Controlling Light Oxidation Flavor in Omega-3 Fatty Acid Enriched 2% Milk by Packaging Films

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Abstract

Milk is often packaged in translucent containers providing little protection against flavor degradation from light. Addition of omega-3 fatty acid sources into milk increases the risk of light-initiated degradation of nutrients and sensory quality.

The effectiveness of iridescent film materials in reducing light-induced oxidation of extended shelf-life omega-3 fatty acid enriched milk (2% total fat) was studied. Film selections were targeted to provide product visibility and control product exposure at targeted riboflavin excitation wavelength regions. Effectiveness was determined by sensory evaluation and measuring changes in volatile compounds on days 1, 7, 14, and 21 when stored under fluorescent light at 4 ℃. Five packaging treatments (films overwrapped on glass bottles) were evaluated: 446nm block, 570 nm block, broad spectrum block with 4% transmission (BS4T), light-protected (foil overwrap) control, and light-exposed (no overwrap) control. Experienced panelists (n=12) rated oxidized flavor intensity (0-9; 9=extreme) for milk samples. Light-protected milk was lower in oxidized flavor (mean score less than 3) throughout the storage period. Oxidized flavor in milk with BS4T film overwraps was not different compared to light-protected milk ($p>.05$) at the later stage (21 days), suggesting some level of protection to milk flavor. Milk without fish oil (milk fat only) shows relatively larger peak areas for 2-butanone on day 14, compared to other milk samples, suggesting antioxidants in the fish oil can prevent light oxidation. Overall, packaging that provides a complete light block is still the best way to prevent light-oxidized flavor in milk.

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CHAPTER I

INTRODUCTION

Omega-3 polyunsaturated fatty acids include α-linolenic acid (ALA, 18:3ω3), eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3). The health benefits associated with these fatty acids have been studied extensively for over 30 years. Omega-3 fatty acids affect inflammation (Cleland and others 2003), reduce the risk of cardiovascular disease (Zyriax and Windler 2000), play a role in the treatment of diabetes (el-Seweidy and others 2002; Gavia and others 2003), and reduce the risk of certain cancers, such as colorectal cancer and pancreatic cancer (Nkondjock and others 2003; Gogs and others 1998). Omega-3 fatty acids may also reduce risk of depression and suicide (Frasure-Smith and others 2004). Omega-3 fatty acids also help infants in developing retina tissue and brain function. Research demonstrating the health benefit of omega-3 fatty acids has led to increased public awareness of the value of these fatty acids in the diet.

The World Health Organization (WHO) recommends 800 to 1100 mg/day of ALA and 300 to 500 mg/day of EPA+DHA (Anonymous 2008). The main sources of omega-3 fatty acids are fish, seeds, and nuts, but overall the American diet has a relatively low level of omega-3 fatty acids. Dairy products, such as milk, which is a popular functional food in the market, are ideal vehicles for incorporation of DHA and EPA to deliver these nutrients to consumers. There are already several milk products launched in the market, such as: Smart Balance Omega Plus Buttery Spread (Smart Balance, Inc., Paramus, NJ), Breyers Smart DHA Omega-3 yogurt (Breyers Yogurt Company, North Lawrence, NV), Horizon Organic DHA Omega-3 milk (Horizon, Boulder, CO), and Silk Plus Omega-3 DHA Soy Milk (WhiteWave Foods, Broomfield, CO).

In the U.S. market, milk is commonly packaged in polyethylene terepthalate (PET) and high density polyethylene (HDPE). It is inexpensive to add titanium dioxide to HDPE to make the package opaque and prevent light oxidation. However, consumers prefer to actually see the products within packages when they make purchase decision (Chapman and others 2002; Doyle 2004). Therefore, milk is also packaged in clear glass or translucent PET bottles, which increases exposure of milk to light and photo-oxidation. Photo-oxidation of milk can cause nutrient loss and off-flavor development, with the potential for subsequent economic loss. White and Bulthaus (1982) indicated that 59% of retail milk samples were reported to have light-oxidized flavor defects. Barnard (1973) found that over 86% of milk in blow-molded plastic containers HDPE had light-oxidized flavor.

Milk enriched with omega-3 fatty acids is easily light-oxidized. Riboflavin is the main photosensitizer in milk, absorbing light in the ultraviolet (UV) and visible light regions. Riboflavin is very sensitive to light, and can cause light-oxidation in milk and degrade the quality of milk. For omega-3 fatty acids, the main issue is to protect EPA and DHA because they are highly unsaturated and are more susceptible to oxidation. Many different strategies have been studied to prevent oxidation in milk enriched with omega-3 fatty acids, such as change in oil types and quality, emulsifiers, package materials that block specific light wavelengths, and addition of antioxidants (Let and others 2006; Webster and others 2009).

The overall objectives of this study were:

1. To determine the effectiveness of iridescent film materials, as overwrap on transparent packages, in reducing or preventing light-induced oxidation in extended shelf-life omega-3 fatty acid fortified 2% milk. Effectiveness was determined by measuring changes in

volatile compounds and riboflavin destruction over 21 days at 4°C when stored under fluorescent light.

2. To determine sensory intensity of the oxidation flavor of extended shelf-life (21 days at 4 ℃) omega-3 fatty acid fortified 2% milk packaged with different light-blocking films.
3. To determine whether the antioxidants in fish oil (added as protection of the source oil) can prevent or reduce the oxidation in omega-3 enriched 2% milk when stored without any films and foils at 4 °C over 21 days under fluorescent light.

REFERENCES

Anonymous. Population nutrient intake goals for preventing diet-related chronic diseases. 2008 [cited 2011, June 17]; Available from: http://www.who.int/nutrition/topics5_population_nutrient/en/index13.html.

Barnard SE. 1973. Importance of shelf life for consumers of milk. Journal of Dairy Science 55: 134-6.

Chapman KW, Whited LJ, Boor KJ. 2002. Sensory threshold of light-oxidized flavor defects in milk. Journal of Food Science 67(7): 2770-2773.

Cleland LG, James MJ, Proudman SM. 2003. The role of fish oils in the treatment of rheumatoid arthritis. Drugs 63: 845–853.

Doyle M. 2004. Consumers have long list of packaging wishes and pet peeves. Food & Drug Packaging 68(8): 24-8.

