tocopherol analysis was conducted with samples that were previously frozen (-20^0 C) for various time periods prior to lipid extraction, therefore, tocopherol contents in the egg yolk varied. For instance, α-tocopherol concentrations in the 8^0 C trial were higher in comparison to the other two trials performed at 20^0 C or -20^0 C. This difference can be attributed to 4 months shorter storage period. For example, Vidal-Valverde and Ruiz (1993) suggested that frozen storage from 4 to 8 months could result in losses in cow milk α-tocopherol. However, variation in tocopherol content could also result from difference in tocopherol deposition in egg yolk. For instance, fresh regular egg yolk from the retail outlet was analyzed to contain 55.38 ppm α-tocopherol, which was less than the value of 94.17 ppm in regular egg yolk from the 8^0 C trial after approximately 4 months frozen storage. Therefore, tocopherol level in egg yolk related to storage temperature and duration as well as its deposition in egg yolk still require further study.

6.0 CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

Based on the results of this study, it can be concluded that tocopherols exerted minimal effect on the growth and/or survival of selected pathogens and aerobic plate counts, and functional properties of omega-3 eggs when compared to regular eggs. Specifically, the growth and/or survival patterns of *S. typhimurium* and *E. coli* were similar for both omega-3 and regular eggs at 22^0 C, 8^0 C, and -20^0 C. In addition, growth of *S. typhmurium* either under aerobic or anoxic conditions at 22^0 C in egg yolk modified with approximately 63-240 ppm α tocopherol was not affected. Moreover, *S. typhimurium*, when heated in egg yolk at 56.5^0 C showed similar heat resistance (D values) regardless of α-tocopherol concentration (55-713 ppm) or total tocopherol (92-1238 ppm).

It is recommended that additional work in regards to the microbiological properties of omega-3 eggs is not warranted from a food safety perspective.

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APPENDICES

Appendix 1. Aerobic plate counts (SPC) in regular and omega-3 whole eggs.

Storage Temperature	Sample	Storage Time	SPC (log_{10}CFU/g) Trial 1		SPC (log_{10}CFU/g) Trial 2		SPC Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	2.02	1.88	2.02	1.89	1.95	0.08
	whole	24 hr	5.05	5.12	4.66	5.23	5.02	0.25
	egg	48 hr	6.91	7.07	6.97	6.83	6.94	0.10
		72 hr	7.88	8.08	7.89	7.88	7.93	0.10
	Omega-3	0 hr	1.26	1.30	1.60	1.52	1.42	0.17
	whole	24 hr	3.87	4.63	3.25	3.62	3.84	0.58
	egg	48 hr	5.20	5.20	5.00	4.86	5.06	0.16
		72 hr	6.74	6.63	6.41	6.30	6.52	0.20
8^0 C	Regular	0 wk	2.11	2.12	2.26	2.24	2.18	0.08
	whole	2 wk	4.05	4.06	3.40	3.30	3.71	0.41
	egg	4 wk	3.88	3.82	3.74	3.97	3.85	0.10
		6 wk	5.94	5.96	6.08	6.16	6.04	0.10
	Omega-3	0 wk	2.12	2.03	2.10	2.26	2.13	0.10
	whole	2 wk	3.83	3.79	3.67	3.41	3.67	0.18
	egg	4 wk	3.28	3.53	3.59	3.38	3.45	0.14
		6 wk	2.92	3.07	2.91	3.02	2.98	0.08

Appendix 2. Aerobic plate counts in regular and omega-3 egg yolk.

Storage Temperature	Sample	Storage Time	SPC (log_{10}CFU/g) Trial 1		Trial 2		SPC Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	2.11	2.12	1.97	2.05	2.06	0.07
	egg yolk	24 hr	6.78	6.90	7.02	6.98	6.92	0.11
		48 hr	8.44	8.31	8.20	8.31	8.32	0.10
	Omega-3	0 hr	2.96	2.88	2.57	2.40	2.70	0.26
	egg yolk	24 hr	7.32	7.26	7.18	7.24	7.25	0.06
		48 hr	8.29	8.35	8.37	8.40	8.35	0.05
8^0 C	Regular	0 wk	1.72	1.88	1.90	1.80	1.83	0.08
	egg yolk	2 wk	2.85	1.60	3.11	2.79	2.59	0.67
		4 wk	3.80	3.40	2.36	3.32	3.22	0.61
		6 wk	4.90	4.92	4.33	4.23	4.60	0.36
	Omega-3	0 wk	0.70	0.70	0.70	0.48	0.64	0.11
	egg yolk	2 wk	0.48	1.36	1.96	2.04	1.46	0.72
		4 wk	2.99	3.67	2.69	2.36	2.93	0.56
		6 wk	5.97	5.73	6.30	6.06	6.02	0.24

Appendix 3. Aerobic plate counts in regular and omega-3 egg albumen.

