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SHELF-LIFE EXTENSION STUDIES ON AN OMEGA-3 ENRICHED BREAKFAST CEREAL

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Abstract

Shelf-life Extension Studies on an Omega-3 Enriched Breakfast Cereal

Numerous nutritional studies over the past 20 years have found omega-3 fatty acids (ω3FA), which include linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, to be health-promoting. However, a shelf-life problem exists with all ω3FA since they are highly susceptible to lipid oxidation (or oxidative rancidity). The application of techniques for slowing this deteriorative process is essential if omega-3 enriched products are to be successful in the marketplace.

A granola-type, omega-3-rich breakfast cereal prototype was developed using ground flaxseed as the principal source of linolenic acid (1.8% w/w). Other ingredients included rolled oats, yellow sugar, honey, sliced almonds, and canola oil. The focus of the research investigation was to apply and study the addition of an antioxidant (AO) and the use of Modified Atmosphere Packaging (MAP) on shelf-life extension of the enriched cereal. Granola samples, both with and without AO (300ppm of 70% mixed tocopherols), were packaged in air, and under the two atmospheres achieved by nitrogen flushing (MA_1) and an oxygen scavenger (MA_2). Samples were stored at either 21 or 35°C.

Shelf-life was terminated when products reached a thiobarbituric acid (TBA) value of 4.0 mg malonaldehyde/kg corresponding to a sensory score of 5 (on a hedonic scale of 10). Based on these criteria, the shelf-life of air-packaged products was terminated after 6 and 2 weeks (without AO) and after 9 and 3 weeks (with AO) at 21 or 35°C respectively. When the air-packaged product reached a TBA value ≈ 4.00 (mg malonaldehyde/kg), the linolenic acid content decreased to 0.68% (w/w), a 62% decrease. However, using either MA_1 or MA_2, at either 21 or 35°C, a shelf-life of one year was possible. The addition of AO only controlled autoxidation during initial heat processing and did not contribute to shelf-life extension under MA_1 and MA_2. In control experiments, it was determined that the ground flaxseed was the principal contributor to overall rancidity. Rolled oats, almonds, canola oil, honey and yellow sugar did not contribute significantly to the termination of shelf-life caused by oxidative rancidity in the prototype cereal.

Résumé

Études sur la prolongation de la durée de conservation à l'étalage d'une céréale du matin enrichie en acides gras oméga-3

Au cours des 20 dernières années, plusieurs études nutritionnelles ont démontré que les acides gras oméga-3 (AGω3) (qui incluent l'acide linolénique, l'acide eicosapentanoïque, et l'acide docosahexaénoïque) sont bénéfiques pour la santé. Cependant, il existe un problème de conservation avec les AGω3, car ils sont très susceptibles à l'oxydation lipidique (le rancissement). Pour que les produits enrichis en AGω3 aient du succès dans le marché, il faut trouver un moyen pour ralentir ce processus de détérioration.

Une céréale prototype enrichie d'AGω3, de type granola, a été développée en utilisant les grains de lin moulus comme source principale d'acide linolénique (1,8 % p/p). Les autres ingrédients utilisés étaient: les flocons d'avoine, la cassonnade, le miel, les amandes tranchées, et l'huile de canola.Le focus de la recherche était d'appliquer et d'étudier sur le prototype l'ajout d'un antioxydant (AO; 300 ppm de tocophérols assortis) et l'utilisation de l'Emballage sous Atmosphère Modifiée (EAM). Les échantillons de granola, avec et sans AO, ont été emballés dans l'air et dans deux types d'atmosphère modifiée obtenus par balayage d'azote (AM_1) et par l'ajout d'un absorbeur d'oxygène (AM_2). Les échantillons ont été conservés à 21 °C ou à 35 °C.

La durée de vie du prototype a pris fin quand les échantillons ont atteint un niveau d'acide thiobarbiturique (ATB) de 4,0 mg de malonaldéhyde / kg qui correspond à une note sensorielle de 5 (sur une échelle hédonistique de 10). En tenant compte de ces critères, la durée de vie des échantillons emballés dans l'air a pris fin après 6 et 2 semaines (sans AO) et après 9 et 3 semaines (avec AO) à 21 °C ou à 35°C, respectivement. Lorsque les échantillons emballés dans l'air ont atteint un niveau d'ATB de 4,0 mg de malonaldéhyde / kg, le niveau d'acide linolénique a diminué à 0,68 % (p/p), une diminution de 62%. En utilisant soit AM_1 ou AM_2, à 21 °C ou à 35°C, une durée de vie d'un an a pu être obtenue. Dans les échantillons emballés par AM_1 et AM_2, l'ajout de l'AO a seulement contrôlé l'autooxydation durant le processus initial de réchauffement et n'a pas contribué à la prolongation de la durée de vie. Lors d'expériences contrôlées, les grains de lin moulus ont fortement contribué au rancissement complet du prototype. Les flocons d'avoine, la cassonnade, le miel, les amandes tranchées, et l'huile de canola n'ont pas contribué de façon significative à la réduction de la durée de vie causée par le rancissement du prototype.

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1. INTRODUCTION

Heading towards the year 2020, Sloan (1998) recommended that food manufacturers and marketers should focus on emerging trends in the health and supplement industries as these trends become mainstream food opportunities. One of these trends is the switch to healthier fats: more and more consumers want a higher degree of unsaturation and fewer "trans" fatty acids (Sloan, 1997). The polyunsaturated fatty acids, in particular the omega-3 variety, have received the most focus as countless nutritional studies over the past 20 years have found that they are health-promoting. Although the omega-3 trend is only now becoming popular in North America, Pszczola (1998a; 1998b) reported that in European markets "omega-3 fatty acids have been incorporated into breakfast cereals, infant formulas, margarine and low-fat spreads, beverages, breads, and other products." Hence, the direction of this research was to develop an omega-3-rich food product which could be easily incorporated into the average North American's diet. Since ready-to-eat breakfast cereals are a staple in most households and already have an established "good-for-you" image (Grider, 1996), they were an obvious choice for omega-3 supplementation. However, a shelf-life problem exists with all omega-3 fatty acids – a high susceptibility to lipid oxidation. Techniques for slowing this deteriorative process must be applied if an omega-3-rich product is to be successful in the marketplace. The focus of this research was to apply and study the addition of an antioxidant and the use of Modified Atmosphere Packaging on shelf-life extension of the enriched cereal prototype. The techniques were compared qualitatively, using sensory analysis, and quantitatively, using the thiobarbituric acid test, in order to determine a standard of acceptability. The shelf-life extension possible using the aforementioned techniques was ultimately determined.

2. LITERATURE REVIEW

2.1 Omega-3 Fatty Acids

Before the 1970s, unsaturated fatty acids were little understood nutritionally, except that they were deemed "essential" (Leat, 1989). Research on them was limited, and the term "unsaturated" was not a part of the common vernacular. Since then, however, there has been sustained publicity and solid scientific research regarding the health benefits of both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), with more emphasis on the latter (Nettleton, 1995). The PUFA consist of two families of fatty acids, omega-3 and omega-6, designated by the location of the first double bond counting from the methyl end of the fatty acid molecule (Figure 2.1) (Simopoulos, 1996a). Alpha-linolenic acid (LNA) is the parent fatty acid of the omega-3 family and linoleic acid (LA) is the parent fatty acid of the omega-6 family; both LNA and LA are essential fatty acids as they cannot be synthesized by human beings.

While original research focused on the omega-6 fatty acids for their blood cholesterol lowering properties, an investigation of the Inuit diet served as a turning point for research on the omega-3 family (Dyerberg *et al.*, 1978). According to Nettleton (1995), the work of Dyerberg and colleagues linking omega-3 fatty acids to reduced heart disease "ushered in a new era of fatty acid research." The more omega-3 fatty acids have been studied, the more nutritionally and physiologically fundamental they have been discovered to be in our diet.

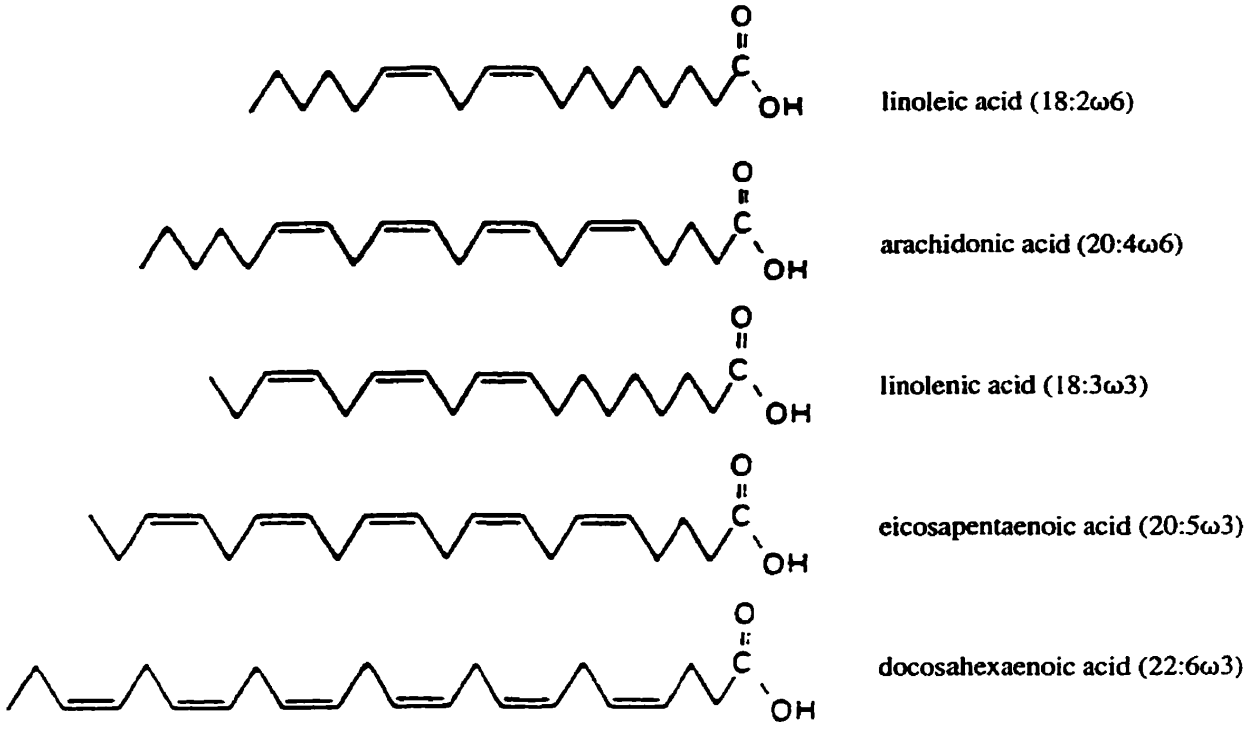


Figure 2.1 Structural Formulas of the Principal ω6 and ω3 Fatty Acids (Simopoulos, 1996a)

2.1.1 Natural Sources

The essential fatty acids LNA and LA cannot be synthesized by humans, hence they need to be obtained from the diet. In nature, both are plentiful. LNA is found mostly in the chloroplast of green leafy vegetables, except for flaxseed and canola where a significant proportion is also found in the seeds; and LA is found in the seeds of most plants, with the exception of coconut, cocoa, and palm (Hunter, 1989). In animals, both LNA and LA are metabolized to longer chain fatty acids of 20 and 22 carbon atoms, increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl end of the fatty acid molecule. By using deuterated material, it has been shown that LNA is metabolized, although inefficiently, to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), and LA to arachidonic acid (AA) (Figure 2.1.1) (Simopoulos, 1996a; Kinsella, 1990).

Omega-3 and omega-6 fatty acids compete for the desaturation and elongation enzymes, but both delta-5 and delta-6 desaturases prefer omega-3 to omega-6 fatty acids. However, a dietary imbalance in omega-6 to omega-3 fatty acids, favoring omega-6, interferes with the metabolism of LNA to EPA and DHA (Kinsella, 1990). This imbalance currently exists in the average North American diet for several reasons: (i) the increased consumption of oils from oilseeds low in omega-3 fatty acids (corn, safflower, and cottonseed), (ii) modern agriculture and aquaculture methods have reduced the use of omega-3-rich feeds, (iii) the decreased consumption of cod liver oil as a supplement, and (iv) the partial selective hydrogenation of LNA in soybean and canola oils for organoleptic reasons (Simopoulos, 1996a). This imbalance can be corrected by either reducing the omega-6 intake, or by increasing the omega-3 intake, especially by

eliminating the conversion step and directly consuming EPA and DHA (i.e., most commonly from fatty fish and fish oils) (Garcia, 1998).

Both omega-3 and omega-6 fatty acids are important components of plant and animal cell membranes and are distributed selectively among lipid classes. LNA and LA are found mostly in triglycerides, in cholesteryl esters, and in smaller amount in phospholipids. EPA is found mostly in phospholipids and in cholesteryl esters, and in smaller amounts in triglycerides. DHA and AA are found mostly in phospholipids. Depending on the plant or oilseed, the LNA and LA content can vary dramatically (see Table 2.1.1). AA is the principal PUFA in grain-fed animals, while EPA and DHA are the prinicpal PUFA in fatty fish (Simopoulos, 1996a). Since the diet of the average North American contains a disproportionate amount of omega-6 fatty acids, it is necessary to supplement the diet with omega-3 fatty acids. Health and Welfare Canada (1990) recommended that the ratio of omega-6 to omega-3 fatty acids in the daily diet fall between the range of 4:1 to 10:1; and that 3% of daily energy come from omega-6 fatty acids (4.0 g / 5000 kJ or 3.3 g / 1000 kcal) and 0.5% from omega-3 fatty acids (0.7 g / 5000 kJ or 0.55 g / 1000 kcal).