El-seweidy MM, El-swefy SE, Ameen RS, Hashem RM. 2002. Effect of age receptor blocker and/or anti-inflammatory coadministration in relation to glycation, oxidative stress and cytokine production in stz (streptozotocin) diabetic rats. Pharmacological Research 45: 391–398.

Frasure-Smith N, Lesperance F, Julien P. 2004. Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biological Psychiatry 55: 891–896.

Gavia MH, Couto RC, Oyama LM. 2003. Diets rich in polyunsaturated fatty acids effect on hepatic metabolism in rats. Nutrition 19: 144–149.

Gogos AC, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos CN, Kalfarentzos F. 1998. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for reversibly ill patients with generalized malignancy. Cancer 82: 395–402.

Let MB, Jacobsen C, Meyer AS. 2006. Preventing oxidation in milk enriched with omega-3 fatty acids. Lipid Technology 18(4): 77-81.

Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. 2003. Specific fatty acids and human colorectal cancer: an overview. Cancer Detection and Prevention 27: 55–66.

Webster JB, Duncan SE, Marcy JE, O'Keefe SF. 2009. Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelengths by interference. Journal of Food Science 74(9): 390-398.

Wishner LA. 1964. Light induced oxidation in milk. Journal of Dairy Science 47:216–21.

Zyriax BC, Windler E. 2000. Dietary fat in the prevention of cardiovascular disease – a review. European Journal of Lipid Science and Technology 102: 355–365.

CHAPTER II

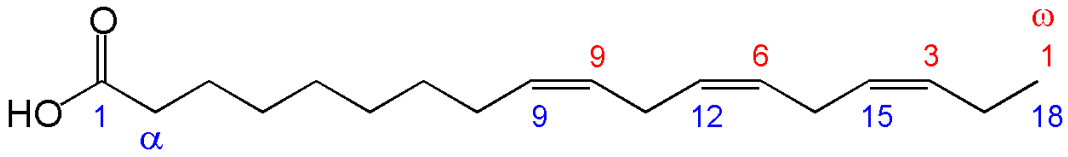
LITERATURE REVIEW

Omega-3 Fatty Acids and Health Benefits

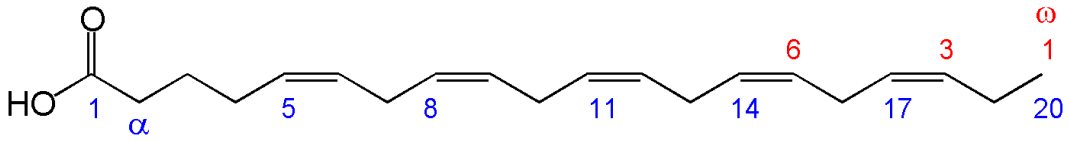
Omega-3 fatty acids are a group of essential unsaturated fatty acids that have the final carbon-carbon double bond in n-3 position, which is the third bond from the methyl end of the fatty acids. The long chain polyunsaturated fatty acids (PUFA) (C18, C20, and C22) include two essential fatty acids: linoleic acid (LA) and α-linolenic acid (ALA). Omega-3 PUFAs and omega-6 PUFAs are derived from these two different C18 fatty acids: the n-3 series are derived from ALA, and the n-6 series are derived from LA. Omega-3 PUFAs as well as omega-6 PUFAs are essential fatty acids because the human body cannot synthesize them, and thus must be supplied via the diet.

Nutritionally important n-3 PUFAs include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaneoic acid (DHA) (Figure 2.1). The primary source in the diet for DHA and EPA are fish, and the main sources in the diet for ALA are vegetable oils. Flax seed and fatty fish are main dietary sources for omega-3 fatty acids.

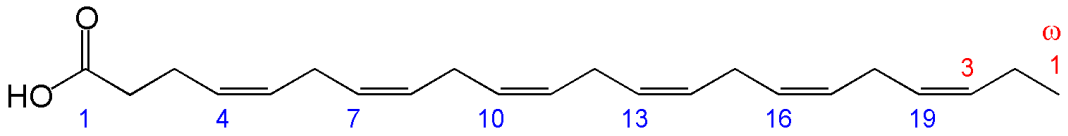
Omega-3 fatty acids have many health benefits. They have anti-inflammatory effects and can be used in treatment of inflammatory diseases such as eczema, psoriasis, and inflammatory bowel disease (IBD) (Cleland and others 2003). Omega-3 fatty acids also can reduce the risk of cardiovascular disease. Dietary cholesterol is one of main factors related to induce atherosclerosis. Omega-3 fatty acids can reduce plasma cholesterol and then prevent



ALA



EPA



DHA

Figure 2.1: Structures of ALA, EPA, and DHA. Adapted from:

http://en.wikipedia.org/wiki/Omega-3

myocardial infarction (Zyriax and Windler 2000). Moreover, study shows that PUFA may have roles in the treatment of diabetes because they can reduce blood sugar levels and the hazards associated with inflammation (el-Seweidy and ohers 2002; Gavia and others 2003). Omega-3 fatty acids also have protective effects on cancers, such as colorectal cancer and pancreatic cancer (Nkondjock and others 2003; Gogs and others 1998), while n-6 PUFA can increase the risk of tumor promotion (Wood and others 1996). Omega-3 fatty acids may also be related to human mental health. Study shows that intake of omega-3 fatty acids can reduce the risk of depression and suicide. Frasure-Smith and others (2004) tested the relationship between omega-3

serum levels in patients at the age of 54 years. Depressed patients had significant lower concentrations of total n-3 PUFAs.

n-3 PUFAs also play a role in eye health. One study, based on 3000 participants and conducted over 8 years, showed those participants who had higher consumptions of omega-3 fatty acids, EPA and DHA, were 25 % less likely to get age related macular degeneration (Chiu and others 2009). n-3 PUFAs also have functions in brain development. They can enable fluidity in neuronal membranes and help regulate neurotransmitters; both are important for the developing brain (Yehuda and others 1999). In 2004, the U.S. Food and Drug Administration (FDA) allowed manufacturers to use qualified health claims for omega-3 fatty acids for reduced risk of coronary heart disease in conventional foods that contain EPA and DHA. Scientific and popular press media information of the role of omega-3 fatty acids related to heart health and other health values inspire consumers to include food and supplement sources in their diet.