Storage Temperature	Sample	Storage Time	SPC (log_{10}CFU/g) Trial 1		Trial 2		SPC Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	1.97	2.17	1.86	1.99	2.00	0.13
	egg	24 hr	1.45	1.52	1.52	1.60	1.52	0.06
	albumen	48 hr	1.30	1.18	1.54	1.30	1.33	0.15
		72 hr	1.08	1.30	1.37	1.01	1.19	0.17
	Omega-3	0 hr	1.94	2.08	2.07	1.89	2.00	0.09
	egg	24 hr	2.08	2.08	1.93	2.10	2.05	0.08
	albumen	48 hr	1.83	1.94	1.83	1.90	1.88	0.06
		72 hr	1.48	1.58	1.85	1.72	1.66	0.16
8^0 C	Regular	0 wk	1.60	1.68	2.00	1.99	1.82	0.21
	egg	2 wk	1.11	1.00	1.40	1.30	1.20	0.18
	albumen	4 wk	0.95	0.78	1.04	1.08	0.96	0.13
		6 wk	0.48	0.60	1.48	0.00	0.64	0.62
	Omega-3	0 wk	0.48	1.00	ND*	1.18	0.88	0.36
	egg	2 wk	0.48	0.70	0.48	0.48	0.53	0.11
	albumen	4 wk	0.00	ND*	0.30	0.30	0.20	0.17
		6 wk	0.00	0.00	0.00	ND*	0.00	0.00

ND*: Not detection by direct plating.

Appendix 4. Growth and/or survival of *S. typhimurium* in regular and omega-3 whole eggs.

Storage Temperature	Sample	Storage Time	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	2.23	2.11	2.00	2.18	2.13	0.10
	whole	24 hr	5.03	5.10	5.63	5.48	5.31	0.29
	egg	48 hr	7.95	8.09	8.25	8.19	8.12	0.13
		72 hr	9.24	9.26	9.28	9.14	9.23	0.06
	Omega-3	0 hr	2.20	1.60	2.00	1.90	1.93	0.25
	whole	24 hr	4.41	4.15	5.59	5.38	4.88	0.71
	egg	48 hr	8.29	7.97	8.48	8.45	8.30	0.23
		72 hr	9.28	9.27	9.16	9.29	9.25	0.06
8^0 C	Regular	0 wk	3.16	3.06	3.00	3.06	3.07	0.06
	whole	1 wk	2.86	2.89	2.96	3.21	2.98	0.16
	egg	2 wk	3.44	3.34	3.18	3.45	3.35	0.12
		3 wk	3.08	3.27	2.77	3.06	3.05	0.21
		4 wk	4.00	4.16	2.79	2.71	3.42	0.77
		6 wk	6.56	6.61	5.00	5.17	5.84	0.87
	Omega-3	0 wk	3.00	3.00	3.01	3.08	3.02	0.04
	whole	1 wk	3.06	3.02	2.96	3.13	3.04	0.07
	egg	2 wk	3.61	3.59	3.84	3.84	3.72	0.14
		3 wk	4.12	4.13	3.23	3.28	3.69	0.50
		4 wk	3.07	3.05	4.12	4.10	3.58	0.61
		6 wk	6.00	5.94	6.70	6.68	6.33	0.41
-20^0 C	Regular	0 wk	2.23	2.11	2.18	2.04	2.14	0.08
	whole	2 wk	1.62	1.34	1.51	1.18	1.41	0.19
	egg	4 wk	1.45	1.26	1.18	1.00	1.22	0.19
		6 wk	1.41	1.38	1.45	1.38	1.41	0.03
		8 wk	1.34	1.20	1.20	1.20	1.24	0.07
	Omega-3	0 wk	2.20	1.60	2.00	1.90	1.93	0.25
	whole	2 wk	1.89	1.85	1.93	1.78	1.86	0.07
	egg	4 wk	1.65	1.70	1.70	1.40	1.61	0.14
		6 wk	1.81	1.83	1.70	1.75	1.77	0.06
		8 wk	1.58	1.56	1.78	1.45	1.59	0.14

Appendix 5. Growth and/or survival of *E. coli* in regular and omega-3 whole eggs.