Linolenate Series	**Linoleate Series**
18:3ω3 α-linolenic acid (LNA)	18:2ω6 linoleic acid (LA)
↓ Δ^6 desaturase	↓ Δ^6 desaturase
18:4ω3	18:3ω6
↓ elongase	↓ elongase
20:4ω3	20:3ω6
↓ Δ^5 desaturase	↓ Δ^5 desaturase
20:5ω3 eicosapentanoic acid (EPA)	20:4ω6arachidonic acid (AA)
↓ elongase	↓ elongase
22:5ω3	22:4ω6
↓ Δ^4 desaturase	↓ Δ^4 desaturase
22:6ω3 docosahexaenoic acid (DHA)	22:5ω6

Figure 2.1.1 Essential Fatty Acid Metabolic Desaturation and Elongation (Kinsella, 1990)

Table 2.1.1 Comparison of ω-3 and ω-6 Fatty Acid Composition of Some Dietary Oils

	% ω-3 and ω-6 Fatty Acid Content (normalized to pure oil)					Ratio
Oil	LNA	EPA	DHA	LA	AA	ω-6:ω-3
Flaxseed[a]	58	-	-	13	-	0.2:1
Canola[b]	10	-	-	26	-	2.6:1
Soybean[b]	7	-	-	54	-	7.7:1
Sunflower[b]	-	-	-	69	-	-
Olive[b]	1	-	-	8	-	8:1
Peanut[b]	-	-	-	34	-	-
Walnut[c]	10	-	-	50	-	5:1
Milkfat[b]	2	-	-	2	-	1:1
Cod liver[c]	1	6	27	2	-	0.1:1
Menhaden[c]	1	14	12	2	2	0.1:1
Shark liver[c]	-	4	30	4	4	0.2:1

[a] (Carter, 1993)
[b] (Giese, 1996b)
[c] (Kinsella, 1990)

2.1.2 Beneficial Health Effects

Epidemiological investigations of health and disease in human populations have shown that omega-3 fatty acids are beneficial to human health. Dyerberg *et al.* (1978) found that Arctic Inuit, whose diet consisted primarily of raw marine mammals and fish, were far less likely to develop heart disease, psoriasis, asthma, diabetes, thyrotoxicosis, multiple sclerosis, certain cancers, and ulcers than Europeans and Americans. Similarly, studies on the traditional Japanese diet, primarily marine-based, have shown that higher omega-3 levels in total plasma fatty acids and phospholipids resulted in lower rates of heart disease (Kagawa *et al.*, 1982; Hirai *et al.*, 1980). Subsequently, it has been shown that as Japanese dietary habits have been changing towards Western eating patterns, there has been a concomitant increase in heart disease and diabetes mellitus in the Japanese population (Simopoulos, 1996b; Nettleton, 1995; Hennekens *et al.*, 1990).

Although there are many other factors which may contribute to heart disease (e.g., genetics, smoking, lack of exercise, alcohol), it has been shown that the development of atheroschlerosis (arteriosclerosis) is principally responsible. This condition involves the combination of the arteries becoming less elastic (sclerosis) and the build-up of lipid-rich plaques (atherosis). The lipids in these plaques come from circulating lipoproteins, mainly low-density lipoproteins (LDL). It was once believed that diets rich in saturated fat and cholesterol were solely responsible for high LDL levels, but it has been shown with the Arctic Inuit studies that so long as a diet rich in saturated fat and cholesterol is also rich in omega-3 fatty acids, heart disease can be minimized (Nettleton, 1995).

Omega-3 fatty acids have been shown to reduce blood viscosity by increasing red blood cell deformability and by affecting platelet activity. Increased blood viscosity is

associated with coronary artery diseases, peripheral vascular disease, atheroschlerosis, and diabetes. Thrombosis is the formation of a blood clot which is essential to repair tissue and vascular injury, but which is also potentially fatal if a large clot is formed in, or delivered to, the blood vessels of the heart, lung, and brain (Nettleton, 1995). Harker *et al.* (1993) found that a diet rich in omega-3 fatty acids decreased platelet aggregation and reduced the production of tissue factor, both essential to clot formation, and as a result, nearly eliminated vascular thrombus formation.

Since individuals with either type I or type II diabetes mellitus are at increased risk for the premature development of atherosclerosis, dietary supplementation with omega-3 fatty acids has been tested and shown to reduce its incidence (Hennekens *et al.*, 1990). However, since these individuals are very sensitive to slight changes in blood lipid and glucose levels, treatment with omega-3 fatty acids must be done under the careful observation of a physician. Nettleton (1995) reported that the regular consumption of fish has been shown to discourage the development of type II diabetes mellitus.

While omega-3 fatty acids in the diet have been shown to have a significant clinical effect, an understanding of the biochemical pathways is necessary to determine how they exert their beneficial effects. Just as there is competition between omega-6 and omega-3 fatty acids for the delta-5 and delta-6 desaturases, there is also competition between AA and EPA for the production of eicosanoids (prostanoids and leukotrienes). AA and EPA compete for the oxidative enzymes cyclo-oxygenase and 5-lipoxygenase to be converted to their respective prostanoids, and leukotrienes. In other words, if an individual's diet is rich in omega-6 fatty acids but low in omega-3, there will be a disproportionate amount of AA within the body, and hence a disproportionate amount of

AA metabolites. This disproportion favours increased blood viscosity, increased platelet aggregation, and the resulting atheroschlerosis and thrombosis. The prostanoids and leukotrienes formed from EPA are antithrombotic, antichemotactic, antivasoconstrictive, and antiinflammatory (Simopoulos, 1996a). Because of this knowledge, it can be predicted that omega-3 fatty acids may be beneficial for other diseases and ailments, such as asthma, rheumatoid arthritis, multiple schlerosis, immune system weakness, psoriasis, and inflammatory bowel disease. Recent studies, although not entirely conclusive, have shown that these conditions improve with omega-3 supplementation (Nettleton, 1995; Spector & Kaduce, 1989).

Omega-3 fatty acids have also been heavily investigated regarding their importance in the neural and retinal development of the human fetus and child. The 22-carbon DHA is the important omega-3 fatty acid in this case, since it is specifically incorporated into the membrane phospholipids of the retina and brain (Nettleton, 1995; Carlson, 1989; Neuringer & Connor, 1989). However, since LNA and EPA are more abundant in nature and can be elongated in the human body, it has been recommended that pregnant and lactating women and children consume diets rich in omega-3 fatty acids of any type; and that infant formula be supplemented with omega-3 fatty acids (particularly EPA and DHA) (Nettleton, 1995; Health & Welfare Canada, 1990).

2.1.3 Toxicity

Although the literature tends to emphasize the positive effects of omega-3 fatty acids on human health, the possible toxic consequences of their addition to foods also needs to be addressed. Beare-Rogers (1989a; 1989b) warned of elevated levels of blood

glucose in non-insulin dependent diabetic individuals due to glucose homeostasis; and of the effects of membrane peroxidation, something which can be countered by ensuring adequate levels of tocopherols (vitamin E), selenium, and sulphur-containing amino acids in the diet. Increased bleeding time, due to reduced platelet production of thromboxane A_2, is also a concern since it can lead to hemorrhaging (Nettleton, 1995; Illingworth & Ullmann, 1990).

2.1.4 Omega-3-rich Food Ingredients

Flaxseed is probably one of the oldest and richest plant sources of LNA, having been used for food in Europe and Asia since 5000 B.C. It is the seed of the annual flax plant (*Linum usitatissimum* L.), a plant most commonly associated with linen cloth. Flaxseed is high in LNA with between 45 and 58 percent of its fatty acid composition consisting of LNA (Carter, 1993; Hunter, 1989). It also has high levels of soluble and insoluble fibre (between 36 and 46 percent dry weight) including plant lignans, which have also been shown to be beneficial to human health. With between 26 and 31 percent (on a dry weight basis) nutritionally complete protein and a potassium level (700 mg / 100 g) similar to that of banana, flaxseed consumption has few disadvantages (Stitt, 1989).There are only three known antinutritional components of flaxseed: phytic acid, which can affect zinc levels; linatine, which can act as a vitamin B_6 antagonist; and cyanogenic glycosides, which can raise thiocyanate levels in the blood (Carter, 1993). However, these are only noticeable with extremely high doses of flaxseed in the diet.

Traditionally, whole flaxseed has been used in porridge and bread products to impart a nutty flavour. Today, along with these traditional products, ground flaxseed is

being used in bakery products (bread, bagels, cookies, and muffins) since grinding releases components from the seed and facilitates their absorption in the intestinal tract. Refrigerated, cold-pressed flaxseed oil is available in most health food stores. By modifying laying hens' diets to contain up to 20% flaxseed, many North American egg producers are producing and marketing high LNA, EPA and DHA eggs (Ferrier *et al.*, 1994). Garcia (1998) reported that The NutraSweet Kelco Co. was producing omega-3 eggs high in DHA by feeding the hens microalgae rich in DHA.

As was previously discussed (2.1.1), consuming EPA and DHA directly, instead of relying on the conversion of LNA within the body, has been shown to be a more efficient and effective method of benefitting from omega-3 fatty acids. This direct consumption can most easily be accomplished by either taking fish oils as a supplement, or supplementing foods with them. The obvious concern with using fish oils in foods is the potential for a "fishy" flavour imparted by the fish oil. By refining and taking the "fish" out of fish oils, some companies are now marketing "bland, almost odourless" EPA- and DHA-rich oils. In North America, there are currently three companies marketing these refined fish oils: Roche Vitamins Inc., Nutley, NJ; Omega Protein Inc., Reedville, VA; and BASF Corp., Mt. Olive, NJ (Pszczola, 1998a, 1998b). To further "mask" any objectionable flavours and to protect from lipid oxidation, these companies also offer dry, microencapsulated forms of fish oils. There are two dry products from BASF Corp., each containing 35 percent omega-3 fatty acids, but in different ratios. One, called "18:12" and intended for foods, has 18 percent EPA, 12 percent DHA, and 5 percent others (e.g., LNA); the other, called "5:25" and intended for infant formula, has 5 percent EPA, 25 percent DHA, and 5 percent others (e.g., LNA) (BASF Corp., 1998).

Although significant effort has been made to take the "fish" out of fish oils, consumer acceptance of foods fortified with fish oil is uncertain. "When Western people think of fish, there is a strong association with strong fishy odors and flavors. It is difficult for the consumer to get past the stereotypical reaction to the presence of something fishy in a sweet or bland product, such as ice cream or cake." (Garcia, 1998) Another hurdle to overcome with fish oils is consumers' increased awareness of environmental contaminants (e.g., heavy metals and PCBs) and bioaccumulation of these contaminants in fish. BASF Corp. (1998) claimed that their products "originate from species taken from deep, clean waters where even traces of pesticide residue are absent," but convincing consumers at the supermarket aisle about this could prove to be difficult.

Becker & Kyle (1998) reported that Martek Biosciences Corp., Columbia, MD produced a DHA-rich oil using marine algae of the *Crypthecodinium cohnii* strain – a strain which produces large amounts of DHA. Under controlled fermentation conditions, algae are grown using an ideal medium, then harvested, dried, and treated with hexane to extract the oil. After desolventization and winterization, it is refined, bleached and deodorized before being diluted with sunflower oil to bring the DHA level to 40%. Becker & Kyle (1998) also reported that this oil was deemed to be GRAS by expert toxicologists and found to have better oxidative stability than fish oil, sand eel oil, and tuna head oil.

2.1.5 Shelf-life Stability

Unsaturated fatty acids of any variety (MUFA or PUFA) are susceptible to oxidative rancidity, and the more unsaturated the fatty acid is (e.g., LNA, EPA, and DHA), the more susceptible it is to rancidity. Rancidity and its prevention are discussed in detail later (Section 2.3).

Whole flaxseed is stable when stored in a cool, dry place. However, once it is pressed for oil or ground for processing, care must be taken to prevent its exposure to heat, light, oxygen, and moisture. Generally, products containing ground flaxseed have a shelf-life of three to four months if stored at or above room temperature (Carter, 1993).

In Europe, fish oils are used to manufacture shortenings and margarines. However, due to their high oxidative instability they need to be hydrogenated which makes these products poor sources of omega-3 fatty acids (Hunter, 1989). Like flaxseed, fish oils require cool, dark, and dry storage to slow the effects of oxidative rancidity. The algal oil produced by Martek Biosciences Corp. was found to be more stable than fish oils; however, off-flavours and odours are still produced when oxidation inevitably sets in (Becker & Kyle, 1998).

Antioxidants are also recommended to extend shelf-life – tocopherols, phenol antioxidants (e.g. BHA and BHT), and ascorbic acid have all been shown to slow rancidity (Carter, 1993). The fish oil products from BASF Corp. contain combinations of sodium ascorbate and/or ascorbyl palmitate, tocopherols, and/or lecithin as antioxidants (BASF Corp., 1998).

2.2 Breakfast Cereals

2.2.1 History, Marketing, and Current Trends

Breakfast cereals have been consumed in some form or other since the cultivation of cereal crops and up until the 20th Century, they consisted of hot, porridge-types. Industrialization, a changing workforce, and marketing resulted in the development of "ready-to-eat" cereals — the add milk and eat variety (Kent & Evers, 1994). The proliferation of brands and types of breakfast cereals began in the 1950s, due to creative advertising, marketing, and technological developments. The top five players in the North American breakfast cereal market are, in order of highest market shares: Kellogg's, General Mills, Philip Morris, Quaker Oats, and Ralston (Grider, 1996).

Grider (1996) reported that the North American breakfast cereals market is saturated, with little increase in sales growth. However, manufacturers are responding by lowering prices, improving manufacturing efficiencies, and changing their marketing attitudes. With a market value in excess of 10 billion dollars per year in North America alone, even if a new product entering captures only 1% of the market, this translates into 100 million dollars in revenue (Grider, 1996). However, with hundreds of existing breakfast cereal products on the supermarket shelf, attaining even 1% of sales can be a formidable task as the leading cereals each hold less than 5% of the market (Grider, 1996; Roellig, 1994).

Roellig (1994) stressed the importance of defining a target audience and marketing scheme for new products: adult cereals, children's cereals, all-family cereals, and "good-for-you" cereals. With these "good-for-you", or healthful, cereals, the packaging must balance the nutritional advantages with great taste in order for the

product to succeed (Pszczola, 1999; Roellig, 1994; Shukla, 1993). Beyond bran, products containing and/or fortified with beneficial phytochemicals and nutraceuticals have been gaining in market share, as health-conscious consumers want to return to eating "real" food (Shukla, 1993).