Omega-3 Fatty Acid Enriched Milk in the Market

In the typical Western diet, the consumption of long chain PUFAs of the n-3 family is inadequate. In the United States, intake of n-3 fatty acids is approximately 1.6 g, of which 1.4 g is ALA and 0.1-0.2 g is EPA and DHA (Kris-Etherton 2000). The World Health Organization (WHO) recommends 800 to 1100 mg/day of ALA and 300 to 500 mg/day of EPA+DHA (Anonymous 2008a). The American Heart Association (AHA) recommends 1.5 to 3 gram/day of ALA plus 500 to 1800 mg/day of EPA+DHA for beneficial health effects (Anonymous 2008b). WHO and AHA also recommend consuming fish (particular fatty fish) twice a week.

Milk is an ideal vehicle to carry healthy functional ingredients for several reasons. First, milk is a functional food because it is an excellent source of essential nutrients including protein, calcium,

phosphorus, potassium, riboflavin, vitamin B_5, vitamin B_{12}, iron as well as other vitamins and minerals. It also contains biologically active substances, such as immunoglobulins, enzymes, antimicrobial peptides, hormones, cytokines, and growth factors (Donovan 2006).
Second, milk has been associated with health benefits for many years. Calcium in milk helps in building strong bones and reduces the risk of bone disease. Drinking milk can also slow the rate of bone loss and help maintain bone density. Milk provides special benefits in weight management (Zemel 2002). Casein and whey protein in milk may help rebuild muscles after physical activities (Lemon 2000). Alpha-lactalbumin in milk may improve sleep quality and next-day alertness, and prevent insomnia (Markus and others 2005).

However, milk has a high proportion of saturated fatty acids, and is low in n-3 omega PUFAs. n-3 Fatty acids comprise approximately 0.66% of the FA in conventional milk, and around 1.1% of FA in organic milk; organic milk has higher proportion of n-3 fatty acids because of farming system, farm managements, and cow nutrients (Ellis and others 2006). To meet recommendations of consumption of PUFA, especially EPA and DHA, many companies try to incorporate marine oil into food products. A variety of milk products already are in the market, such as milk, yogurt, and cheese, which are fortified with omega-3 fatty acids. One of the potential problems for milk enriched with omega-3 fatty acids is increased risk of oxidation. DHA and EPA are very susceptible to oxidative deterioration because of the high degree of unsaturation.

Lipid Oxidation

Both milk and omega-3 fatty acids are susceptible to lipid oxidation and production of off-flavors. Lipid oxidation is a free radical oxidation, which has three basic steps: initiation, propagation, and termination. During the initiation step, a lipid loses a hydrogen radical initiated by catalysts, such as heat, light, or metals, and become a lipid free radical. The lipid free radical reacts with oxygen and turns into peroxy free radical. Peroxy radicals are extremely active. During the propagation step, the peroxy radical attracts a hydrogen atom from nearby unsaturated lipid, and forms a new lipid free radical and lipid hydroperoxide. The newly formed lipid free radical propagates the chain reaction, yielding more hydroperoxides, which are the primary oxidation products. In the termination step, free radicals react with other free radical species and form non-reactive secondary products. The primary oxidation hydroperoxide product is highly unstable, and easily interacts to form secondary products. The secondary oxidation products, such as aldehydes, ketones, and alcohols, in milk will cause fishy and other off flavors.

Volatile Compounds in Milk with Fish Oil

The oxidation reactions in milk enriched with fish oil can cause strong off-flavors, nutrition loss, and the reduction of product shelf life. Hexanal and pentanal are very typical secondary oxidation products, which form in the oxidation of unsaturated lipids in the milk (Rysstad and others 1998; Marsili 1999).

Proteins and amino acids, such as cystine, methionine and histidine, in milk also can undergo oxidative changes, cause nutrients loss, and form volatile compounds such as methionine sulfoxide and dimethyl disulfide (Jadhav and others 1996; Cadwallader and Howard 1998).

Dimethyl sulfide, 2-methylpropsanal, n-pentanal, n-hexanal, dimethyltrisulfide, and 1-octen-3-ol are identified as important oxidation compounds in milk (Cadwallader and Howard 1998). Because of the high degree of unsaturation of DHA and EPA, these fatty acids undergo rapid deterioration, which can cause problems in a fish oil-enriched food system. Highly unsaturated PUFAs lipids are more sensitive to oxidation than milk fat, which has more saturated fatty acids. In a complex food system, many factors can affect the initiation and propagation of oxidation, such as processing conditions, interfacial area, the types of fish oil and quality, transition metals, storage conditions, process condition, and type of emulsifier used (McClements and Decker 2000; Frankel 2005; Let and others 2006).

Solid phase microextraction/ gas chromatography (SPME/GC) is a good tool for monitoring volatile organic compounds in milk enriched with fish oil. Milk samples mixed UHT skimmed milk (95 vol-%) and cod oil (5 vol-%) was transferred into headspace vials and stored at 24 ℃ in the dark for 15 days. The concentration of hexanal, 1-penten-3-ol, *E*-2-hexanal, pentanal, *Z*-4-heptenal, heptenal, heptanal, and 3,5-octadien-2-one increased based on analysis by SPME/GC. None of these volatile compounds was detected in the milk without fish oil during storage (Jimenez-Alvarez and others 2008). (*E*,*E*)-2, 4-heptadienal, (E, Z)-2,6-nonadienal, and 1-penten-3-one were identified as important substances in related to the formation of fishy off-flavor (Let and ohers 2004).

The presence of certain compounds can influence the development of volatile compounds. Protein can inhibit lipid degradation by chelating metals, scavenging radicals and reacting with volatile compounds (Dalsgard and others 2005; Villiere and others 2005). Milk fat affects the formation of off-flavors. When milk fat increases from 0.5% to 3.4%, pentanal, heptanal, and

hexanal in the milk increase significantly while dimethyl disulfide concentration did not change (Lee and others 2002).

Riboflavin and Light Oxidation

Milk and dairy products are sensitive to oxidation initiated by light energy (photooxidation). Dairy products contain six types of photosensitizers: riboflavin, protoporphyrin, hematoporphyrin, a chlorophyll a-like compound, and 2 unidentified tetrapyrroles (Wold and others 2006). These photosensitizers absorb light in the UV and violet light region, but at different wavelength regions. The most studied photosensitizer is riboflavin.