Storage Temperature	Sample	Storage Time	*E. coli* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation
22° C	Regular	0 hr	2.08	2.11	2.11	2.20	2.13	0.05
	whole	24 hr	5.62	5.58	5.70	5.85	5.69	0.12
	egg	48 hr	7.90	8.46	8.48	8.43	8.32	0.28
		72 hr	8.81	8.96	9.08	9.15	9.00	0.15
	Omega-3	0 hr	2.15	2.12	2.06	2.13	2.11	0.04
	whole	24 hr	5.81	5.83	5.40	5.34	5.59	0.26
	egg	48 hr	8.38	8.34	8.18	8.40	8.32	0.10
		72 hr	8.88	8.96	8.52	8.32	8.67	0.30
8° C	Regular	0 wk	2.91	2.85	2.86	2.83	2.86	0.03
	whole	1 wk	4.15	4.12	4.21	4.30	4.19	0.08
	egg	2 wk	6.96	7.01	5.02	4.95	5.98	1.15
		3 wk	7.21	7.26	7.48	7.42	7.34	0.13
		4 wk	6.62	6.38	7.25	7.24	6.87	0.44
		6 wk	7.05	6.98	7.05	7.00	7.02	0.03
	Omega-3	0 wk	2.91	2.81	2.81	2.87	2.85	0.05
	whole	1 wk	4.17	4.32	3.95	4.03	4.12	0.16
	egg	2 wk	5.96	5.70	6.63	6.65	6.23	0.48
		3 wk	7.62	7.59	7.53	7.47	7.55	0.07
		4 wk	7.32	7.40	7.35	7.30	7.35	0.04
		6 wk	6.90	6.97	7.39	7.43	7.17	0.28
-20° C	Regular	0 wk	2.08	2.11	2.11	2.20	2.13	0.05
	whole	2 wk	1.91	1.93	1.94	1.99	1.94	0.03
	egg	4 wk	1.96	1.95	1.88	1.92	1.93	0.04
		6 wk	1.90	1.91	1.92	1.94	1.92	0.02
		8 wk	1.92	1.92	2.03	1.97	1.96	0.05
	Omega-3	0 wk	2.15	2.12	2.06	2.13	2.11	0.04
	whole	2 wk	1.95	1.99	2.00	1.96	1.98	0.02
	egg	4 wk	1.97	1.93	1.18	1.15	1.56	0.46
		6 wk	1.97	1.98	1.85	1.76	1.89	0.10
		8 wk	1.81	1.84	1.73	1.81	1.80	0.05

Appendix 6. Survival of *E. coli* in regular and omega-3 egg albumen.

Storage Temperature	Sample	Storage Time	*E. coli* (log_{10}CFU/g) Trial 1		Trial 2	
22^0 C	Regular egg albuen	0 hr	1.48	1.45	1.52	1.72
		24 hr	0.00	0.00	0.00	0.00
		48 hr	NDP[1]	NDP[1]	NDP[1]	NDP[1]
		72 hr	DE[2]		NDE[3]	
	Omega-3 egg albumen	0 hr	1.48	1.70	1.36	1.54
		24 hr	0.00	0.00	0.00	NDP[1]
		48 hr	NDP[1]	0.00	NDP[1]	NDP[1]
		72 hr	NDP[1]	0.00	DE[2]	
8^0 C	Regular egg albumen	0 wk	2.92	2.93	2.83	2.90
		1 wk	0.90	0.48	NDP[1]	NDP[1]
		2 wk	DE[2]		NDE[3]	
		3 wk	NDE[3]		NDE[3]	
		4 wk		NP*		
		6 wk		NP*		
	Omega-3 egg albumen	0 wk	2.89	2.83	2.83	2.89
		1 wk	0.70	NDP[1]	0.70	NDP[1]
		2 wk	NDE[3]		NDE[3]	
		3 wk		NP*		
		4 wk		NP*		
		6 wk		NP*		
-20^0 C	Regular egg albumen	0 wk	1.48	1.45	1.52	1.72
		2 wk	NDE[3]		NDE[3]	
		4 wk		NP*		
		6 wk		NP*		
		8 wk		NP*		
	Omega-3 egg albumen	0 wk	1.48	1.70	1.36	1.54
		2 wk	NDE[3]		NDE[3]	
		4 wk		NP*		
		6 wk		NP*		
		8 wk		NP*		

NDP[1]: Not detected by direct plating.

DE[2]: Detected by enrichment procedure.

NDE[3]: Not detected by enrichment procedure

NP*: Not performed.

Appendix 7. Growth and/or survival of *S. typhimurium* in regular and omega-3 egg albumen.

Storgae Temperature	Sample	Storage Time	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	2.04	2.11	1.90	1.60	1.92	0.23
	egg	24 hr	1.90	1.88	1.94	2.01	1.93	0.06
	albuen	48 hr	1.60	1.60	2.49	1.85	1.89	0.42
		72 hr	2.25	1.72	1.99	1.85	1.95	0.23
	Omega-3	0 hr	1.70	1.85	1.60	1.90	1.76	0.14
	egg	24 hr	1.97	1.94	1.97	1.99	1.97	0.02
	albumen	48 hr	2.04	2.38	2.34	3.20	2.49	0.50
		72 hr	2.58	2.90	2.05	2.62	2.54	0.36
8^0 C	Regular	0 wk	2.86	2.85	2.79	2.79	2.82	0.04
	egg	1 wk	2.07	2.08	2.03	1.97	2.04	0.05
	albumen	2 wk	1.45	1.54	1.65	1.68	1.58	0.11
		3 wk	1.26	1.36	1.40	1.18	1.30	0.10
		4 wk	0.90	1.00	0.90	0.00	0.70	0.47
		6 wk	0.30	0.85	0.48	0.90	0.63	0.29
	Omega-3	0 wk	3.05	3.00	3.00	3.01	3.01	0.03
	egg	1 wk	2.09	1.93	1.68	1.88	1.89	0.17
	albumen	2 wk	1.36	1.26	1.30	1.36	1.32	0.05
		3 wk	1.00	1.26	1.40	1.30	1.24	0.17
		4 wk	0.70	0.60	ND*	0.00	0.43	0.38
		6 wk	0.70	0.48	0.48	0.30	0.49	0.16
-20^0 C	Regular	0 wk	2.02	1.94	1.90	1.91	1.94	0.05
	egg	2 wk	1.40	1.48	1.23	1.26	1.34	0.12
	albumen	4 wk	1.00	0.90	0.48	0.30	0.67	0.33
		6 wk	ND*	0.30	0.60	ND*	0.45	0.21
		8 wk	0.00	ND*	0.30	ND*	0.15	0.21
	Omega-3	0 wk	1.90	1.93	1.73	1.79	1.84	0.09
	egg	2 wk	1.52	1.65	1.28	1.38	1.46	0.16
	albumen	4 wk	1.11	1.11	1.11	1.04	1.10	0.04
		6 wk	0.70	0.48	0.78	0.48	0.61	0.15
		8 wk	0.70	0.00	0.30	0.48	0.37	0.30