2.2.2 Manufacture

Breakfast cereals are generally manufactured from cereal grains as flakes, shreds, extruded shapes, puffs, or granola clusters. Flaked cereals are produced by breaking whole grains into pieces about one-third to one-half the size of the original whole grain, steaming these pieces with other ingredients as required, breaking the steamed mass into small segments, and then drying and flaking the segments between steel rollers. Shredded cereals are manufactured similarly, except that one of the steel rollers is corrugated to form small strands. The strands exiting the rollers are cut into the pillow shaped products and then dried or baked. Extruded cereals also use a steamed cereal mass, but with finer ground grains. The mass is sent through, under high pressure, an extrusion die corresponding to the shape of cereal desired. Exposure of the extruded hot cereal to atmospheric pressure causes instantaneous puffing and the puffed product is then immediately dried. Similarly, puffed grains are produced by cooking whole grains, drying the surface, and then puffing them at temperatures as hot as possible short of scorching the grains. Granola cereals are clusters of whole rolled grains and non-cereal ingredients such as nuts, coconut, oil, dried fruits, and spices that are toasted and held together with a sugar matrix (Kent & Evers, 1994; Tribelhorn, 1991; Fast, 1990).

Generally, plain flaked, shredded and extruded cereals have very little flavour, the high temperatures used to process the cereals can distill many of the natural flavours from them, making flavour addition or enhancement necessary for product acceptability. Hence, many of these cereals are coated with a flavoured, sugar-based coating. A variety of processing problems (e.g., cereal collapse, clumping, crystallization, and equipment fouling) in applying a "wet" sugar syrup to a dry cereal have been overcome by using invert sugars, high pressures and temperatures, and gums and starches (Burns & Fast, 1990; Daniels, 1974).

2.2.3 Fortification

Within the last century, great strides have been made in defining nutrient deficiencies and their consequences. As a result, "there has been a strong movement toward the modification of the food supply to contain increased levels of nutrients that have been shown to have positive correlations with the decreased incidence of certain disease states" (Davies, 1995a). Breakfast cereals were among the first foods to be fortified with vitamins and minerals, dating back to the 1950s. Ever since, consumers have expected good nutritional value in every bowl of cereal.

The degree of fortification of any cereal depends primarily on the desired nutrient claim to accompany the cereal's marketing. Other product characteristics to consider are: product formulation, pH, moisture content, processing temperatures, storage temperatures and times, storage oxygen levels, and packaging. These characteristics can all have deleterious effects on the nutritional value of the cereal depending on each vitamin's, or nutraceutical's, individual stability. For example, heat-labile vitamins, such as vitamin A,

vitamin C and thiamine, are commonly sprayed onto the product after processing steps involving heat. Conversely, minerals and more stable vitamins (e.g., niacin, riboflavin, vitamin B6, and vitamin E acetate) are usually added to the basic formula mix (Johnson *et al.*, 1988). Significant "overage" (overcompensation) is generally employed to make up for processing and storage losses; the degree of overage depends on each vitamin's level of stability, but is usually between 15 and 50% (Borenstein *et al.*, 1990). Either addition of nutrients is generally made using a premix or blend which has been either prepared in-house or purchased from a supplier.

In addition to considerations of chemical and physical stability, micronutrients must be selected based on flavour, odour and colour considerations. For example, although thiamine is heat sensitive and application via a spray method would minimize losses, the potential for negative sensory characteristics in its spray application favours its addition to the basic mix (Davies, 1995a). Similarly, vitamins A and D cannot be added directly to the basic mix since they are both oxygen and temperature sensitive. However, spraying also maximizes their exposure to oxidative degradation so protection in the form of antioxidants (e.g., BHT) and carbohydrates (e.g., sucrose) added to the spray solution can significantly control the oxidation problem (Johnson *et al.*, 1988). Recently, new technologies, such as microencapsulation, have also been introduced to ease fortification losses (Stauffer & Caldwell, 1990).

Although fortification is commonly thought of exclusively in terms of vitamins and minerals, Toma & Curtis (1989) recommended fortification of breakfast cereals with proteins and beneficial fats, as both are present in very small amounts in cereal grains and their addition to cereals would make them a better-balanced breakfast. Shukla (1993)

suggested that high-nutrition breakfast cereals, containing phytochemicals and nutraceuticals, will be part of "a new consumer-driven movement regarding health maintenance by diet management and modification."

Succinctly, Borenstein *et al.* (1990) reduced the challenges of fortification technology to three key points: 1) the product must not be negatively affected in odour, flavour, or colour; 2) the added nutrients are acceptably stable, and sufficient overage is added to compensate for losses in processing and storage; and 3) the process remains practical and economically viable.

2.2.4 Packaging

Retail stores first began selling packaged breakfast cereals around 1910. The cereals were packaged by hand into waxed glassine bags, sealed with a double fold and a wax heat seal, and inserted into printed paperboard boxes (Monahan & Caldwell, 1990). Over the years, as packaging machinery became more efficient and package material costs were reduced, breakfast cereal companies were able to focus more energy and resources on marketing and distribution.

Monahan (1988) identified four main objectives of breakfast cereal packaging as: product protection, product identification, consumer attraction when purchasing, and consumer appeal throughout the use of the package. Firstly, the package must protect the contents from breakage during storage and distribution, from the entry of moisture, gases and contaminating odours, and from the loss of or change in flavour. Secondly, the package must clearly identify the contents, link itself with commercial advertising, and provide the list of ingredients and nutrition labelling. Thirdly, the package should try to

attract consumers on the supermarket shelf by portraying how the product will look when served, highlighting any premium offers, and conveying whatever distinctive concept the product provides. And finally, the package needs to satisfy the consumer in his or her own use of the product: how easily the package opens, how well the consumer can control the pouring of the product into a serving bowl, how efficiently the package recloses to protect the opened product and to store neatly on the kitchen shelf, how effectively the printed graphics and information on the carton draw attention to the product within the consumer's home, and for how long the package can extend the shelf-life once opened and the cereal partially consumed (Monahan & Caldwell, 1990).

Traditionally, materials used to package breakfast cereals include printed paperboard cartons, protective liners, corrugated shipping containers, and the necessary adhesives. However, recently some manufacturers, most notably the Quaker Oats Company, have started marketing their cereal lines in stand-alone laminates (Buss, 1997; Hartman, 1997). The conventional paperboard carton, though, has been widely accepted over the years for several reasons: the original and historical breakfast cereal packaging concept, the paperboard provides the rigidity needed to protect the contents, the paperboard keeps the product in darkness (slowing oxidation), the paperboard provides for excellent print quality (either by lithography or rotogravure), the consumer is accustomed to the paperboard cartons on the supermarket shelf, and most of the existing packaging equipment in breakfast cereal plants can only use paperboard boxes (Monahan & Caldwell, 1990). Although the paperboard carton serves many valuable purposes, it is the lining material which is the real workhorse. Firstly, the liner must be able to form a good seal and be strong enough to resist puncture on the packaging line and during

storage and distribution. Secondly, it must be relatively impervious to moisture, to the loss of desired product aromas, and to the entrance of foreign odours. Finally, the liner should open cleanly and offer some degree of reclosability to the consumer. Although modern glassines (waxed papers) can outperform plastics in terms of barrier protection and reclosability, and are still used for some products today, most cereal companies have changed over to plastic film to reduce packaging costs. High density polyethylene (HDPE) coextruded with ethyl vinyl acetate (EVA) is a typical combination; the HDPE provides a good barrier against moisture and odour transfer, while EVA allows for a low-temperature seal which is easily opened by the consumer (Monahan & Caldwell, 1990).

Buss (1997) reported that market research has shown that the largest complaint consumers have with breakfast cereals is packaging: cartons that neither open without ripping nor close properly, liners that are not peelable, and liners that do not roll down adequately to "reseal" the cereal. The innovative, zippered, printed laminate breakfast cereal bags used to package products from the Quaker Oats Company is long overdue, and appears to answer some of the above consumer complaints. The Quaker Oats Company was so ecstatic with consumer response to this package that they planned to market all of their cereal products in these packages by the end of 1998 (Buss, 1997; Hartman, 1997). Although there is a capital cost to installing packaging equipment capable of handling these laminated packages, Buss (1997) reported that there should be significant savings since the laminates were cheaper than the traditional liner in carton arrangement and also since this bagged arrangement could reduce shipping costs. Apparently, the big three cereal manufacturers (Kellogg's, General Mills, and Philip

Morris) are waiting to see the results of the Quaker Oats experiment and their own test-marketing before taking the plunge to convert existing packaging equipment.

Another innovative packaging solution was recently test-marketed in Canada; Kraft Canada Inc. (Philip Morris) packaged two of their cereals in tapered, reusable plastic containers wrapped in printed vinyl sleeves (Lingle, 1997). The package resembled the traditional paperboard carton, but there was no liner inside, and the lid facilitated opening, reclosing and pouring. Once the original contents were consumed, it can be refilled by the consumer with more cereal or other foods, or recycled. The downside to this type of packaging is the increased costs in its manufacture and higher distribution costs of a rigid plastic container.

2.2.5 Shelf-life

A food is said to have reached the end of its shelf-life when, upon storage for a certain period of time, one or more quality attributes have reached an undesirable state, making the food unsuitable or unappealing for consumption (Singh, 1994). Breakfast cereals are inherently stable and have a "long" shelf-life. Because of this, the requirements for determining shelf-life are different from products having a short shelf-life (i.e., years for cereals versus days for chilled foods). The factors affecting the shelf-life are also different: the deterioration will probably not affect the safety of a breakfast cereal but will affect consumer satisfaction. Therefore, the manufacturer needs to identify the parameters most critical to consumer satisfaction and ensure that these are preserved throughout the life of the product (Howarth, 1994).

The ultimate shelf-life value for a breakfast cereal is difficult to measure since it is dependent on many variables, most notably, raw materials, processing, packaging, storage conditions, distribution conditions, retailing conditions, and individual consumer storage and consumption habits. Traditionally, manufacturers have tried to guarantee a "fresh" product at the point of purchase, since they can neither control nor predict what the consumer does with the breakfast cereal upon leaving the supermarket (Howarth, 1994; Stauffer & Caldwell, 1990). However, market research has shown that consumers think reclosability, "Best Before" dates, and "staling" are important issues, so it would appear that some consideration, at least, is needed in predicting shelf-life after the point of purchase (Buss, 1997). Howarth (1994) reported that major retailers are now demanding that 75% of the shelf-life is still available when the product is delivered to them.

The control of moisture in the final product is critical to maintaining its integrity. Stauffer & Caldwell (1990) reported that moisture levels in excess of 3% during packaging accelerated vitamin C degradation, and that levels below 1% could promote cereal breakage and rancidity. Breakfast cereals can spoil in a number of ways. Those containing significant fat levels, either naturally from the grain or added from other sources, are subject to rancidity. Fortified products lose their vitamin potency over time. In order to control rancidity, antioxidants may be added to packaging materials or even directly to the cereals and oxygen can be flushed out during package sealing. Vitamin overage and techniques discussed in the previous section (2.2.3) can be used to guarantee nutrition claims on the package.

The use of high-quality, consistent raw materials ensures that both the desired quality and required shelf-life will be met. Processing conditions can affect shelf-life by initiating lipid oxidation and significantly degrading vitamins, so care needs to be taken to minimize the heat treatment and severity of processing, yet still obtain the desired results. The packaging used needs to protect the cereal from physical damage; from moisture, oxygen and flavour/odour transfer; and also from light (Howarth, 1994).

Storage, distribution, and retailing conditions are also important considerations regarding shelf-life since maintaining ideal conditions is almost impossible. An understanding of the mean temperature and relative humidity at each location is useful, but it cannot predict the effects of poor rotation, poor warehousing, and abusive transportation conditions. Hence, to ensure product confidence, a regular monitoring program is advisable to track product in the actual stream of events (Howarth, 1994). Regular inspection of warehouses and transportation facilities can be used to spot problems; consumer complaints can be studied and acted upon (Stauffer & Caldwell, 1990).

2.3 Techniques for Slowing Lipid Oxidation and Extending Shelf-life

As previously discussed in Section 2.2.5, the shelf-life of breakfast cereals is limited by vitamin degradation and lipid oxidation (or oxidative rancidity). When the vitamin levels remaining in the breakfast cereal fall below the nutritional claim made on the package, the shelf-life of the product has expired. Similarly, when the extent of the lipid oxidation renders the breakfast cereal sensorily "unacceptable", the shelf-life of the

product has expired (Dougherty, 1988). Since the present research is concerned with the addition of omega-3 fatty acids (highly susceptible to oxidation) to a breakfast cereal, the determination of shelf-life using lipid oxidation as an indicator is of principal importance.

2.3.1 Lipid Oxidation

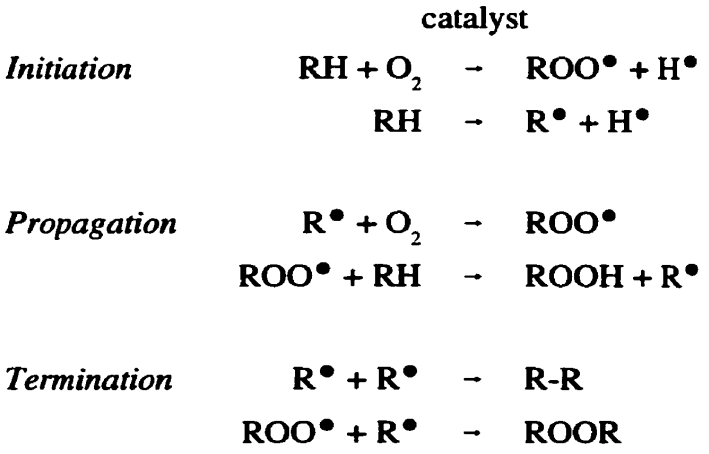
Lipid oxidation is the most significant of three chemical processes that lead to the undesired rancidity – the other two processes are lipolysis and flavour reversion. Lipid oxidation (also referred to as "oxidative rancidity" and "autoxidation of unsaturated fatty acids") is irreversible and proceeds by a free radical chain mechanism that results in hydroperoxide formation. A subsequent decomposition reaction produces a variety of secondary oxidative products (Zapsalis & Beck, 1986). The initial hydroperoxide formation is a three step mechanism involving initiation, propagation, and termination (Figure 2.3).