Dairy products are sensitive to light because of the presence of riboflavin, which is both a vitamin (Vitamin B_2) and a photosensitizer. Riboflavin can be found in lean meats, eggs, milk, and vegetables, such as broccoli, mushroom and avocado (Szczesniak and others 1971). Riboflavin content in milk varies from 100 ug to 180 ug/100g (USDA 2011). Riboflavin plays a role in the production of energy and helps convert carbohydrates into sugar to fuel the body. It also helps in processing fat and amino acids for biological energy in the electron-transport system. Riboflavin is important for healthy hair, skin, eyes, nail growth, thyroid activity, and repair of tissues, and healing of the injuries. It is a yellowish, water-soluble compound, and is relatively stable in thermal or nonthermal food processing and storage. However, it is very sensitive to light, because it is easy to reduce or oxidize by accepting hydrogen and donating hydrogen or an electron.

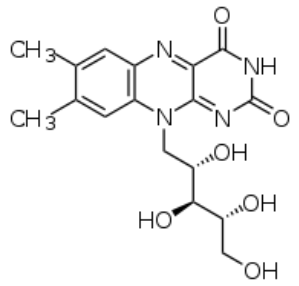


Figure 2.2: Riboflavin structure. Adapted from: http://en.wikipedia.org/wiki/Riboflavin

Riboflavin is a 7,8-dimethyl-10-(1.-D-ribityl) isoalloxazine; it has many conjugated double bonds and a nitrogen molecule in the ring structure (Figure 2.2). The cationic riboflavin is non-fluorescent, whereas the neutral riboflavin is fluorescent the anionic riboflavin is weakly fluorescent (static fluorescent quenching) (Drossler and others 2003).

The light sensitivity of food depends on its composition, particularly the presence of riboflavin, which acts as a photosensitizer. Riboflavin can absorb visible and UV light, and then transfer it into reactive forms of oxygen such as singlet or triplet oxygen (Min and Boff 2002). The dissolved oxygen in milk acts as a source of activated oxygen (singlet oxygen, superoxide anion radicals, hydroxy radical, and hydrogen peroxide), and amino acids, fatty acids, carbohydrates, and vitamins can serve as reactants in different chemical process. Riboflavin can photodegrade into lumichrome, lumiflavin, and others (Ahmad and others 2004; Ansari and others 2004). The wavelengths between 415 and 455nm are responsible for the degradation of riboflavin (Sattar and others 1977). Light at 455 nm is the most destructive to riboflavin.

Riboflavin reactions are described as follows:

1st Phase

Riboflavin + light ⟶ Excited riboflavin

Exited riboflavin + DH_2 (electron donor) $\longrightarrow$ Riboflavin-H_2 + D

Riboflavin –H_2 + O_2 $\longrightarrow$ Riboflavin + H^+ +O_2^-

2^{nd} Phase

Riboflavin –H_2 + O_2 $\longrightarrow$ Riboflavin + H_2O_2

O_2^- + H_2O_2 $\longrightarrow$ OH^- + HO^-+O_2

Riboflavin + O_2^- $\longrightarrow$ Riboflavin decomposition

(Bosset and others 1994; Toyosaki and others 1987).

Riboflavin loss by light depends on the wavelength, the light intensity, exposure time, packaging materials, and food processing (Choe and others 2005). Since riboflavin is very sensitive to light, unprotected milk can lose 30% of its riboflavin concentration when exposed to sunlight for 30 min (Wishner 1964).

Light Wavelengths

Intensity of the emission spectrum of light, the duration of light exposure, and the light transmittance of packaging materials are factors affecting the deterioration of food products (Hansen and others 1975; Bosset and others 1994). Kiermeier (1969) indicated that milk packaged in clear glass exposed to sunlight longer than 2 hours would reduce riboflavin content by more than 50%. Light also can induce the photooxidation of protein and amino acids. Sunlight and fluorescent light may provoke the hydrolysis of peptides. Cystine, methionine,

histidine, tyrosine are principal amino acids that can be affected by light (Jadhav and others 1996; Cadwallader and Howard 1998).

The visible wavelengths are between 380 and 780 nm, and ultraviolet (UV) light is between 200 and 380nm. For practical purposes, the range of wavelength of ultraviolet-visible radiation affecting food extends from 200 to 780nm (Rosenthal 1994). It is recognized that wavelengths between 420 to 520 nm can cause problems to food products, especially those containing riboflavin, but emerging research indicates that some molecules may also be photoresponsive and function as photoinitiators at wavelengths above 550 nm (Wold and others 2005). UV light is almost entirely absorbed by food packages, such as glass, polyethylene, and polyethylene-terephthalate. UV light has high energy, which can split certain chemical bonds (Bosset and others 1994).

In retail food markets, milk products are exposed to fluorescent light, which has a lower amount of energy compared to sunlight and, therefore, yet still causes riboflavin excitation and milk quality deterioration. In 1974, the average light intensity of display cabinets of 105 supermarkets was about 2000 lux (Dimick 1982).

Some authors found that blocking wavelengths between 400-500 nm could protect against riboflavin destruction, while blocking UV light did not protect against riboflavin destruction (Fukumoto and Nakashima 1975; Sattar and others 1977). However, Hansen and Skibsted (2004) found that light-induced formation of peroxides in a water-in-oil emulsion, such as dairy spreads, increased when the wavelength decreased from 436 nm to 405 nm, and then to 366 nm. Andersen and others (2008) found that riboflavin in Danbo cheese was degraded by 436 nm light wavelength when the cheese was exposed to light at wavelengths of 366, 436, or 546 nm.

Riboflavin has maximum absorption at 370 and 436 nm, and does not absorb at 546 nm. Riboflavin has three absorption bands: the first is in the UVB region (280-320 nm, the absorption maxima is 275nm), the second band is in the UVA region (320-380 nm, and the absorption maxima is 370 nm), and the third band in the visible region (blue to green, broad maximum at 430-460 nm), which is the main band responsible for the photo-oxidation of food, especially for the dairy products (Borle and others 2001; Drossler and others 2003). Lennersten and Lingert (2000) found that wavelengths from 410 nm to 450 nm had the greatest effect on color changes of mayonnaise with β-carotene added as colorant. The red and yellow fluorescent light, which had wavelength shorter than 470 nm did not affect color changes. Lipid oxidation was accelerated most by 365 nm, followed by 405 nm, and 435 nm.