ND*: Not detected by direct plating.

Appendix 8. Growth of *S. typhimurium* and α-tocopherol content in regular and omega-3 egg yolk at 22^0 C.

Sample	Storage Time (hr)	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation	α-tocopherol/egg yolk (ppm) Trial 1	Trial 2	Average (ppm)	Standard Deviation
Regular	0	3.06	3.06	2.97	3.17	3.06	0.08	54.34	51.08	52.71	2.31
egg yolk	24	8.59	8.34	8.48	8.23	8.41	0.16	56.01	54.43	55.22	1.12
	48	9.44	9.41	9.26	9.44	9.39	0.09	50.37	45.77	48.07	3.25
Omega-3	0	2.84	2.89	2.83	2.82	2.84	0.03	388.52	385.35	386.94	2.24
egg yolk	24	7.91	8.09	8.30	8.19	8.12	0.16	389.35	391.58	390.47	1.58
	48	9.34	9.36	9.31	9.20	9.30	0.07	423.30	398.01	410.66	17.88

Appendix 9. Survival of *S. typhimurium* and α-tocopherol content in regular and omega-3 egg yolk at -20^0 C.

Sample	Storage Time (wk)	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation	α-tocopherol/egg yolk (ppm) Trial 1	Trial 2	Average (ppm)	Standard Deviation
Regular egg yolk	0	3.15	3.27	3.13	3.19	3.18	0.06	54.34	51.08	52.71	2.31
	2	2.48	2.44	2.31	2.56	2.45	0.11	59.27	55.48	57.38	2.68
	4	1.97	2.04	1.86	1.83	1.93	0.10	53.64	57.67	55.66	2.85
	6	1.82	1.84	1.96	1.90	1.88	0.06	54.73	51.62	53.18	2.20
	8	1.76	1.68	1.68	1.79	1.73	0.05	54.36	63.74	59.05	6.63
Omega-3 egg yolk	0	2.97	3.23	3.19	3.14	3.13	0.11	388.52	385.35	386.94	2.24
	2	2.23	2.34	1.98	2.10	2.16	0.16	414.42	376.63	395.53	26.72
	4	1.97	1.86	2.10	1.93	1.96	0.10	405.44	282.31	343.88	87.07
	6	1.89	1.77	1.88	1.92	1.87	0.07	334.39	333.54	333.97	0.60
	8	1.53	1.76	1.70	1.74	1.68	0.10	335.98	331.31	333.65	3.30

Appendix 10. Growth of *S. typhimurium* and α-tocopherol in regular and omega-3 egg yolk at 8^0 C.

Sample	Storage Time (wk)	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation	α-tocopherol/egg yolk (ppm) Trial 1	Trial 2	Average (ppm)	Standard Deviation
Regular egg yolk	0	3.11	3.05	3.16	3.23	3.14	0.07	93.37	98.64	96.01	3.73
	1	2.91	3.00	3.17	3.09	3.04	0.11	92.42	101.58	97.00	6.48
	2	3.45	3.53	3.76	3.63	3.59	0.13	93.46	85.94	89.70	5.32
	3	3.38	3.37	3.96	4.02	3.68	0.36	97.15	98.12	97.64	0.69
	4	4.09	4.09	3.94	4.14	4.06	0.09	91.17	86.32	88.75	3.43
	6	4.45	4.42	4.78	4.65	4.57	0.17	100.29	91.59	95.94	6.15
Omega-3 egg yolk	0	3.11	3.08	3.20	3.05	3.11	0.06	490.98	521.86	506.42	21.84
	1	2.97	2.79	2.86	2.79	2.85	0.08	505.13	501.90	503.52	2.28
	2	3.36	3.30	3.03	3.15	3.21	0.15	468.84	475.89	472.37	4.99
	3	3.08	3.37	3.24	3.20	3.22	0.12	471.72	480.78	476.25	6.41
	4	3.53	3.53	4.13	4.00	3.80	0.31	479.14	483.93	481.54	3.39
	6	5.20	5.30	4.20	4.18	4.72	0.61	508.87	381.69	445.28	89.93

Appendix 11. Growth and/or survival of *E. coli* in regular and omega-3 egg yolk.