The reaction rates for autoxidative processes are influenced by the presence of prooxidants, such as trace metals; antioxidants, either indigenous or added; ultraviolet radiation; available oxygen levels; and temperature conditions (Gordon, 1990; Zapsalis & Beck, 1986). Once autoxidation has been initiated (formation of free radicals) and is left unchecked, it progresses at an ever increasing rate (Dougherty, 1988). Also, as the degree of fatty acid unsaturation increases, the rate of autoxidation proceeds more rapidly.

Hydroperoxides are unstable, colourless, odourless and flavourless. The real cause of the off-flavours and off-odours associated with lipid oxidation are the products of the decomposition of the hydroperoxides – the cleaving of the residues and/or the termination of the autoxidative process to form aldehydes, alcohols, ketones, esters, lactones,

aromatics, acids, and/or hydrocarbons (Hamilton, 1994; Gordon, 1990). The flavour threshold of these oxidation products can be as low as 1 ppm (Schuler, 1990). Due to the random complexity of product formation and hundreds of possible end-products, the decomposition products cannot easily be quantified, so sensory evaluation must also play an important role in evaluating shelf-life (Hamilton, 1994).

Although not intended to form a part of the present research, lipolysis and flavour reversion may be responsible for unexpected results and errors when attempting to quantify and qualify the rancidity effects in breakfast cereals. Hence, they may need to be considered when analyzing results. Lipolysis is the hydrolytic reaction in which fatty acids split from the glycerol backbone in triglycerides. The uptake of a molecule of water is promoted either by the action of lipase or by heat. Lipolysis should not be a serious problem in breakfast cereals since plant lipases are degraded during processing and the fatty acid residues common in cereals are long-chain, which contribute little to the aroma and flavour of the cereal. Flavour reversion is poorly understood, but is defined as the development of off-odours and off-flavours (fishy, painty, and beany) as a result of the oxidation of polyunsaturated fatty acids with only a small amount of oxygen (approximately 1% of the oxygen required for autoxidation) (McWilliams, 1997; Zapsalis & Beck, 1986).



Where: RH = unsaturated lipid, $R^{\bullet}$ = lipid radical, and $ROO^{\bullet}$ = lipid peroxy radical

Figure 2.3 Initiation, Propagation, and Termination Steps in Hydroperoxide Formation (Hamilton, 1994)

2.3.2 Control of Processing and Storage Conditions

Garcia (1998) recommended that process optimization to minimize PUFA oxidation be implemented to limit oxidation during product manufacture. Changing the order of addition of ingredients, adjusting the ingredient formulation, and modifying the processing operations to minimize exposure to oxygen, heat, and light were all recommended.

The most conventional technique to extend the shelf-life of any food product is to control the storage environment (i.e., temperature, relative humidity, and light in the storage area). Generally, with low moisture foods such as breakfast cereals, their "storage in tightly closed containers in a cool, dark place is helpful in slowing the onset and continued development of oxidative rancidity" (McWilliams, 1997). These controls can be implemented in the manufacturers' own premises as required, but once the product leaves the storage and distribution facilites of the manufacturer, the degree of control is less. Although information kits and special training can be given to warehouse and retail operators, secondary packaging can clearly advise on proper storage and handling, and primary packaging can also advise the consumer on proper home storage. However, it is still the responsibility of the manufacturer to predict possible abuses and counter them with innovative shelf-life extending solutions.

2.3.3 Antioxidants

Antioxidants are capable of delaying, retarding, and/or preventing oxidation processes (Schuler, 1990). They protect against oxidative rancidity by blocking the autocatalytic continuation of autoxidation in providing a hydrogen atom from their own

molecule to react with a free radical of a fatty acid or with a peroxide that has already been formed (McWilliams, 1997). Buford Coulter (1988) summarized that antioxidant technology is critical in preserving fats, oils and lipid-containing foods from: (1) the development of objectionable odours and flavours; (2) the formation of potentially toxic autoxidative decomposition products; (3) the loss of nutritional value from the degradation of vitamins (A, C, D, E, and folate), essential fatty acids (linoleic and linolenic acids), and essential amino acids (methionine, cystine, histidine, lysine, and tryptophan); and (4) the bleaching by active peroxides of labile pigments (anthocyanins, carotenoids, chlorophyll, and browning reaction products). Since antioxidants can neither reverse the action of autoxidation nor regenerate a rancid product, attention must be given to adding antioxidants to freshly produced fats, oils or lipid-containing foods before the autoxidation reaction has a chance to begin (Schuler, 1990). Currently, there is an increasing interest in antioxidants for their nutritional and health benefits since they also function within the human body (Giese, 1996a).

2.3.3.1 Natural

Originally, all antioxidants were from natural sources — one of nature's defence mechanisms. Buford Coulter (1988) reported that Native American Indians once used tree barks (rich in phenolic antioxidants) to preserve bear fat. Naturally occurring fats and oils contain indigenous antioxidants that protect the unsaturated fatty acids from autoxidation in their native vegetative and animal sources. The highest concentrations of naturally occurring antioxidants can be found in vegetable oils (Zapsalis & Beck, 1986).

The most commonly occurring natural antioxidants in plants are the tocopherols (vitamin E); they are derivatives of 6-chromanol and cannot be sythesized by animals, but dietary consumption ensures the maintenance of adequate tissue levels (Megremis, 1990; Zapsalis & Beck, 1986). Other common natural antioxidants are lecithin, rosemary extract, gum guaiac, propylgallate, and ascorbic acid (Dougherty, 1988). Since tocopherols occur naturally in vegetable oils, they are often used to slow rancidity in vegetable oils and fat-containing foods. They are extracted from tocopherol-rich deodorizer distillates, which are by-products from steam stripping of vegetable oils during their final processing step (Giese, 1996a; Dougherty, 1988). Due to their high molecular weight, tocopherols are capable of surviving high temperature processing and hence carry through into the final product to impart protection. If used, they must be added directly to breakfast cereal mixes before processing because they do not migrate into the package headspace or onto the packaging material (Megremis, 1990; Dougherty, 1988).

There are four epimers (varieties) of tocopherols: alpha-, beta-, gamma-, and delta-tocopherols (Fig. 2.3.3.1) (Eitenmiller, 1997). Alpha-tocopherols are the most abundant and exhibit antioxidant potency, but the gamma- and delta-tocopherols are considered to be the most effective antioxidants. It is for this reason that tocopherol antioxidant products usually contain a minimum 80% gamma- and delta-tocopherols (Dougherty, 1988).

Megremis (1988) showed that adding mixed tocopherols to wheat flake cereal mix at levels of 50 and 250 ppm significantly protected the finished product from rancidity. However, care must be taken not to exaggerate their addition to foods in the hope of

extending the shelf-life indefinitely since they can also act as pro-oxidants if their concentrations are too high (i.e., > 500 ppm of fat weight) (Dougherty, 1988; Zapsalis & Beck, 1986). Tocopherol antioxidant effects can be enhanced using a variety of synergists, e.g., citric, ascorbic, and phosphoric acids as well as ethylenediamine-tetraacetic acid (EDTA). Whereas tocopherols inhibit oxidative free radical chain mechanisms, these synergists act as metal chelators, deactivating the oxidation and reduction reactions between the organic peroxides and metal ions. Of all the metal ions, copper is one of the most undesirable prooxidants, followed by iron, manganese, chromium, nickel, vanadium, zinc and aluminum (Zapsalis & Beck, 1986). Commercial tocopherol products are marketed in different forms: pure alpha-tocopherol, mixed tocopherols (with all epimers), and synergistic mixtures composed of tocopherols, ascorbyl palmitate, lecithin, citric acid and carriers (usually vegetable oils) (Schuler, 1990).

2.3.3.2 Artificial

The two most effective antioxidants currently used to slow lipid oxidation in breakfast cereals are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Fig. 2.3.3.2) (Giese, 1996a). Both BHA and BHT cause no changes in the colour, odour, or flavour of food products; both are soluble in fats, oils, propylene gycol, paraffin, and ethanol; but not in water. BHA is normally used as a mixture of two isomers: 3-tertiary butyl-4-hydroxyanisole and 2-tertiary butyl-4-hydroxyanisole. BHT is known chemically by two names: 2,6 di-tertiary butyl-*p*-cresol or 2,6 di-tertiary butyl-4-methyl-phenol (Kikugawa *et al.*, 1990; Buford Coulter, 1988). BHA and BHT can be

used directly in food formulations, or, as is commonplace with breakfast cereals, they can be applied to the interior of liner films since they vaporize and migrate into the breakfast cereal. In either case, they are Generally Recognized As Safe (GRAS) when the total content of antioxidant does not exceed 0.02% of the fat content of the food (Buford Coulter, 1988). When used together, or with other antioxidants, the resulting synergism can provide extra product longevity.

There have been controversies involving the toxicology of BHA and BHT: some long-term animal studies have shown carcinogenicity and reduced blood clotting with their usage. However, contradictory and comparative studies reported that these results were inconclusive at the present time and that long-term human studies were required to prove that they are sufficiently toxic (Barlow, 1990). The situation all boils down to marketing image of a given product: if the target consumer group is aware of potential BHA/BHT toxicity, alternatives should be used. Sloan (1998) reported on food industry trends that "natural" has become the "norm" and "artificial" is "out" unless the end benefit is so appealing that it cannot be achieved any other way: hence, the benefits of using BHA and BHT would have to far outweigh those of natural antioxidants in order for them to be used in a new product.

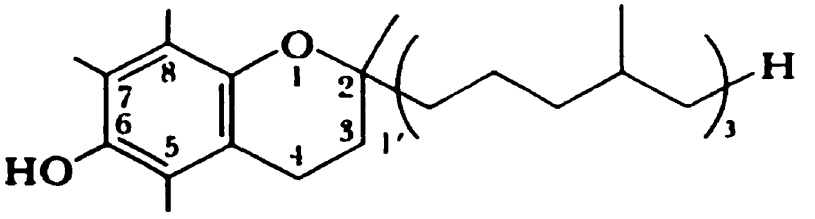


Figure 2.3.3.1 Basic α-tocopherol Structure; β-tocopherol replaces methyl group at C_7 with H, γ-tocopherol replaces methyl group at C_5 with H, and δ-tocopherol replaces methyl groups at both C_5 and C_7 with H (Zapsalis & Beck, 1986)

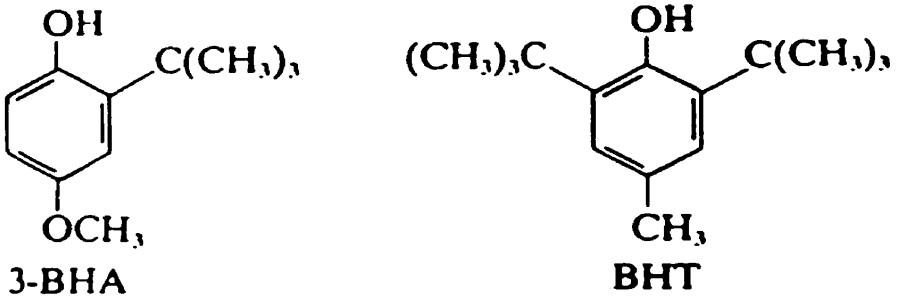


Figure 2.3.3.2 Structures of Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) (Barlow, 1990)

2.3.4 Modified Atmosphere Packaging

As today's consumer is becoming increasingly demanding and discriminating when it comes to food quality, safety, diet, additives, and product labelling, Modified Atmosphere Packaging (MAP) for the shelf-life extension of food has seen tremendous growth (Parry, 1993; Sakamaki *et al.*, 1988). Although MAP is most often associated with high-moisture foods, where microbial safety is the primary issue, it has also been used with intermediate and low moisture foods to slow chemical deterioration (Brody, 1995). Since breakfast cereals are low moisture and susceptible to lipid oxidation and loss of vitamin potency, an effective MAP system for breakfast cereals should (throughout storage and perhaps after opening and re-storage): (a) maintain the "ideal" moisture level, (b) maintain low oxygen levels, and (c) prevent ultraviolet light from entering the package.

2.3.4.1 Film Barriers and Gas Flushing

Vacuum packaging was the earliest form of MAP developed commercially; it involves the packaging of product in low oxygen permeable film and the evacuation of the air, but the resulting deformation of the product makes it unsuitable for breakfast cereals. Standard MAP technology involves: (i) removal of air from the package; (ii) its replacement with a single gas or mixture of gases (depending on the product); and (iii) the continuous flux of the gaseous atmosphere within the package due to product respiration and chemical and biochemical changes in the product (Parry, 1993).

The film barriers that can be used with MAP breakfast cereals must be, ideally, impermeable to moisture, oxygen, and ultraviolet radiation. Also, the seal formed must be

peelable and the opened package must be reclosable (either by folding or a zipper).

Greengrass (1993) reported that the films most impermeable to moisture were found to be high density polyethylene (HDPE), oriented polypropylene (OPP), coated OPP, and polyvinylidene chloride copolymer (PVdC). However, HDPE does not seal well, so it is often used coextruded with a sealable polymer (e.g., ethylene vinyl acetate (EVA) or a different polyethylene). Greengrass (1993) also reported that the films most impermeable to oxygen were found to be nylon-6 (PA-6), coated OPP, polyacrylonitrile, and PVdC. Metallized films, using a thin layer of aluminum, provided excellent protection from ultraviolet radiation, as well as oxygen and moisture protection (Ishitani, 1995; Church, 1993).

Since no single film material can provide a good moisture, oxygen and ultraviolet barrier as well as being peelable, the obvious solution is to use a coextruded laminate for the package. By sandwiching the desired barrier materials together, a laminate is formed which should deliver the required protection. Also, the lamination process allows for sandwich printing, wherein the print is trapped between two protective films; providing a high surface gloss and no print contact with the sealing interface (Greengrass, 1993).

Regarding which gas should be used to flush the breakfast cereal package, the obvious answer is nitrogen. Nitrogen is inert, plentiful and economical; can effectively "displace oxygen" from the package; and acts as a "filler" to prevent package collapse (Parry, 1993). Carbon dioxide, which is commonly used in high- and intermediate-moisture foods to control microbial spoilage, is not required with breakfast cereals due to their low water activity (i.e., < 0.4).