Webster and others (2009) used iridescent films targeted to block different riboflavin excitation wavelengths as overwraps on glass packaging of milk. The overwrap treatments targeted two single visible riboflavin excitation wavelengths (400 nm, 446 nm) and all visible riboflavin excitation wavelengths; all treatments blocked UV riboflavin excitation wavelengths. Milk packaged in these different treatments were exposed under fluorescent light for up to 35 days at 4°C. They showed that blocking all visible and UV riboflavin excitation wavelength reduced oxidation flavor better than blocking only a single visible riboflavin excitation wavelength and UV riboflavin excitation. They demonstrated that even blocking specific wavelengths can reduce oxidation off-flavor but the best way to prevent oxidation off-flavor is to completely block light.

Bosset and others (1994) indicated that cold light (mainly in violet (380-440 nm) and blue (440-480 nm) light) is more harmful than warm light (mainly in yellow (580-600 nm), orange (600-620 nm), and red (620-750 nm) light) regarding to the deterioration of the milk. Hansen and others (1975) indicated that yellow and green (560-580 nm) filters gave the best protection

against the off-flavor in homogenized milk packaged in transparent polyethylene packages. Intawiwat and others (2010) found that milk samples stored under noncolored and orange (520-800 nm) filters were highly oxidized and red (570-800 nm), green (500-800 nm), and amber (500-800 nm) filter offered better protection against photooxidation.

Exposure of dairy products to different wavelengths can alter production of oxidation products. Lennersten and Lingert (2000) exposed low-fat mayonnaise under ultraviolet radiation, which was longer than 340 nm with the main irradiance at 365 nm, and found that mayonnaise oxidized very rapidly; this demonstrated that 365 nm was very effective in causing lipid oxidation. They also found that hexanal concentration for mayonnaise increased rapidly when exposed to blue light with emission peaks at 365, 405, and 435 nm, and between 410 nm to 470 nm, and the rate slowed when exposed to 365 nm alone. Mortenson and others (2003) exposed Havarti cheeses under monochromatic light of wavelengths 366 nm, 405 nm, and 436 nm, and found that no hexanal and 1-pentanol were found when exposed to 366 nm. The amount of hexanal and 1-pentanol was significantly lower when the cheese exposed at 405 nm than 436 nm.

Temperature of Milk Storage

Refrigerated storage of dairy products is a basic requirement for microbiological preservation. Moreover, the storage temperature should be as low as possible to limit the energy transmission and lower the rate of photooxidation. However, the solubility of oxygen in milk increases when the temperature becomes lower.

One study showed that the degree of oxidation was delayed in the milk enriched with omega-3 fish oil stored at 2 ℃ compared to those stored at 5 ℃ and 8 ℃, when measured by peroxide

and volatiles (Aparicio and McIntyre 1998). Recent research shows that the storage temperature between 2 to 9 ℃ affects the oxidative stability during storage, but only in the emulsions (milk enriched with fish oil) based on the oil with a peroxide value (PV) of 0.1 meg kg^{-1}, and does not affect the emulsions with PVs of 0.5, 1.0, and 2.0 meg kg^{-1}. However, the increasing temperature can promote the formation of volatiles (Let and others 2005).

Antioxidants

Milk oxidation can be affected by antioxidants, such as tocopherol, ascorbic acid, carotene, unsaturated fatty acid, riboflavin, EDTA. Tocopherols (Figure 2.3) are a class of chemicals that may have vitamin E activity. They are antioxidants, which can prevent or delay the oxidation, and be used as a food additive. Alpha-tocophorol can terminate free-radical chain reaction by donating hydrogen or electrons to free radical and converting them to more stable products (Frankel 2005). Tocopherols can reduce the rate of the initiation reaction in the free-radical chain and function at very low concentrations, 0.01% or less (Madhavi and others 1996).

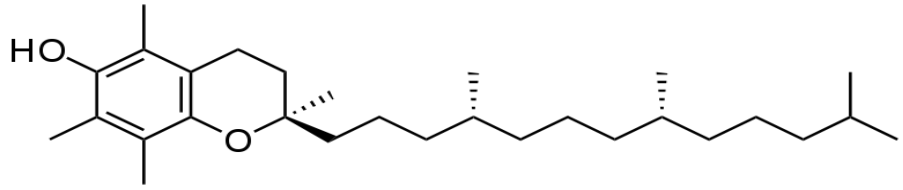


Figure 2.3: d-α-Tocopherol structure.

Adapted from: http://en.wikipedia.org/wiki/Tocopherol

Canola oil can function as an antioxidant, because it contains α-, β-, and the γ-tocopherol, while fish oil only has the α-tocopherol homologue. At lower concentrations (100ppm), the antioxidant

activity of different tocopherol homologues is $\alpha > \gamma > \beta$ in fish oil (Kulas 2001). Trolox is an α-tocopherol derivative, also commonly used as an antioxidant food additive and as an active ingredients in cosmetic products. Trolox also can donate hydrogen to food radicals and terminate the oxidation. Antioxidants in polymers are used to terminate polymeric reactions and incidental release into contained food may provide some protective effects.

Metal chelators in milk, such as milk protein lactoferrin also serve to provide protection against oxidation. EDTA is a metal chelating agent that can prevent the contact between transition metals and the unsaturated lipid or hydroperoxide. EDTA (5 mg/kg milk, water soluble) was able to retard oxidation in milk enriched with fish oil when PV of the fish oil was 1.5 meq/kg (Let and others 2003).

Milk Packaging

Packaging can prevent the development of light-induced off-flavor by preventing light and oxygen transmission. Generally, milk is stored in refrigerated cases and exposed to fluorescent light to attract consumers' attention. Packaging material is an important factor in the protection of riboflavin from photo-degradation in milk (Hoskin and Dimick 1979). Packaging materials that blocked all light were found to be best at protecting riboflavin from degradation. Hoskin (1988) found that aluminum and oriented polypropylene film protected milk from riboflavin degradation and off flavor development. Farrer (1983) found that paperboard was most effective at protecting against riboflavin destruction, followed by HDPE with TiO_2, and HDPE without TiO_2 (Table 2.1). Mestdagh and others (2005) found that milk packaged in a 3-layered 1 L PET

bottle consisting of white-black-white layers had no significant loss of riboflavin when exposed to 2500 lux fluorescent lighting at 18-25 ℃ for up to 2 months.

In order to eliminate the light transmission into food products, the ideal packaging material should be opaque or strongly light scattering. Tinted red-brown is another way to protect the light oxidation by minimizing the light absorption by riboflavin (Bosset 1994). To avoid light oxidation, light barrier properties can be used instead of amber (red-brown) coloration.