Storage Temperature	Sample	Storage Time	*E. coli* (log_{10}CFU/g) Trial 1		Trial 2		Average (log_{10}CFU/g)	Standard Deviation
22^0 C	Regular	0 hr	1.99	1.95	2.09	1.97	2.00	0.06
	egg yolk	24 hr	7.91	7.90	7.89	7.74	7.86	0.08
		48 hr	9.05	8.96	9.11	9.00	9.03	0.07
	Omega-3	0 hr	2.03	1.89	1.94	1.97	1.96	0.06
	egg yolk	24 hr	7.89	7.91	7.68	7.90	7.84	0.11
		48 hr	8.94	9.03	8.94	8.82	8.93	0.09
8^0 C	Regular	0 wk	3.10	3.01	2.95	2.96	3.01	0.07
	egg yolk	1 wk	6.67	6.67	6.74	6.81	6.72	0.07
		2 wk	7.94	7.88	7.88	8.02	7.93	0.07
		3 wk	7.95	8.00	7.89	7.86	7.93	0.06
		4 wk	7.91	7.93	7.93	7.95	7.93	0.01
		6 wk	7.46	7.75	7.63	7.63	7.62	0.12
	Omega-3	0 wk	2.94	3.08	3.16	3.01	3.05	0.09
	egg yolk	1 wk	6.48	6.47	6.53	6.53	6.50	0.03
		2 wk	7.80	7.91	7.84	7.87	7.85	0.05
		3 wk	7.76	7.89	7.81	7.79	7.81	0.05
		4 wk	7.81	7.89	7.79	7.86	7.83	0.05
		6 wk	7.54	7.85	7.34	7.43	7.54	0.22
-20^0 C	Regular	0 wk	1.99	1.95	2.09	1.97	2.00	0.06
	egg yolk	2 wk	2.05	1.94	1.93	1.89	1.95	0.07
		4 wk	1.68	1.81	1.72	1.81	1.76	0.07
		6 wk	1.89	1.78	1.72	1.67	1.76	0.10
		8 wk	1.32	1.30	1.11	1.34	1.27	0.11
	Omega-3	0 wk	2.03	1.89	1.94	1.97	1.96	0.06
	egg yolk	2 wk	1.79	1.75	1.84	1.78	1.79	0.04
		4 wk	1.74	1.71	1.58	1.62	1.66	0.07
		6 wk	1.49	1.68	1.48	1.46	1.53	0.10
		8 wk	1.23	1.36	1.18	1.32	1.27	0.08

Appendix 12. Growth of *S. typhimurium* in regular egg yolk fortified with vitamin E at 22^0 C under aerobic and anoxic conditions.

Growth Condition	Sample	Storage Time (hr)	*S. typhimurium* (log_{10}CFU/g) Trial 1		Trial 2		Average	Standard Deviation
Aerobic	Regular egg	0	3.23	3.30	3.23	3.25	3.25	0.03
	yolk (control)	24	9.14	9.14	9.17	9.18	9.16	0.02
	Regular egg	0	3.26	3.22	3.21	3.20	3.22	0.03
	yolk (RY1)	24	9.08	9.06	9.15	9.04	9.08	0.05
	Regular egg	0	3.31	3.15	3.37	3.35	3.29	0.10
	yolk (RY2)	24	9.01	9.06	9.08	9.12	9.07	0.05
	Regular egg	0	3.24	3.26	3.21	3.28	3.25	0.03
	yolk (RY3)	24	9.01	9.07	9.12	9.08	9.07	0.05
Anoxic	Regular egg	0	1.72	1.76	1.78	1.74	1.75	0.02
	yolk (control)	24	7.85	7.80	8.09	8.09	7.96	0.16
		48	9.24	9.40	9.30	9.26	9.30	0.07
	Regular egg	0	1.80	1.63	1.58	1.40	1.60	0.17
	yolk (RY1)	24	7.79	7.90	8.00	7.90	7.90	0.09
		48	9.30	9.24	9.24	9.29	9.27	0.03
	Regular egg	0	1.76	1.60	1.68	1.80	1.71	0.09
	yolk (RY2)	24	7.74	7.79	7.90	7.92	7.84	0.09
		48	9.43	9.29	9.37	9.33	9.36	0.06
	Regular egg	0	1.65	1.80	1.63	1.70	1.70	0.07
	yolk (RY3)	24	7.90	7.72	7.76	7.72	7.78	0.08
		48	9.25	9.29	9.31	9.19	9.26	0.05