2.3.4.2 Scavengers

Developed as an extension and improvement to the standard MAP arrangement (i.e., selective barriers and gas flushing), scavengers, generators, and dessicants are used to control the levels of gases and vapours within the package after sealing. For the breakfast cereal in this project, which will be susceptible to oxidative rancidity, oxygen scavengers and moisture dessicants could possibly be used to control the levels of oxygen and water vapour within the package (2.3.1).

Oxygen scavengers were first developed in Japan in the 1970s with the first commercially available product being Ageless®, marketed by the Mitsubishi Gas Chemical Co. Inc. (Smith, 1993). Since then, other companies have produced them and they have been successful primarily in Japan (Smith *et al.*, 1995). The scavengers usually consist of small sachets containing various reducing agents, such as powdered iron oxide, ferrous carbonate, ferrous compounds, or ascorbates (Smith, 1993). The sachets come in various sizes, are capable of absorbing between 20 and 2000 mL of headspace oxygen, and can reduce the oxygen level to less than 0.01 percent within one to four days at room temperature (Smith *et al.*, 1995). Ideally, the barrier film or material used in a packaging system employing oxygen scavengers should be highly impermeable to oxygen transmission to keep the amount of scavenger material needed to a minimum and to maintain oxygen levels low throughout the life of the product (Rooney, 1995). Mitsubishi Gas Chemical Co. Inc recommended the KON laminate, which consists of nylon (PA) / ethylene vinyl alcohol (EVOH) / low density polyethylene (LDPE), for packaging dry, susceptible-to-oxidation foods (Smith *et al.*, 1995). In a study on the oxidative stability of an oat breakfast cereal during storage, Sakamaki *et al.* (1988) reported that, by including

an oxygen absorber and using a high oxygen barrier film (PVdC-coated PP/PE), it was possible to extend the shelf-life three-fold over a control standard consisting of nitrogen gas flushed and low oxygen barrier film (PE). Similarly, Subramaniam (1993) reported that studies on coffee (a commodity highly susceptible to oxidation) have shown that even the slightest change in residual oxygen levels can have a dramatic effect on the shelf-life. When the residual oxygen level increased to 1% from 0.5% in coffee having 4% moisture content and stored at 21°C, the shelf-life was reduced by a third. Brody (1995) reported that Kraft General Foods' DiGiornio brand of pasta used nitrogen flush and an internal oxygen scavenger to maintain a "zero" oxygen interior. Hence, the use of an oxygen scavenger could prove to be effective in extending the shelf-life of the omega-3-rich breakfast cereal. The oxygen level within the package is not the only concern for shelf-life extension, but the moisture content is also. The oxygen scavenger selected will have to be of the self-reacting type (e.g., Ageless® Type E) for the breakfast cereal should be stored with a moisture content between one and three percent (Smith *et al.*, 1995; Stauffer & Caldwell, 1990). If a moisture desiccant is included in the packaging, it should be selected so as not to interfere with the functioning of the oxygen scavenger. In order to reduce costs, a "composite function type" scavenger could be used combining the desiccant material, most commonly the non-toxic and non-corrosive silica gel, with the oxygen scavenger material in the same sachet (Miltz *et al.*, 1995; Smith *et al.*, 1995).

2.3.4.3 Active Films

Active films are a relatively new and expensive technology which eliminates the need for the sachets to contain the active materials by incorporating them directly into the

packaging film or laminates (Parry, 1993). These films can be used as closure wads, enamels in cans, labels, layers in liquid cartonboard, or as packages in their own right (Rooney, 1995; Smith *et al.*, 1995). By incorporating them into the film itself, the elimination of the scavenger/desiccant sachet would diminish consumers' fear of the possibility of ingestion of potentially toxic active materials (Davies, 1995b; Smith *et al.*, 1995). Oxygen scavenging films are manufactured by dissolving or dispersing low molecular weight active materials into a highly permeable resin (e.g., PVC or PE); desiccant films are prepared by placing one or more humectants (e.g., propylene glycol and carbohydrates) between two layers of a plastic film which is highly permeable to water vapour (Rooney, 1995). The commercial development of these active films is still in its infancy, and commercial release has been delayed by regulatory, safety, and environmental impact concerns (Rooney, 1995).

2.4 Objectives

Based on the previous review, the objectives of this research were:

1. To develop a potentially consumer-acceptable and commercially viable omega-3 enriched breakfast cereal prototype;
2. To determine a standard of acceptability based on sensory scores and chemical values for rancidity for the enriched prototype;
3. To monitor the shelf-life of the prototype, based on lipid oxidation, packaged in air and under modified atmosphere packaging (MAP), with and without an antioxidant, and stored at 21 or 35°C.

3. FORMULATION OF THE OMEGA-3 ENRICHED BREAKFAST CEREAL PROTOTYPE

3.1 Introduction

In the highly competitive and lucrative breakfast cereal industry, manufacturers are constantly searching for novel products and line extensions to improve their market positioning. Since breakfast cereals are regarded as an important part of a wholesome diet, and since they can be enhanced to make them "functional foods", their enrichment with omega-3 fatty acids would help manufacturers find a new market niche for their products among health-conscious consumers. A ready-to-eat, granola-type breakfast cereal was chosen for this study since it was hypothesized that it could be easily fortified with omega-3 fatty acids and since breakfast cereals already have an established good-for-you image (Grider, 1996). The prototype had to be enriched with a significant amount of omega-3 fatty acids, yet still be sensorally acceptable and commercially viable.

3.2 Materials & Methods

3.2.1 Ingredient Selection

The granola prototype was developed to meet several criteria: to have an omega-3 fatty acid level of greater than 1% (w/w), so that a 100 g serving would provide at least 1 g of omega-3 fatty acid (Health and Welfare Canada, 1990); to consist of a core set of ingredients commonly used in commercial formulations, in order to limit variables; and to be sensorally acceptable. Flaxseed was chosen as the source of omega-3 fatty acid (LNA, 18:3ω3) because of its affordability, its acceptance in breakfast cereals (versus fish or algal oils), its high LNA content (45-58% of its fatty acid composition) (Carter, 1993), and its other health-promoting features (e.g., dietary fibre, lignans, potassium) (Stitt,

1989). The flaxseed was freshly ground prior to granola production, because whole flaxseed is poorly digested, hence leading to reduced LNA absorption (Jenkins, 1995). The remaining formulation ingredients, and their levels, were commonly used granola ingredients as reported by Rombauer & Becker (1995): rolled oats (granola grade), yellow sugar, honey, sliced almonds, and canola oil (Table 3.2.1). All ingredients were obtained fresh from wholesale suppliers (Table 3.2.1). Basing the formulation on Rombauer & Becker's (1995) recipe, the levels of ingredients used were modified by first estimating the amount of flaxseed and canola oil required to give the target LNA level of > 1% (w/w), and then by adjusting the amount of honey-sugar syrup to obtain a granola that would hold together in clusters.

Table 3.2.1 Omega-3 Enriched Granola Prototype Formulation

Ingredient	% (w/w)	Source
Rolled oats (granola grade)	44.0	Robin Hood, Montreal
Yellow sugar	16.0	Lantic, Montreal
Ground flaxseed	15.0	Rudolph Sales, Montreal
Honey (Canada No. 1)	10.0	Doyon & Doyon, Montreal
Canola oil	8.0	Proctor & Gamble, Toronto
Sliced almonds	7.0	Rudolph Sales, Montreal
Total	100.0	

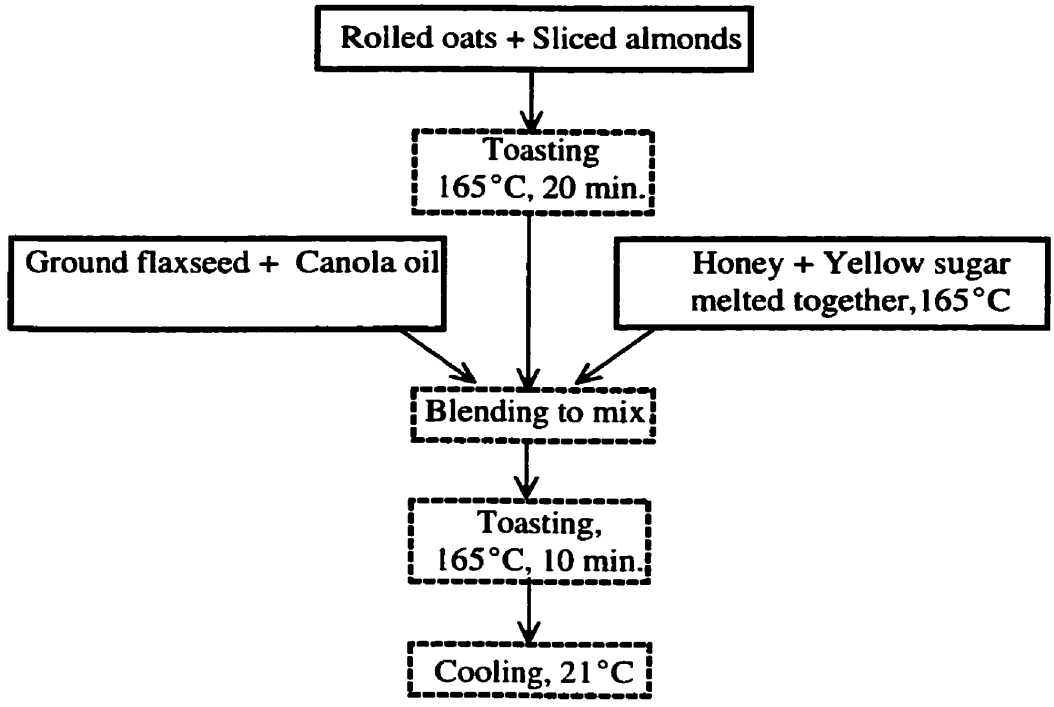
3.2.2 Manufacturing Procedure

One kg batches of granola were prepared (four at a time) using a convection oven (Garland Convection Oven TE-3, 4-CH, Commercial Ranges Ltd; Mississauga, ON) at 165°C. A flow process diagram of the unit operations in the production of the prototype granola is shown in Figure 3.2.2. The rolled oats and sliced almonds were toasted for 20 min, stirring every 5 min. Then, the freshly ground flaxseed (200 g at a time, 15 seconds on setting "Blend", Osterizer®, 4071-5 with attachment 4937, Sunbeam Corp. Ltd., Toronto, ON), the canola oil, and the hot sugar-honey syrup (heated on a rangetop just until sugar was dissolved, 165°C) were blended into the hot oat-almond mixture. The hot granola mixture was returned to the convection oven for an additional 10 min, stirring once after 5 min. The granola was then transferred from hot to cool trays, cooled to room temperature, and then immediately stored in large polymer tubs to minimize exposure to light. The granola was then frozen (to inhibit oxidation), and kept frozen until proximate and sensory evaluations.

3.2.3 Proximate Composition and Fatty Acid Profile of Prototype

Five hundred g of fresh granola prototype was delivered frozen (i.e., to limit lipid oxidation) to SGS Bio-Lalonde Food Laboratory, Pointe-Claire, QC, and evaluated for proximate composition according to AOAC procedures as well as for its fatty acid profile using AOAC procedures 963.22 (41.1.29) and 969.33 (41.1.28).

Figure 3.2.2 Flow Diagram of Heat Processing and Assembly of Prototype



3.2.4 Sensory Evaluation

In order to ensure that the enriched prototype developed would be sensorally acceptable to the average consumer, a sensory evaluation was performed. The enriched prototype was compared against Quaker Harvest Crunch Original (Quaker Oats Company, Peterborough, ON) using a sensory panel consisting of 9 non-smoking individuals (ages: 21 to 40). The samples were evaluated for three attributes, namely, appearance, taste, and overall acceptability. A 10-point hedonic scale was employed; where, 10 = excellent, 7 = good, 5 = fair, 3 = poor, and 1 = very poor. The questionnaire used was modified from Warner (1995) (Figure 3.2.4). A three-digit random number was assigned to each sample, to reduce bias (Cochran & Cox, 1957). Panelists evaluated 100 g samples of granola at room temperature and under white fluorescent lighting. The samples were placed in a cereal bowl-like, lidded polymer container (Ziploc, #6714009400). Panelists were provided with a plastic spoon for tasting. Milk was not served, as standardizing it would have been difficult and since granola can be eaten dry, as a snack food. Panelists were provided with distilled water with which to rinse their mouths between samples.

Figure 3.2.4 Questionnaire for Sensory Evaluation of Enriched Granola and Quaker Harvest Crunch Original

Name_____

Granola Quality Evaluation

Directions: Rate each sample for three attributes: appearance, taste, and overall acceptability using a 10-point scale (where 10 corresponds to "excellent", 7 to "good", 5 to "fair", 3 to "poor", and 1 to "very poor".

		Scores									
#	attribute	10	9	8	7	6	5	4	3	2	1
471	appearance	___	___	___	___	___	___	___	___	___	___
	taste	___	___	___	___	___	___	___	___	___	___
	acceptability	___	___	___	___	___	___	___	___	___	___
835	appearance	___	___	___	___	___	___	___	___	___	___
	taste	___	___	___	___	___	___	___	___	___	___
	acceptability	___	___	___	___	___	___	___	___	___	___
		10	9	8	7	6	5	4	3	2	1

3.3 Results & Discussion

3.3.1 Proximate Composition and Fatty Acid Profile of Prototype

The results of the proximate composition are reported in Table 3.3.1.1. The prototype was rich in carbohydrates (60.1%), had a total protein content of 13.4%, and a total fat content of 22.3%. It also had a low moisture content (2.4%). The fatty acid profile (Table 3.3.1.2) indicated that the prototype is low in saturated fats (5.4%), and high in both monounsaturates (53.6%) and polyunsaturates (41.0%). Of the polyunsaturates, 8.1% was LNA and 28.3% linoleic acid (omega-6). Hence, taking into consideration the total fat content of 22.3% and 8.1% of this in the form of LNA, the omega-3 target level of > 1% was achieved with an LNA content of 1.8% (w/w). The omega-6:omega-3 ratio was 3.5:1; as a result this enriched granola could be classified as a functional food since it betters Health and Welfare Canada's (1990) recommendation that the omega-6:omega-3 ratio in the daily diet fall between the range of 4:1 to 10:1.