Table 2.1: Fluorescent light transmittance of different milk packaging materials

Packaging Materials	Thickness (mm)	Light Transmittance (%)
Clear flint glass	3.4	91
Clear polycarbonate	1.5	90
Tinted polycarbonate	1.5	75
Non returnable polyethylene	0.5	70
High density polyethylene	1.7	57
Unprinted fiberboard	0.7	4

Source: Dimick 1982

Polyethylene terephthalate (PET) and high density polyethylene (HDPE) are common package materials used in dairy packages. PET is a linear, transparent thermoplastic. It is tough, stiff, and strong in the glassy state. Crystallized PET containers have a higher degree of crystallinity, and are opaque white. PET films have outstanding proprieties as food packaging, including excellent chemical resistance, light weight, elasticity and stability at room temperature. PET is used in the milk industry as an effective product packaging.

HDPE is a nonpolar, linear thermoplastic, and has up to 90% crystallinity. Compared to low density polyethylene (LDPE), HDPE has better moisture barrier, and stiffer and harder, but is more opaque. HDPE film has a white, translucent appearance, and tends to compete more with paper in the market (Robertson 2005). However, HDPE has high oxygen permeability and low light barrier, which can cause nutritional losses and off-flavor in food products, such as milk (Drennan 1983; Nicolas 1995).

Lennersten and Lingert (2000) found that polyethylene naphthalate (PEN) and PEN/PET copolymer had a better protection for low-fat mayonnaise than PET regarding to color changes and lipid oxidation. However, none of PET, PEN, and PEN/PET offered sufficient protection against color changes. van Aardt and others (2001) found that the oxidation off-flavor of milk was significantly lower when packaged in amber PET containers, which block wavelength below 450 nm, compared to containers made of glass, HDPE, clear PET, and clear PET with UV block (PET-UV) when milk was stored at 4 ℃ and exposed to light of 1100 to 1300 lux for up to 18 days. Milk packaged in HDPE had significantly higher level of oxidation off-flavor than milk packaged with PET- UV. The amber PET blocked light from 300 to 400 nm and partially blocked light from 400 to 700 nm. PET-UV blocks light between 300 and 350 nm.

Pigmented glass bottles have been used in a lot of research for protection of the light-induced flavor in milk. Ruby glass bottle was found to be most protective for sunlight flavor, followed by amber, paper and clear glass bottles (Herreid and others 1952).

Sensory Changes

Light-oxidized flavors in milk are derived from two different reaction sequences. One reaction sequence involves protein and amino acids. The photodecomposition of methionine to methional

and other sulfur compounds can cause the milk to have off-flavors, which are described as burnt protein, burnt cabbage, mushroom, sulfur flavor, or plastic-like. This reaction happens quickly, usually within 10-15 minutes exposed to sunlight. The second reaction sequence involves unsaturated fatty acids. This reaction takes longer time, usually in 1-2 days. These off-flavors are characterized as old vegetable oil, cardboard, and metallic flavor.

Milk enriched with fish oil is an oil-in-water emulsion. The oxidative deterioration of fish oil can generate off-flavors even at low levels of oxidation (Karahadian and Lindsay 1989; Hartvigsen and others 2000). Pure menhaden oil when slightly oxidized has an initial aroma of green and cucumber-like, followed by the development of fishy and rancid flavor (Stansby and Jellinek 1965). t,c-2,6-Nonadienal has been identified as the primary compound that causes the green and cucumber-like aroma, and t-2-hexenal, 1,5-octadien-3-one and low concentrations of t,t,c,-2,4,7-decatrienal may give heavier green aroma. The rancid odors are caused by saturated and unsaturated aldehydes, including hexanal and heptadienals (Karahadian and Lindsay 1989).

The incidence of light oxidized milk is common. In an early study, 59% of retail milk samples were reported to have light-oxidized flavor defects (White and Bulthaus 1982). The threshold for light oxidation in reduced fat milk (2%) in HDPE containers and exposed to fluorescent light at 6 ℃ was between 54 minutes and 2 hours; for a trained sensory panel to detect flavor defects was between 15 to 30 minutes of light exposure (Chapman and others 2002). Chapman and others (2002) also suggested that approximately 50% of the plastic containers would remain in dairy cases exposed to fluorescent light for at least 8 hours, providing opportunity for the majority of milk in light-transmissible container on the market to undergo light oxidation and develop light-oxidized flavor.

REFERENCES

Anonymous. 2008a. Population nutrient intake goals for preventing diet-related chronic diseases. [cited 2011, June 17] Available from: http://www.who.int/nutrition/topics/ 5_population_nutrient/en/index13.html.

Anonymous. 2008b. Fish and omega-3 fatty acids. [cited 2011, June 17] Available from: http://www.americanheart.org/presenter.jhtml?identifier=4632.

Ahmad I, Fasihullah Q, Vaid FHM. 2004. A study of simultaneous photolysis and photoaddition reactions of riboflavin in aqueous solution. Journal of Photochemistry and Photobiolology B: Biology 75: 13-20.

Aparicio R, McIntyre P. 1998. Food authenticity: issue and methodologies, Eurofins Scientific, Nantes, France. 214p.

Andersen C, Andersen L, Hansen A, Skibsted L, Petersen M. 2008. Wavelength dependence of light-induced lipid oxidation and naturally occurring photosensitizers in cheese. Journal of Agricultural and Food Chemistry 56(5): 1611-1618.

Ansari IA, Vaid FHM, Ahmad I. 2004. Chromatographic study of photolysis of aqueous cyanocobalamin solution in the presence of vitamins B and C. Pakistan Journal of Pharmaceutical Sciences 17:19-24.

Bosset JO, Gallmann PU. and Siebe, R. 1994. Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products- a review In: Mathlouthi, M. (Ed.), Food Packaging and Preservation. London: Blackie Academic and Professional, 222-268.

Borle F, Sieber R, Bosset JO. 2001. Photo-oxidation and photoprotection of foods, with particular reference to dairy products: An update of a review article (1993–2000). Sciences des Aliments 21:571–590.

Cadwallader KR, Howard CL. 1998. Analysis of aroma- active components of light activated milk. In: Flavor Analysis: Developments in isolation and characterization/ACS Symposium Series. No. 700. American Chemical Society, Washington, DC. 343–358p.