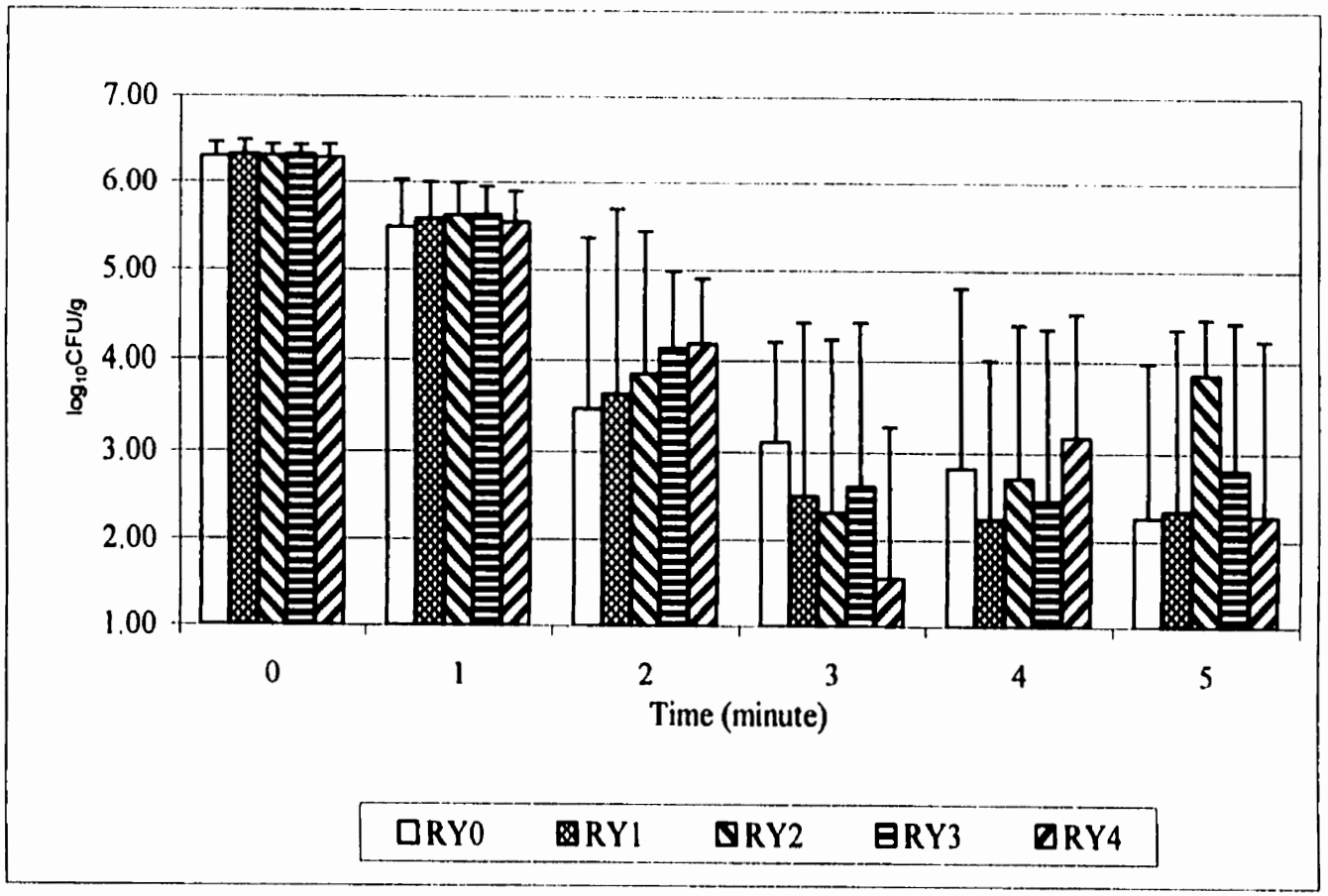
Appendix 13. Thermal inactivation of *S. typhimurium* in regular and omega-3 egg yolk at 56.5^0 C.

Sample	Time (min)	*S. typhimurium* (log_{10}CFU/g)												Average	Standard Deviation
		Trial 1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6			
Regular egg yolk	0	6.60	6.60	6.47	6.41	6.11	6.20	6.13	6.20	6.38	6.31	6.39	6.43	6.35	0.17
	1	5.57	5.63	5.68	5.62	5.83	5.90	5.90	5.83	5.97	6.00	6.07	6.07	5.84	0.18
	2	3.60	3.60	3.94	3.88	4.30	4.54	4.58	4.51	4.80	4.85	4.99	5.02	4.38	0.52
	3	4.07	3.98	4.28	4.25	3.45	3.18	2.95	1.78	3.48	3.45	4.22	4.28	3.61	0.74
	4	4.69	4.73	3.95	3.96	1.78	1.78	3.43	3.33	2.89	2.82	3.53	3.56	3.37	0.95
	5	3.64	3.65	4.22	4.14	3.98	3.97	3.59	3.21	4.33	4.35	1.78	1.78	3.55	0.89
Omega-3 egg yolk	0	6.49	6.51	6.45	6.63	6.38	6.35	6.30	6.22	6.33	6.37	6.42	6.38	6.40	0.11
	1	5.65	5.64	5.27	5.25	6.05	6.05	6.05	6.02	6.08	6.09	6.05	6.02	5.85	0.32
	2	4.12	4.08	2.60	2.60	4.58	4.21	4.26	4.20	5.20	5.17	5.09	5.06	4.26	0.89
	3	3.75	3.72	2.08	2.48	4.35	4.36	2.48	2.56	2.92	3.18	3.78	3.72	3.28	0.77
	4	4.26	4.14	3.24	3.30	3.41	3.97	3.59	3.21	4.07	4.00	3.38	3.49	3.67	0.39
	5	4.54	4.51	3.32	3.19	3.50	3.48	3.60	3.33	3.06	3.26	2.08	1.78	3.30	0.80