Table 3.3.1.1 Proximate Composition of Omega-3 Enriched Granola Prototype

Constituent	%
Carbohydrate	60.1
Protein	13.4
Fat	22.3
Moisture	2.4
Ash	1.8
Total	100.0

Table 3.3.1.2 Fatty Acid Profile of Omega-3 Enriched Granola Prototype

Constituent		%
Saturates		5.4
Monounsaturates		53.6
Polyunsaturates		41.0
Linolenic acid	8.1	
Linoleic acid	28.3	
Others	4.6	
Total	41.0	
Total		100.0

3.3.2 Composition and Price Comparison with Quaker Harvest Crunch Original

When compared with the product Quaker Harvest Crunch Original (Table 3.3.2), the prototype was 2.8% higher in total fat, 2.2% lower in total protein, and 0.6% lower in total carbohydrate. In order to verify that the developed prototype could be competitively priced in the marketplace, a price comparison was performed with the popular Quaker Harvest Crunch Original cereal. The estimated retail price (Table 3.3.2) was determined using the prototype formulation, wholesale ingredients prices (Rudolph Sales, Montreal, January 1999), and a 60% retail markup. The estimated retail price for the prototype compared favourably to that of the Quaker Harvest Crunch retail price. The compositions of both Quaker Harvest Crunch Original and the prototype are also shown in Table 3.3.2.

Table 3.3.2 Composition and Price Comparison of Omega-3 Enriched Prototype and Quaker Harvest Crunch Original

Cereal	Constituent (%)			Retail Price
	Protein	Carbohydrate	Fat	($/750g)
Harvest Crunch Original[A]	15.6	60.7	19.5	4.99[B]
Omega-3 Prototype	13.4	60.1	22.3	4.65[C]

[A]constituent information taken from retail Nutrition Information panel,
[B]retail price at Maxi, Pointe-Claire, QC, January 1999, [C]based on a 60% retail mark-up

3.3.3 Sensory Evaluation

Mean scores of the sensory evaluation comparing the omega-3 enriched prototype and Quaker Harvest Crunch Original revealed that the latter was preferred slightly (Table 3.3.3). However, the scores for the prototype show that this new product was ranked "good," and was therefore acceptable. Apart from the scores, some panellists noted that they found the Quaker Harvest Crunch "too pale" in colour, others noted that the prototype was "dark like chocolate" because of the dark brown colour of the flaxseed. In terms of taste, the predominant coconut and cinnamon flavours of the Quaker Harvest Crunch was noted as a positive attribute by some panellists, whereas others commented that they preferred the honey and nutty flavours of the prototype. However, there was no substantial difference ($p < 0.05$) between the prototype and the commercial product.

Table 3.3.3 Sensory Evaluation Comparing Omega-3 Enriched Prototype and Quaker Harvest Crunch Original

Cereal	Mean Attribute Score (x/10)*		
	Appearance	Taste	Overall Acceptability
Harvest Crunch Original	7.50	7.63	7.75
Omega-3 Prototype	6.88	7.13	7.25

* derived from individual scores of 9 non-smoking individuals.

3.4 Conclusion

A potentially consumer acceptable and commercially viable omega-3 enriched granola prototype was developed (Figure 3.4). Even though the product was enriched with omega-3 fatty acid, its estimated retail price was similar to the non-enriched Quaker Harvest Crunch cereal. This enriched prototype was subsequently used in all future storage trials.

Figure 3.4 Photograph of the Omega-3 Enriched Granola Prototype

4. DETERMINATION OF A STANDARD OF ACCEPTIBILITY FOR THE OMEGA-3 ENRICHED GRANOLA PROTOTYPE

4.1 Introduction

While sensory analysis is probably one the most powerful tools for assessing the rancidity of fat-containing foods, it is a costly and time-consuming technique for shelf-life monitoring. As a solution to this problem, Rossell (1994) recommended the correlation of the results gathered from sensory analysis assessing odour acceptibility of a fatty food with the results of a chemical test. The resulting correlation can be used to assign a numerical standard of acceptibility for the food, in this case, the omega-3 enriched prototype. Hence, the objective of this study was to determine an acceptable standard of acceptability based on sensory scores and chemical values for rancidity for the prototype.

4.2 Materials & Methods

4.2.1 Prototype Preparation

The omega-3 enriched prototype was produced as per the formulation outlined in Section 3.2. Since each oven batch produced 4 kg granola, and approximately 3 kg were required for this experiment, only one oven batch was produced. Once the oven batch had cooled to room temperature, it was stored covered, to minimize its exposure to light, in a sturdy plastic bin until packaging.

4.2.2 Packaging & Storage

Samples (100 g) of the omega-3 enriched prototype were packaged in duplicate in foil laminate bags (15x26 cm, Polyester/Polyethylene/Foil/Polyethylene, VF52 Vaporloc Tamper Evident Barrier, LPS Industries, Moonachie, NJ) in air. A double heat seal (TEW Impulse Sealer, TISH-300, Taiwan; Multivac A300/42, Wolfertschwenden, Germany) was

used to reduce the probability of seal failure. Packaged samples were stored at two storage temperatures: 21 °C (representing an air-conditioned supermarket) and 35 °C (representing tropical conditions). Sample monitoring was conducted at the onset of storage and at three week intervals for 12 weeks (i.e., until all samples were completely sensorally unacceptable).

4.2.3 Sensory Analysis

An untrained sensory panel consisting of 9 non-smoking individuals (ages: 21 to 40) was used to determine the odour acceptability of the granola. A 10-point hedonic scale was employed; where, 10 = excellent, 9 = good, 7 = fair, 5 = poor, 3 = very poor. Samples scoring below 5 were considered to be unacceptable. The questionnaire used was modified from Warner (1995) (Figure 4.2.3). In order to reduce panelist bias at each sensory session, samples were assigned three-digit random numbers obtained from a random order table (Cochran & Cox, 1957). Panelists evaluated 100 g samples of granola at room temperature, using 50 g of each duplicate sample for a given treatment. The samples were placed in a cereal bowl-like, lidded polymer container (Ziploc, #6714009400,); each panelist opened the lid in order to evaluate odour. Scores were recorded on the questionnaire. Mean sensory scores were generated from individual panellists' scores to be used in the determination of the standard of acceptibility.

4.2.4 Thiobarbituric Acid (TBA) Test

Rancidity was quantitatively measured using the distillation method of the TBA test (Rossell, 1994; Kirk & Sawyer, 1991) since this test can be readily carried out on whole foods and measures the amount of moderately stable aldehydes formed as a result of the breakdown of the polyunsaturated fatty acids (Przybylski & Eskin, 1995).

Figure 4.2.3 Questionnaire for Sensory Evaluation of Omega-3 Enriched Granola

Name_____

Granola Odour Quality Evaluation

Directions: Smell the sample. Rate each sample for overall odour quality using a 10-point scale (i.e., fresh sample is rated "10"); placing an "x" or a check mark.

Overall Quality Scores

Sample #:		___	___	___	___	___	___	___	___	
10	excellent	___	___	___	___	___	___	___	___	10
9	good	___	___	___	___	___	___	___	___	9
8		___	___	___	___	___	___	___	___	8
7	fair	___	___	___	___	___	___	___	___	7
6		___	___	___	___	___	___	___	___	6
5	poor	___	___	___	___	___	___	___	___	5
4		___	___	___	___	___	___	___	___	4
3	very poor	___	___	___	___	___	___	___	___	3
2		___	___	___	___	___	___	___	___	2
1		___	___	___	___	___	___	___	___	1

Each granola sample was tested for TBA values in duplicate. Ten g of granola were macerated with 50 mL of distilled water for 2 minutes in a blender (Osterizer®, 4071-5 with attachment 4937, Sunbeam Corp. Ltd., Toronto, ON) and the mixture was then washed into a distillation flask with 47.5 mL of distilled water. A 2.5 mL aliquot of 4 N hydrochloric acid was then added, along with a drop of antifoaming agent (Antifoam B, R06436, BDH Inc., Toronto, ON), and a few glass beads. The flask was then heated to a vigorous boil until 50 mL of distillate were collected. Five mL of this distillate were then pipetted into a test tube, and 5 mL of 0.2883% (w/v) TBA (4,6-Dihydroxy-2-mercaptopyrimidine, Acros, NJ) solution in 90% glacial acetic acid were added. Similarly, a blank tube was prepared using 5 mL of distilled water and 5 mL of reagent. The tubes were then stoppered and heated in a boiling water bath for 35 minutes; then cooled in water for 10 minutes. Using a spectrophotometer (Ultrospec® 1000, Pharmacia Biotech, Cambridge, England), the absorbance (D) was measured against the blank at 538 nm using 10 mm cells. The TBA number was then calculated as mg of malonaldehyde per kg of sample, which is equal to 7.8 times D (Kirk & Sawyer, 1991). Since the TBA test was performed on duplicates of each sample, the resulting values were averaged.

4.2.5 Linolenic Acid Content of Unacceptably Rancid Prototype

A sample of omega-3 enriched granola that had reached the end of its shelf-life (as determined by the preceding procedures) when stored at 21 °C, had its fatty acid profile determined using AOAC procedures 963.22 (41.1.29) and 969.33 (41.1.28) by SGS Bio-Lalonde Food Laboratory, Pointe-Claire, QC. This was done in order to report the percentage derease in LNA at the termination of the prototype's shelf-life.

4.3 Results

4.3.1 Sensory Analysis

The panelists detected the rapid deterioration in odour quality of the omega-3 enriched granola prototype when stored in air. Within weeks, almost all of these air-packaged samples were rated "unacceptable" (Figure 4.3.1). When a mean sensory score of 5 was reached, representing "poor" on the hedonic scale, the prototype samples stored at 21 °C had a shelf-life of < 10 weeks while those stored at 35 °C had only a shelf-life of < 6 weeks. Since breakfast cereal manufacturers expect their products to have a shelf-life of one year, air-packaging of the omega-3 prototype is unacceptable.

4.3.2 TBA Test

The enriched, air-packaged granola samples had steadily increasing TBA values at both 21 and 35 °C due to the breakdown of its unsaturated fatty acids. The resulting TBA curves are shown in Figure 4.3.2. Comparing Figure 4.3.1 with Figure 4.3.2, the results of the sensory analysis on the omega-3 prototype granola appeared to have a negative linear correlation with those obtained using the TBA test. That is, as TBA values increased, there was a corresponding decrease in sensory scores.

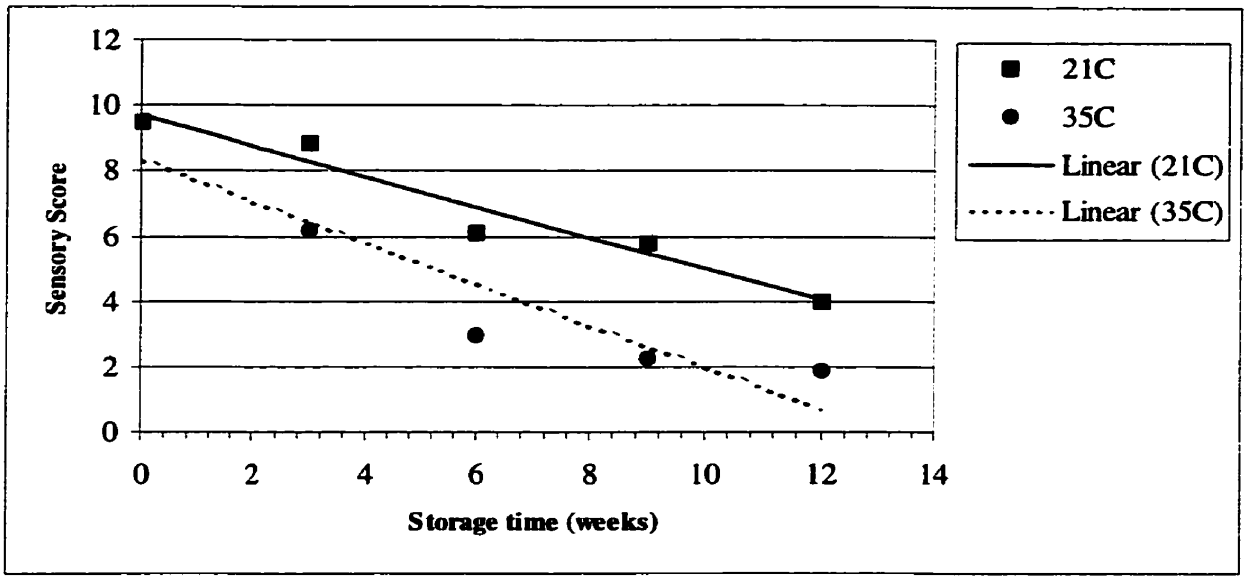


Figure 4.3.1 Sensory Scores for Omega-3 Enriched Prototype (12 weeks, air-storage, omega-3 enriched granola samples at both 21 and 35 ° C)