Chapman KW, Whited LJ, Boor KJ. 2002. Sensory threshold of light-oxidized flavor defects in milk. Journal of Food Science 67(7): 2770-2773.

Choe E, Huang H, Min DB. 2005. Chemical reactions and stability of riboflavin in foods. Journal of Food Science 70(1): 28–36.

Cleland LG, James MJ, Proudman SM. 2003. The role of fish oils in the treatment of rheumatoid arthritis. Drugs 63: 845–853.

Chiu CJ, Klein R, Milton RC, Gensler G, Taylor A. 2009. Does eating particular diets alter the risk of age-related macular degeneration in users of the age-related eye disease. British Journal of Ophthalmology 93: 1–6.

Dalsgard TK, Nielsen JH, Larsen LB. 2005. Characterization of reaction products formed in a model reaction between pentanal and lysine-containing oligopeptide. Journal of Agriculture and Food Chemistry 54:6367–6373.

Dimick PS.1982. Photochemical effects on flavor and nutrients of fluid milk. Canadian Institute of Food Science and Technology Journal 15: 247-256.

Drennan B. 1983. Aseptic bottles. Journal of Food Engineering 55(6):64–5.

Drossler P, Holzer W, Penzkofer A, Hagamann P. 2002. pH dependence of the absorption and emission behavior of riboflavin in aqueous solution. Chemical Physics 282:429–39.

Donovan SM. 2006. Role of human milk components in gastrointestinal development: current knowledge and future needs. Journal of Pediatrics 149: S49-S41.

Kulas E, Ackman RG. 2001. Properties of a-, b- and d- tocopherol in purified fish oil triacylglycerols. Journal of the American Oil Chemists' Society 78: 360–367.

El-seweidy MM, El-swefy SE, Ameen RS, Hashem RM. 2002. Effect of age receptor blocker and/or anti-inflammatory coadministration in relation to glycation, oxidative stress and cytokine production in stz (streptozotocin) diabetic rats. Pharmacological Research 45:391–398.

Ellis KAIG, Grove-White D, Cripps P, McLean WG, Howard CV, Mihm M. 2006. Comparing the fatty acid composition of organic and conventional milk. Journal of Dairy Science 89: 1938-1950.

Farrer KTH. 1983. Light damage in milk: a comparison of the protective properties of paperboard cartons and plastic bottles. Blackburn, Victoria. Farrer Consultants.

Frankel EN. 2005. Lipid oxidation, 2nd ed.; PJ Barnes and Associates: Bridgewater, England.

Frasure-Smith N, Lesperance F, Julien P. 2004. Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biological Psychiatry 55: 891–896.

Fukumoto J, Nakashima K. 1975. Protection of riboflavin in liquid milk from destruction by light using color filters. Japanese Society of Nutrition and Food Science 28: 257-61.

Gaíva M, Couto R, Oyama L, Couto G, Silveira V, Ribeiro E, Nascimento C. 2003. Diets rich in polyunsaturated fatty acids: effect on hepatic metabolism in rats. Nutrition (Burbank, Los Angeles County, Calif.) 19(2): 144-149.

Gogos AC, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos CN, Kalfarentzos F. 1998. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for reversibly ill patients with generalized malignancy. Cancer 82: 395–402.

Hansen AP, Turner LG, Aurand LW. 1975. Fluorescent light-activated favor in milk. Journal of Milk Food Technology 38: 388-392.

Hansen EE, Skibsted LH. 2000. Light-induced oxidative changes in a model dairy spread. Wavelength dependence of quantum yields. Journal of Agricultural and Food Chemistry 48(8):3090-3094.

Hartvigsen KK, Lund PP, Hansen LF, Holmer GG. 2000. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. Journal of Agricultural and Food Chemistry 48(10): 4858-4867.

Herreid EO, Ruskin B, Clark GL, Parks TB. 1952. Ascorbic acid and riboflavin destruction and flavor development in milk exposed to the sun in amber, clear paper and ruby bottles. Journal of Dairy Science 35:772–8. In: Bradley R. 1983. Eliminating light-activated flavor. How processors and grocers can join forces to get results. Dairy Record 84(9): 168.

Hoskin JC. 1988. Effect of fluorescent light on flavor and riboflavin content of milk held in modified half-gallon containers. Journal of food protection 51(1): 10-23.

Hoskin JC, Dimick PS. 1979. Evaluation of fluorescent light on flavor and riboflavin content of milk held in gallon returnable containers. Journal of food protection 42(2): 105-9.

Intawiwat NN, Pettersen MK, Rukke EO, Meier MA, Vogt GG, Dahl AV, Wold JP. 2010. Effect of different colored filters on photooxidation in pasteurized milk. Journal of Dairy Science 93(4): 1372-1382.

Jadhav SJ, Nimbalkar SS, Kulkarni AD, Madhavi DL. 1996. Lipid oxidation in biological and food systems. Pages 5–64 in Food Antioxidants: Technological, Toxicological, and HealthPerspectives. MadhaviDL, Deshpande SS, Salunkhe DK. Marcel Dekker, Inc., New York, NY.

Jimenez-Alvarez D, Giuffrida F, Golay P, Cotting C, Destaillats F, Dionisi F, Keely B. 2008. Profiles of volatile compounds in milk containing fish oil analyzed by HSSPME-GC/MS. European Journal of Lipid Science and Technology 110: 277-283.

Kiermeier F, Waiblinger W. 1969. Effect of fluorescent lighting on ascorbic acid and riboflavin contents of milk in polyethylene packs. Zeitschrift für Lebensmittel-Untersuchung und -Forschung141: 320-331.

Karahadian CC, Lindsay RC. 1989. Evaluation of compounds contributing characterizing fishy flavors in fish oils. Journal of the American Oil Chemists' Society 66(7): 953-960.

Kris-Etherton PMT, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD. 2000. Polyunsaturated fatty acids in the food chain in the United States. American Journal Clinical Nutrition, 71: 179-188.

Lee J. 2002. Photooxidation and photosensitized oxidation of linoleic acid, milk, and lard. PhD dissertation, Columbus, Ohio: The Ohio State University.

Lemon PW. 2000. Beyond the zone: protein needs of active individuals. Journal of the American College of Nutrition 19: 513-521.

Lennersten MM, Lingnert HH. 2000. Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. Lebensmittel-Wissenschaft und Technologie 33(4): 253-260.

Let MB, Jacobsen C, Frankel EN, Meyer AS. 2003. Oxidation flavor deterioration of fish oil enriched milk. European Journal of Lipid Science and Technology 105: 518-528.