Appendix 14. Thermal inactivation of *S. typhimurium* in regular egg yolk fortified with vitamin E at 56.5^0 C (5min duration)

Sample	Time (min)	*S. typhimurium* (log_{10}CFU/g)												Average	Standard Deviation
		Trial1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6			
RY0	0	6.23	6.28	6.45	6.35	6.59	6.38	6.43	6.39	6.13	6.11	6.20	6.05	6.30	0.16
	1	6.24	6.30	6.13	6.14	5.29	5.20	5.27	5.30	4.85	5.08	4.92	5.11	5.49	0.55
	2	5.34	5.25	5.45	5.49	0.00	2.90	2.90	0.00	3.62	3.68	3.58	3.46	3.47	1.89
	3	3.08	3.23	4.56	4.28	1.78	1.78	1.78	2.08	3.14	2.98	4.32	4.35	3.11	1.08
	4	4.24	4.34	1.78	0.00	2.48	2.38	4.96	5.01	4.32	4.34	0.00	0.00	2.82	1.99
	5	2.08	1.78	1.78	0.00	2.26	2.26	3.73	3.71	0.00	0.00	4.77	4.74	2.26	1.72
RY1	0	6.23	6.25	6.43	6.50	6.36	6.48	6.64	6.33	6.22	6.12	6.18	6.18	6.33	0.16
	1	6.13	6.24	6.14	6.04	5.44	5.47	5.34	5.31	5.29	5.18	5.22	5.15	5.58	0.42
	2	5.44	5.43	5.21	5.21	2.60	0.00	5.52	5.37	2.51	0.00	3.11	3.11	3.63	2.07
	3	3.06	3.03	3.94	3.06	0.00	0.00	3.80	3.76	4.62	4.67	0.00	0.00	2.50	1.92
	4	4.48	4.36	0.00	0.00	2.62	2.86	0.00	0.00	2.82	2.38	3.60	3.69	2.23	1.77
	5	2.08	0.00	0.00	0.00	0.95	0.00	4.22	4.44	3.59	3.65	4.53	4.54	2.33	2.02
RY2	0	6.39	6.49	6.43	6.20	6.38	6.38	6.34	6.45	6.15	6.15	6.15	6.20	6.31	0.13
	1	6.13	6.25	6.04	6.05	5.51	5.41	5.49	5.32	5.16	5.30	5.36	5.36	5.62	0.38
	2	5.41	5.29	5.63	5.58	2.60	0.00	3.64	3.64	3.20	3.28	3.90	4.06	3.85	1.59
	3	3.01	3.19	4.50	4.60	0.00	0.00	1.78	2.08	0.00	0.00	4.28	4.26	2.31	1.92
	4	2.78	2.78	2.26	2.56	1.78	0.00	0.00	1.78	4.72	4.75	4.61	4.43	2.70	1.69
	5	4.18	4.15	4.56	4.44	2.92	2.78	3.31	3.35	4.16	4.06	4.22	4.21	3.86	0.60
RY3	0	6.36	6.49	6.44	6.21	6.35	6.21	6.46	6.38	6.26	6.15	6.23	6.31	6.32	0.11
	1	6.16	6.06	6.05	5.96	5.36	5.48	5.35	5.38	5.43	5.39	5.48	5.41	5.63	0.32
	2	5.26	5.28	5.20	5.35	3.08	3.30	3.56	3.75	3.71	3.71	3.74	3.51	4.12	0.87
	3	4.21	4.24	2.78	2.48	2.78	2.82	0.00	2.26	4.89	4.87	0.00	0.00	2.61	1.81
	4	0.00	0.00	4.66	4.31	2.68	2.56	0.00	0.00	3.45	3.23	4.23	4.20	2.44	1.91
	5	1.78	0.00	2.95	2.56	4.55	4.62	3.97	4.03	3.72	3.73	0.00	1.78	2.81	1.62

Sample	Time (min)	*S. typhimurium* (log_{10}CFU/g)												Average	Standard Deviation
		Trial1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6			
RY4	0	6.36	6.24	6.22	6.18	6.40	6.54	6.51	6.38	6.20	6.20	6.04	6.16	6.29	0.15
	1	5.98	5.93	6.01	6.07	5.42	5.44	5.52	5.37	5.21	5.21	5.22	5.07	5.54	0.36
	2	5.38	5.26	4.76	4.21	3.83	3.92	4.58	4.50	3.68	3.28	3.41	3.28	4.17	0.73
	3	2.73	2.56	2.56	2.56	0.00	0.00	0.00	0.00	0.00	0.00	4.12	4.20	1.56	1.72
	4	0.00	1.78	3.74	3.68	4.27	4.41	2.78	2.95	4.56	4.49	2.62	2.82	3.18	1.34
	5	4.32	4.47	2.08	1.78	0.00	0.00	2.56	2.89	0.00	0.00	4.64	4.59	2.28	1.94



Appendix 15. Thermal inactivation of *S. typhimurium* in regular egg yolk fortified with vitamin E at 56.5^0 C (5min duration).