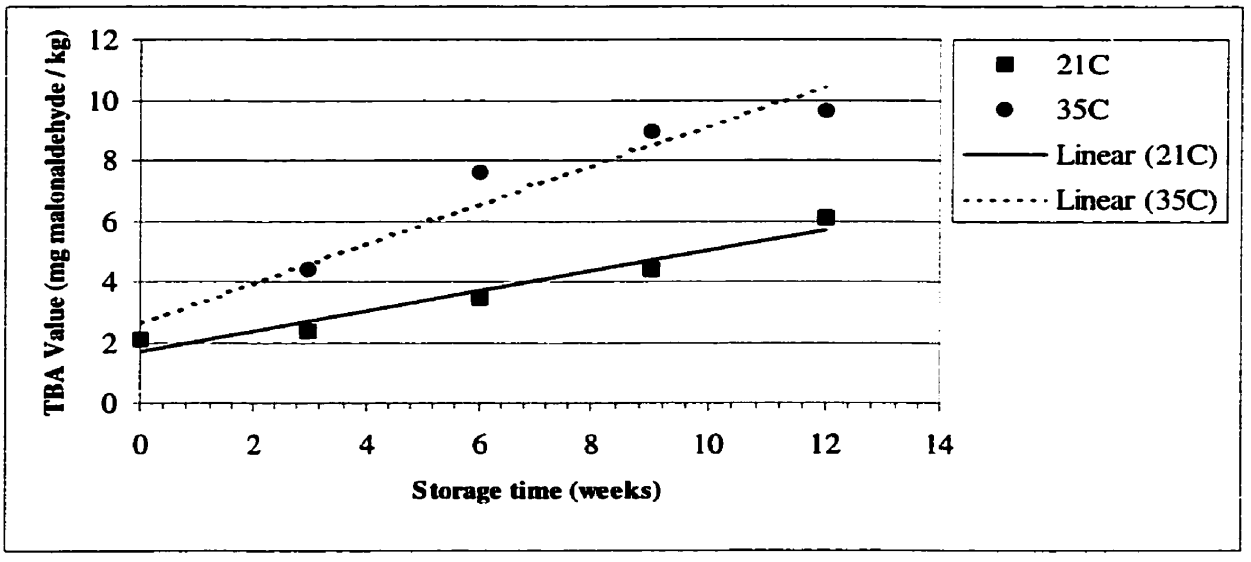


Figure 4.3.2 TBA Scores (mg malonaldehyde / kg) for Omega-3 Enriched Prototype (12 weeks, air-storage, omega-3 enriched granola samples at both 21 and 35 ° C)

4.3.3 Determination of the Standard of Acceptibility

A scatter plot (Figure 4.3.3) of TBA scores and the correlating sensory scores for all granola samples, combining data from both storage temperatures, during the first 12 weeks of storage was generated to determine the standard of acceptability, or TBA cut-off point. Using Microsoft Excel's least squares linear regression, the resulting curve demonstrated that when sensory scores were < 5 (i.e., unacceptable) this correlated with TBA scores > 5.00 mg malonaldehyde / kg. The correlation coefficient, or R^2, was 0.7206, which shows a fair correlation for the data. However, since lipid oxidation is autocatalytic and manufacturers want to guarantee a reasonable degree of "freshness" once the cereal is in the consumer's home, a TBA value of 4.00 mg malonaldehyde was selected as the minimum standard of acceptable quality and termination of shelf-life. Furthermore, from the data collected, it was observed that once the TBA score of a given sample exceeded 4.00 mg malonaldehyde / kg, it would not decrease below this value on continued storage.

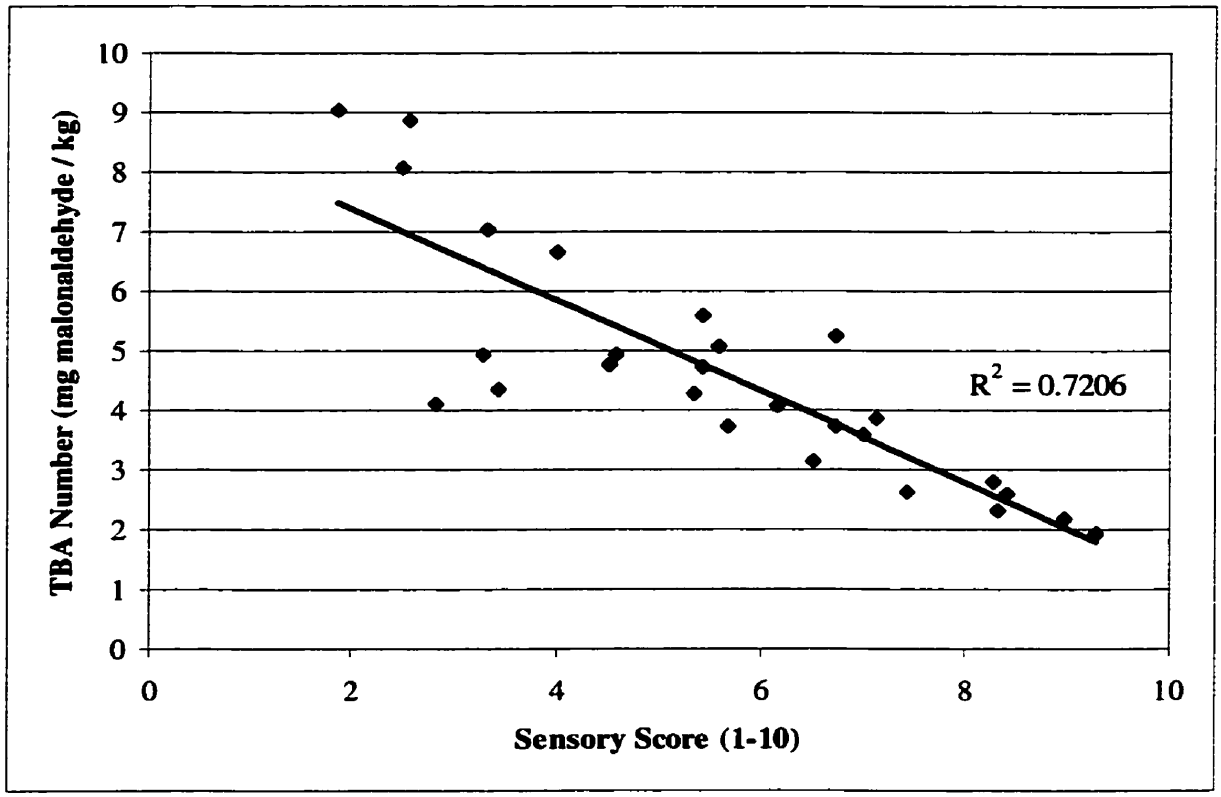


Figure 4.3.3 Scatter Plot with Linear Trendline of TBA Scores (mg malonaldehyde / kg) and the Correlating Sensory Scores (first 12 weeks, omega-3 enriched granola samples stored in air at both 21 and 35 ˚C)

4.3.4 Decrease in LNA Content of Prototype

A comparison of the fatty acid profiles of both fresh and rancid (at termination of shelf-life) prototype samples is shown in Table 4.3.4. It is evident that the levels of mono- and polyunsaturated fatty acids decreased, while the proportion of saturates increased. The polyunsaturates were degraded by more than 50%. The LNA acid level of the prototype decreased 62%, from 1.8% (w/w) to 0.68%, as determined by fatty acid profile analysis (Table 4.3.4). This is an important observation to be considered when developing packaging and marketing strategies for omega-3 enriched products. When health claims are being made on the package or in marketing, the enriched product needs to deliver an either equal or greater amount of omega-3 fatty acids as described on the package.

Table 4.3.4 Fat Analysis Comparison of Fresh and Rancid Omega-3 Enriched Granola

Fat	%	
	Fresh	Rancid*
Saturates	5.4	50.3
Monounsaturates	53.6	33.4
Polyunsaturates	41.0	16.3
- linolenic acid	8.1	4.3
- linoleic acid	28.3	11.9
- others	4.6	0.1

*stored at 21 °C, TBA score of 4.0 mg malonaldehyde / kg

4.4 Conclusion

The results of this study show that air-storage of the omega-3 enriched prototype is unacceptable for long-term storage. A standard of acceptability, based on the sensory scores and the TBA test values, was determined to be 4.0 mg malonaldehyde / kg. This value could then be used in the subsequent shelf-life study. The need to extend the shelf-life of the prototype was further emphasized by the significant decrease in LNA content when the product had reached the end of its shelf-life.

5. SHELF-LIFE INVESTIGATION FOR THE OMEGA-3 ENRICHED GRANOLA PROTOTYPE

5.1 Introduction

Breakfast cereal manufacturers usually require a shelf-life of one year for their products. Therefore, a one year shelf-life was the target for the omega-3 enriched prototype. The methods used to extend shelf-life included the use of a natural antioxidant and packaging under two modified atmospheres. The standard of acceptability determined in Section 4 was used to determine the end of the shelf-life for granola samples studied in this section. Hence, the objective of this study was to monitor the shelf-life of the prototype, based on lipid oxidation, packaged in air and under modified atmosphere packaging (MAP), with and without an antioxidant, and stored at 21 or 35°C.

5.2 Materials & Methods

5.2.1 Prototype Preparation

The omega-3 enriched prototype was produced as per the formulation outlined in Section 3.2. Since each oven batch produced 4 kg granola, and approximately 32 kg were required for the subsequent experiments, 8 oven batches were produced. Once each oven batch had cooled to room temperature, it was stored in a sturdy plastic bin. Each subsequent batch was added to the same bin, and the batches were mixed together in an effort to produce a homogeneous end-product.

5.2.2 Addition of Antioxidant

A natural AO was added to half of the granola prototype produced during manufacture, as it needed to be diluted in the canola oil. The AO used was a natural, food-grade tocopherol blend: Covi-ox® T-70 (Cognis, formerly Henkel Corporation, Fine

Chemicals Division, LaGrange, IL) which consists of 9% d-α tocopherol, 1% d-β tocopherol, 45% d-γ tocopherol, 15% d-δ tocopherol in a 30% vegetable oil diluent. This AO blend was used at a level of 300 ppm, as recommended by Cognis.

5.2.3 Packaging & Storage

Samples (100 g) of granola, with and without AO, were packaged in duplicate in oxygen-impermeable (Oxygen Transmission Rate < 0.1 cc/m^2/day at 0% Relative Humidity and 24°C) metallized laminate bags (15x26 cm, Polyester/Polyethylene/Foil/Polyethylene, VF52 Vaporloc Tamper Evident Barrier, LPS Industries, Moonachie, NJ) under three atmospheres: air, MA_1 (achieved by nitrogen flushing, Multivac A300/42, Wolfertschwenden, Germany) and MA_2 (achieved using an oxygen scavenger: Ageless® ZPT-200E, Mitsubishi Gas Chemical Co., Japan). The Ageless® ZPT-200E scavenger is a self-reacting type (i.e., it can react with oxygen without the need for moisture), and is designed for low water activity and high fat foods. A double heat seal (TEW Impulse Sealer, TISH-300, Taiwan) was used to reduce the probability of seal failure. Packaged samples were stored at two storage temperatures: 21°C (representing an air-conditioned supermarket) and 35°C (representing tropical conditions).

5.2.4 Test Intervals

Sample monitoring was initially conducted every three weeks, but due to the nature of the results obtained for the samples packaged under MAP, this period was extended to four, five, eight, nine, and up to 10 week intervals. When a sample had reached a TBA value of > 4.00 mg malonaldehyde / kg, as determined to be the minimum standard of acceptability

in Chapter 4, the shelf-life was terminated and the treatment no longer monitored for the remainder of the study.

5.2.5 Headspace Oxygen Monitoring

To monitor package integrity and atmosphere changes, prior to sensory and chemical analysis, the packaged granola was sampled for headspace gas composition. Headspace gas was withdrawn using a 0.5 mL gas-tight Pressure-Lok® syringe (Precision Sampling Co; Baton Rouge, LA) through a septum previously attached to the outside of each package. Headspace gas was analysed with a Varian gas chromatograph (model 3300, Varian Canada, Inc; Montreal, QC), fitted with a thermal conductivity detector and using Porapack Q and molecular sieve 5A (80-100 mesh) columns (Chromatographic Specialties; Brockville, ON) in series. Helium at 80 PSI was used as the carrier gas, the column temperature was 60°C, and both the detector and injector were run at a temperature of 100°C. Peaks were recorded and analysed with a Hewlett Packard integrator (model 3390A).

5.2.6 Control Test

Three control samples were prepared and studied to identify the ingredients contributing to lipid oxidation, as well as to determine if a sugar coating on the cereal was protective against autoxidation (Burns & Fast, 1990). The samples were heat-processed as they would be within the prototype. They included: toasted rolled oats (toasted for 30 min. at 165°C), toasted ground flaxseed (toasted for 10 min. at 165°C), and the toasted prototype without the honey and yellow sugar coating. These samples were stored in plastic bags, in air, at 21°C. They were evaluated at the start and at three week intervals for 12 weeks using the TBA test.

5.3 Results

5.3.1 Headspace Oxygen

Air-packaged granola samples consumed headspace oxygen with storage, indicating that autoxidation was taking place (Figure 5.3.1). There was a 4-5% decrease in headspace oxygen in all the samples once the TBA cut-off value of 4.00 mg malonaldehyde / kg was reached. The changes in headspace gas composition were significantly less than those observed by Lyver (1997); where air-packaged surimi nuggets stored at 4°C resulted in a 15% decrease in headspace oxygen due to the growth of aerobic psychrophilic bacteria. In the prototype, it was the chemical reaction of autoxidation which was responsible for diminishing oxygen levels. This decrease could possibly be used as an indicator for shelf-life termination (although no published studies demonstrate this) for air-packaged product. MAP-packaged products were also monitored for changes in atmospheres, but none were detected (i.e., oxygen levels were low/nil at the beginning of storage and remained that way throughout storage).

On three occasions, package leaks were discovered. On each of these occasions, the samples were also rancid. This raises the question: how will the manufacturer, retailer, and/or consumer know that a given package has spoiled due to a leak? This problem could be remedied by installing a leak detector for oxygen (e.g., the Ageless Eye®, produced by the Mitsubishi Gas Chemical Co.) on the package; this detector is in the form of a tablet which changes colour from pink to blue when there is an oxygen leak. However, because a foil laminate bag was used, a space on the final package design would have to be alloted for the leak detector to be visible through the package yet not allow for significant amounts of light to enter the package (e.g., small foil layer cutaway window).