Appendix 16. Thermal inactivation of *S. typhimurium* in regular egg yolk fortified with vitamin E at 56.5^0 C (2.5min duration).

Sample	Time (min)	*S. typhimurium* (log_{10}CFU/g)												Average	Standard Deviation
		Trial 1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6			
RY0	0	6.05	6.02	6.03	5.98	6.05	6.19	6.23	6.12	5.87	5.98	5.97	5.87	6.03	0.11
	0.5	5.81	5.88	5.92	5.94	6.08	6.07	6.00	6.02	5.86	5.87	5.71	5.63	5.90	0.14
	1	5.54	5.59	5.32	5.25	5.25	5.27	5.34	5.33	5.17	5.04	4.83	4.74	5.22	0.25
	1.5	4.90	4.92	5.01	4.97	4.80	4.74	4.69	4.66	3.97	3.85	4.53	4.39	4.62	0.38
	2	4.24	4.13	4.86	4.84	4.26	4.20	4.19	4.23	3.62	3.62	3.41	3.62	4.10	0.46
	2.5	3.61	3.66	3.74	3.73	3.61	3.62	3.65	3.63	3.75	3.73	4.47	4.50	3.81	0.32
RY1	0	6.02	6.11	6.02	6.16	6.18	6.29	6.22	6.21	6.11	5.87	5.81	5.94	6.08	0.15
	0.5	5.84	5.85	5.86	5.85	6.05	5.99	5.95	5.96	5.93	5.83	5.82	5.59	5.88	0.12
	1	5.71	5.73	5.68	5.73	5.30	5.25	5.39	5.45	5.32	5.23	5.17	4.89	5.40	0.27
	1.5	5.13	5.12	5.01	4.80	4.81	4.79	4.85	4.84	4.80	4.76	4.90	4.86	4.89	0.13
	2	4.42	4.50	4.13	4.05	4.28	4.39	4.22	4.28	3.11	3.28	4.44	4.37	4.12	0.45
	2.5	4.39	4.37	3.96	3.94	3.65	3.61	3.68	3.70	4.38	4.34	4.36	4.32	4.06	0.33
RY2	0	6.12	5.94	6.00	6.08	6.29	6.11	6.30	6.21	5.92	6.11	6.02	5.94	6.31	0.13
	0.5	5.93	5.91	5.92	5.98	5.90	5.94	5.93	5.90	5.88	5.80	5.89	5.81	5.90	0.05
	1	5.72	5.66	5.67	5.64	5.18	5.25	5.36	5.41	5.43	5.39	5.46	5.17	5.45	0.19
	1.5	5.01	5.05	5.07	5.06	4.81	4.81	4.80	4.78	4.93	4.83	4.70	4.40	4.85	0.19
	2	4.37	4.29	4.33	4.22	4.14	4.10	4.24	4.14	3.76	3.78	3.71	3.28	4.03	0.33
	2.5	3.14	3.08	2.78	2.92	3.49	3.61	3.66	3.68	4.21	4.21	1.90	1.90	3.22	0.76
RY3	0	6.08	6.11	6.16	6.18	6.24	6.04	6.23	6.31	5.87	5.94	5.81	5.94	6.08	0.16
	0.5	5.97	5.97	5.89	5.91	5.99	5.99	5.95	5.92	5.84	5.80	5.89	5.83	5.91	0.06
	1	5.81	5.77	5.59	5.57	5.27	5.26	5.41	5.39	5.36	5.28	5.39	5.00	5.43	0.23
	1.5	5.20	5.18	5.06	4.86	4.94	4.87	4.95	4.87	4.68	4.60	4.61	4.46	4.86	0.23
	2	4.73	4.78	4.71	4.61	4.30	4.36	4.28	4.24	4.54	4.50	4.24	4.15	4.45	0.22
	2.5	4.36	4.33	3.18	3.18	3.57	3.64	3.68	3.64	1.90	1.90	4.09	4.10	3.46	0.83

Sample	Time (min)	*S. typhimurium* (log_{10}CFU/g)												Average	Standard Deviation
		Trial 1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6			
RY4	0	6.16	6.11	6.02	6.12	6.30	6.21	6.26	6.28	5.97	6.00	5.87	6.13	6.12	0.13
	0.5	5.85	5.92	5.85	5.71	5.93	5.90	5.94	5.92	5.86	5.70	5.88	5.81	5.86	0.08
	1	5.53	5.49	5.28	5.37	5.37	5.29	5.28	5.23	5.32	5.34	5.32	4.98	5.32	0.14
	1.5	4.86	4.78	4.85	4.75	4.81	4.75	4.70	4.76	4.42	4.39	3.51	2.98	4.46	0.60
	2	4.36	4.48	4.47	4.39	4.13	4.18	3.81	4.12	2.51	2.81	2.81	2.98	3.75	0.75
	2.5	3.80	3.83	4.33	4.31	3.62	3.58	3.69	3.67	3.63	3.54	4.40	4.36	3.90	0.35