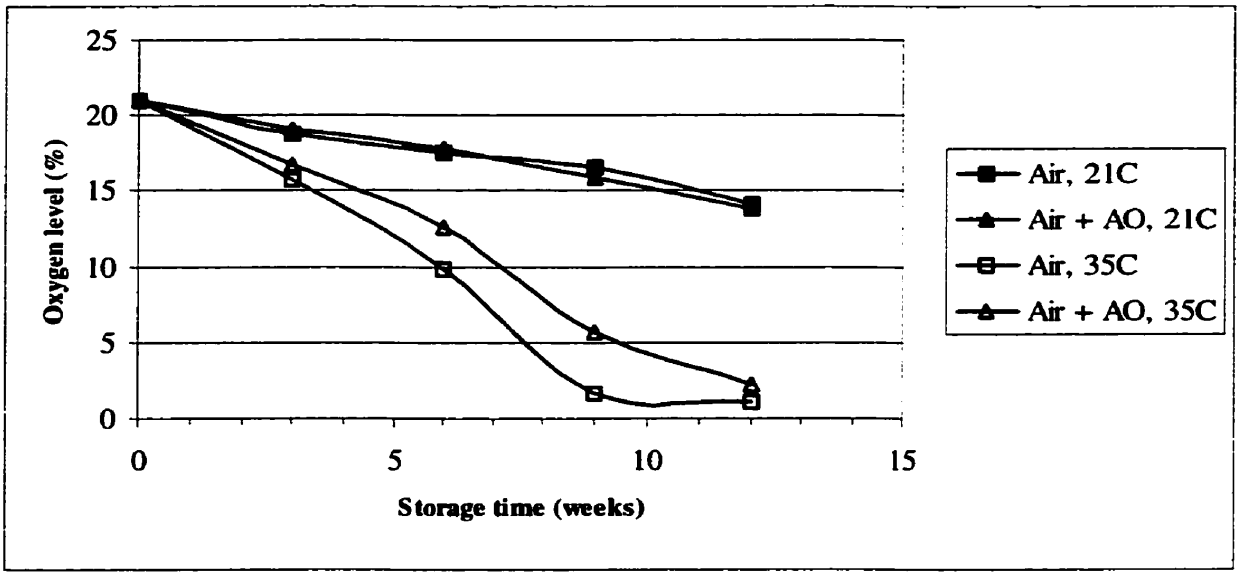


Figure 5.3.1 Headspace Oxygen for Air-Packaged Omega-3 Enriched Prototype (12 weeks of air-storage, with and without AO, at both 21 and 35°C)

5.3.2 Shelf-life Study

The TBA scores of the air-packaged omega-3 enriched granola sampies, with and without AO, stored at either 21 or 35°C, indicated that their shelf-lives were short : 6 and 2 weeks (without AO) and 9 and 3 weeks (with AO) at 21 and 35°C respectively (Figures 5.3.2.1 and 5.3.2.2). However, a shelf-life of one year was possible at these storage temperatures by packaging the granola (with and without AO) under modified atmospheres, MA_1 (nitrogen flushing) and MA_2 (oxygen scavenger) (Table 5.3.2). This shelf-life extension, using MAP to delay autoxidation, compares favourably with the findings of Sakamaki *et al.* (1988) on an oat breakfast cereal and the reportings of Subramaniam (1993) on coffee.The addition of the natural AO, Covi-ox T-70 (Cognis), appeared to protect the granola only during heat processing, as the TBA score for granola without AO was 2.32 versus 1.93 (mg malonaldehyde / kg) for the granola with AO. The protection offered by the AO during storage was insignificant compared to packaging under modified atmospheres (see Figures 5.3.2.1 and 5.3.2.2).

Table 5.3.2 Shelf-life of Omega-3 Enriched Prototype Under Different Atmospheres

Atmosphere ± AO	Shelf-life (months)*	
	21°C	35°C
Air	1.4	0.5
Air + AO	2.1	0.7
MA_1	12	12
MA_1 + AO	12	12
MA_2	12	12
MA_2 + AO	12	12

*based on time to reach a TBA value > 4.00 mg malonaldehyde / kg

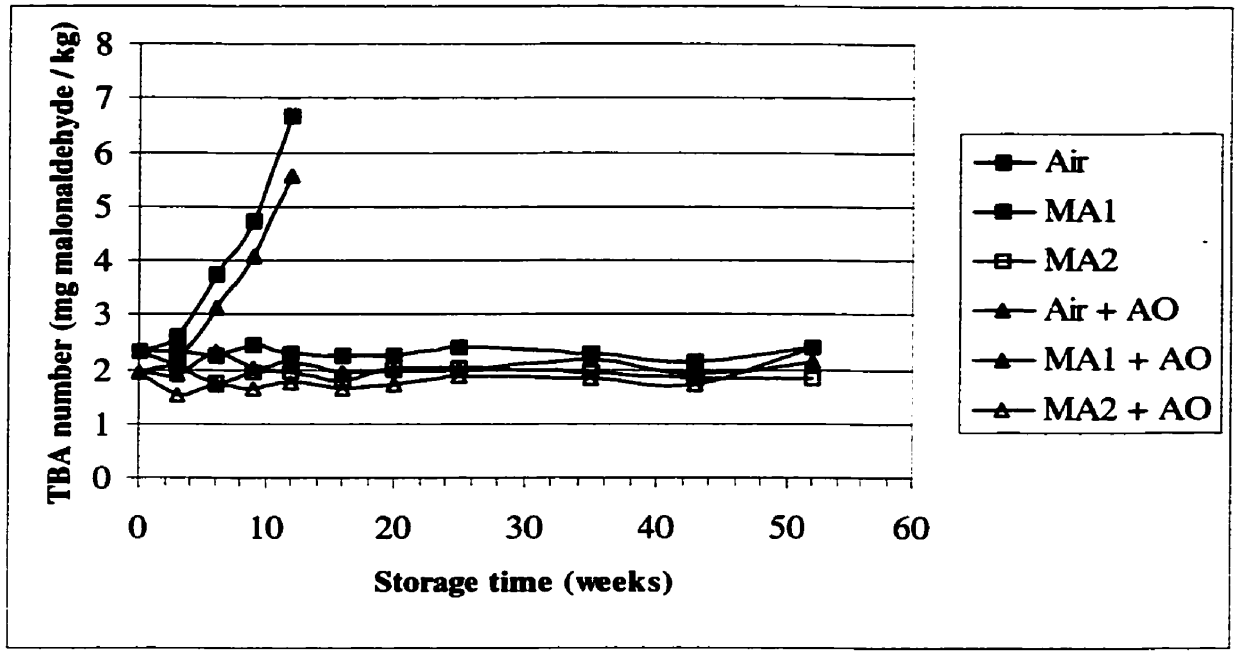


Figure 5.3.2.1 TBA Scores (mg malonaldehyde / kg) for Omega-3 Enriched Granola and Packaging Under 3 Different Atmospheres (air, MA_1, MA_2), With and Without AO, and Stored at 21 ° C

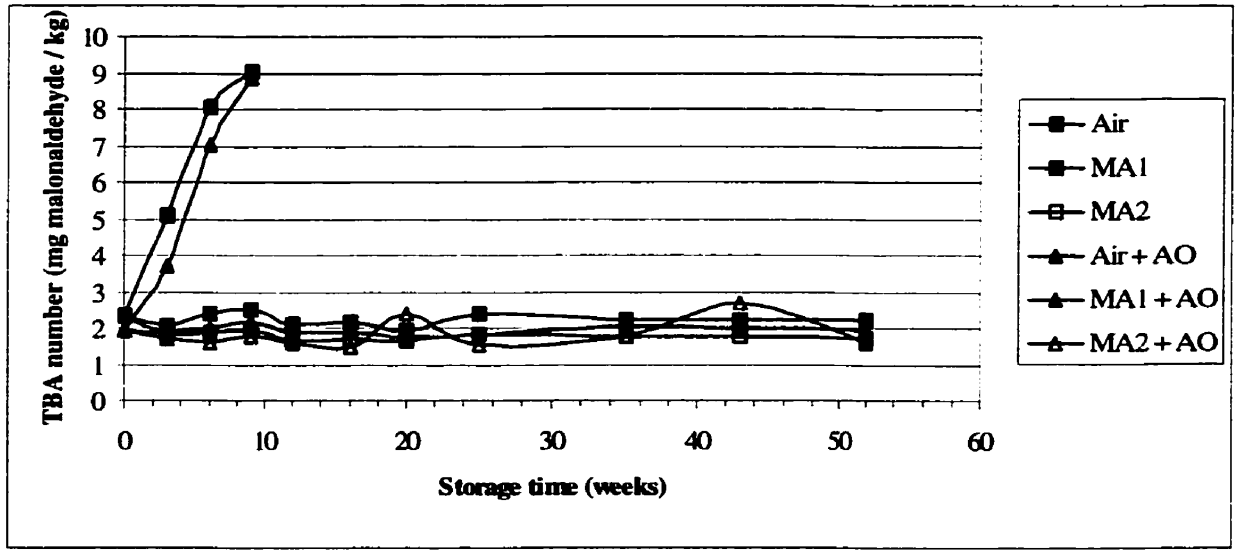


Figure 5.3.2.2 TBA Scores (mg malonaldehyde / kg) for Omega-3 Enriched Granola and Packaging Under 3 Different Atmospheres (air, MA_1, MA_2), With and Without AO, and Stored at 35 ° C

5.3.3 Control Test

The control test demonstrated two points: (i) the ground flaxseed was the principal contributor to overall rancidity in the prototype, and (ii) the honey-sugar coating was protective (Figure 5.3.3). The toasted, ground flaxseed reached a TBA value of 4.00 mg malonaldehyde / kg in 4 weeks. The granola without the honey-sugar coating reached 4.00 mg malonaldehyde / kg in approximately 4.5 weeks; versus 6 weeks for the enriched prototype. Also, since the flaxseed is the principal contributor to overall rancidity and since rancidity is autocatalytic, the need to ensure a fresh supply of flaxseed when preparing an enriched cereal product is of utmost importance. Interestingly, the honey-sugar coating was important in slowing rancidity in this product. The optimization of the even distribution of the honey-sugar coating could be useful if the prototype were to make it to market.

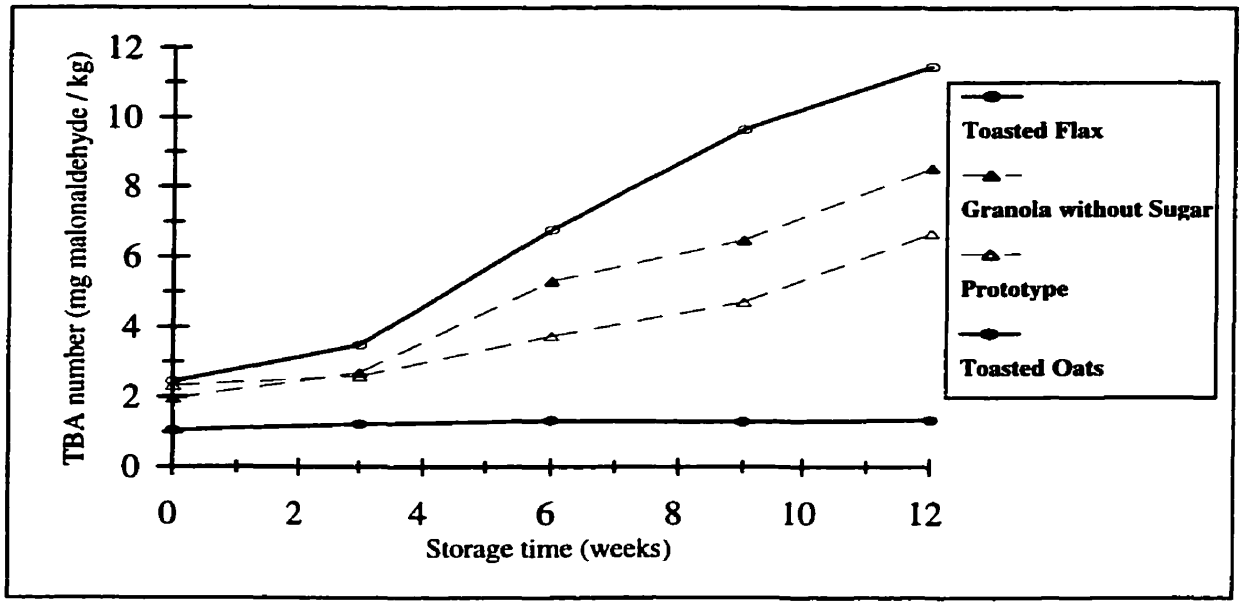


Figure 5.3.3 TBA Values for Air-packaged Toasted Flaxseed, Prototype without Sugar, Prototype, and Toasted Oats, 21 ° C Storage Temperature

6. CONCLUSIONS & RECOMMENDATIONS

In this study, an omega-3 enriched granola (high in LNA) was developed and found to have an LNA content of 1.8% w/w. This prototype was found to be sensorally acceptable to an untrained sensory panel and was composed of ingredients which would make it competitive in the marketplace against current commercial granolas. However, the prototype was susceptible to autoxidation when packaged in air. Since air-packaging was unacceptable, two methods were evaluated to extend shelf-life: the addition of a mixed tocopherol as an antioxidant, and the use of two modified atmospheres, each of which excluded oxygen.

The exclusion of oxygen using modified atmospheres increased shelf-life of product stored at 21 and 35 °C from 2-9 weeks to one year – the shelf-life expected by breakfast cereal manufacturers. Both nitrogen flushing and the use of an oxygen scavenger proved effective in extending the prototype's shelf-life. Provided the oxygen scavenger has a higher scavenging capacity than is required for achieving the initial low-oxygen atmosphere, the oxygen scavenger offers the additional benefit of continued oxygen absorption once the package has been opened and re-sealed using a zipper lock by the end consumer. Previous research in our laboratory have shown that oxygen absorbents retain their scavenging capacity after 3 or 4 openings of a package. After that time, they become saturated with oxygen, leading to a higher headspace oxygen level. This aspect of the packaging solution recommended could be studied further to simulate the life of the package once it is in the consumer's hands.

While the addition of the antioxidant offered negligible shelf-life improvement, it protected the granola during the original heat-processing, a significant observation to consider since rancidity is autocatalytic. The honey-sugar coating offered a protective effect,

another important observation to consider when formulating omega-3 enriched breakfast cereals and snack products. Also, since package leaks are inevitable with MAP products, a leak indicator for oxygen should be considered for all MAP-packaged omega-3 enriched products.

Finally, the use of oxygen absorbent technology offers the cereal industry a relatively simple and cost-efficient means of extending the shelf-life of low-moisture omega-3 enriched products. While the cost of the absorbent would increase the product price by five to ten cents per package, this cost could be offset by the elimination of antioxidants. Furthermore, sales of the product could be enhanced with MAP packaging by marketing the products as "natural and additive/preservative free".

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