Let MB, Jacobsen CC, Meyer AS. 2004. Effects of fish oil type, lipid antioxidants and presence of rapeseed oil on oxidative flavour stability of fish oil enriched milk. European Journal of Lipid Science and Technology 106(3): 170-182.

Let MB, Jacobsen CC, Meyer AS. 2005. Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. International Dairy Journal 15(2): 173-182.

Let MB, Jacobsen CC, Meyer AS. 2006. Preventing oxidation in milk enriched with omega-3 fatty acids. Lipid Technology 18(4): 77-81.

Madhavi DL,Deshpande SS, Salunkhe DK. 1996. Introduction.1–4 in Food Antioxidants: Technological, Toxicological, and Health Perspectives. Madhavi DL, Deshpande SS, Salunkhe DK, ed. Marcel Dekker, Inc. New York, NY.

Markus CR, Jonkman LM, Lammers JM, Deutz NP, Messer MH, Rigtering NN. 2005. Evening intake of α-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. American Journal of Clinical Nutrition 81(5):1026-1033.

Marsili RT. 1999. Comparison of solid phase micro-extraction and dynamic headspace method for the gas chromatographic-mass spectrometric analysis of light-induced lipid oxidation products in milk. Journal of Chromatographic Science 37:17–23.

McClements DJ, Decker EA. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. Journal of Food Science 65 (8): 1270–1282.

Mestdagh FF, Meulenaer B, Clippeleer J, Devlieghere FF, Huyghebaert AA. 2005. Protective influence of several packaging materials on light oxidation of milk. Journal of Dairy Science 88(2): 499-510.

Min DB, Boff JM. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Review in Food Science and Food Safety 1:58–72.

Mortensen G, Sørensen J, Danielsen B, Stapelfeldt H. 2003. Effect of specific wavelengths on light-induced quality changes in Havarti cheese. The Journal of Dairy Research 70(4): 413-421.
Nicolas R. 1995. Aseptic filling of UHT dairy products in HDPE bottles. Food Tech Europe 2(1): 52.

Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. 2003. Specific fatty acids and human colorectal cancer: an overview. Cancer Detection and Prevention 27: 55–66.

Robertson GL. 2005. Food packaging: principles and practice. Boca Raton, FL 33487, USA; CRC Press.

Rysstad G, Ebbesen A, Eggestad J. 1998. Sensory and chemical quality of UHT-milk stored in paperboard cartons with different oxygen and light barriers. Food Additives and Contaminants 15(1):112-122.

Sattar A, deMan JM, Alexander JC. 1977. Wavelength effect on light-induced decomposition of vitamin A and beta-carotene in solutions and milk fat. Canadian Institute of Food Science and Technology. Aliment 10: 56-60.

Stansby M.E, Jellimek G.1965. The Technology of Fish Utilization Contributions from Research, edited by R. Kreuzer, Fishing News Ltd., London, England. 171-176p.

Szcześniak T, Karabin L, Szczepankowska M, Wituch K. 1971. Biosynthesis of riboflavin by Ashbya gossypii. I. The influence of fats of the animal origin on the riboflavin production. Acta Microbiologica Polonica. Series B: Microbiologia Applicata 3(1): 29-34.

Toyosaki TT, Yamamoto AA, Mineshita TT. 1987. Antioxidant effect of riboflavin tetrabutylate in emulsions. Journal of Food Science 52(5): 1377-1380.

[USDA] U.S. Dept. of Agriculture. 2011. USDA Natl. nutrient database for standard reference. Release 16. Nutrient lists. [cited 2011, June 17] Available from: http:// www.nal.usda.gov/fnic/foodcomp/Data/SR16/wtrank/wt_rank.html.

van Aardt M, Duncan SE, Marcy JE, Long TE, Hackney CR. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. Journal of Dairy Science 84(6):1341–7.

Villiere A, Viau M, Bronnec I, Moreau N, Genot C. 2005. Oxidative stability of bovine serum albumin- and sodium caseinate-stabilized emulsion depends on metal availability. Journal of Agricultural and Food Chemistry 53: 1514–1520.

White CH, Bulthaus M. 1982. Light activated flavor in milk. Journal of Dairy Science 65:489-494.

Wold JP, Veberg A,Nilsen A, Iani V, Juzenas P, and Moan J. 2005. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. International Dairy Journal 15:343–353.

Wood OL, Spark A, Spark A. 1996. Medical nutrition therapy guidelines for treating the breast cancer patient. Journal of the American Dietetic Association, 96(6): A35.

Wishner LA. 1964. Light induced oxidation in milk. Journal of Dairy Science 47:216–21.

Yehuda S, Rabinovitz S, Mostofsky DI. 1999. Essential fatty acids are mediators of brain biochemistry and cognitive functions. Journal of Neuroscience Research 56: 565–570.

Zemel MB. 2002. Regulation of adiposity and obesity risk by dietary calcium: Mechanisms and implications. Journal of the American College of Nutrition 21 (2): 146–51.

Zyriax BC, Windler E. 2000. Dietary fat in the prevention of cardiovascular disease – a review. European Journal of Lipid Science and Technology 102: 355–365.

CHAPTER III

CONTROLLING LIGHT OXIDATION FLAVOR IN OMEGA-3 FATTY ACID ENRICHED 2% MILK BY PACKAGING FILMS

INTRODUCTION

Increased consumer awareness of the health benefits of omega-3 fatty acids motivates the food industry to develop more omega-3 fatty acid related food products (Miraglio 2006). Omega-3 fatty acids have anti-inflammatory properties to prevent diseases such as inflammatory bowel disease (IBD), eczema, psoriasis and rheumatoid arthritis (Cleland and others 2003). Additional health benefits include protective effects against cardiovascular disease and diabetes. Omega-3 fatty acids also are associated with improving brain health and preventing neurological disorders. In 2004, the Food and Drug Administration (FDA) approved a health claim for omega-3 fatty acids for reducing the risk of coronary heart disease.

The dairy industry has responded to increased consumer interest for dietary sources of omega-3 fatty acids by marketing omega-3 enriched milk products, including fluid milk (Horizon, Broomfield, CO), Breyer's Smart DHA Omega-3 yogurt (Breyers Yogurt Company, North Lawrence, NY). It is estimated the US market was worth $2 billion for foods and beverages bearing EPA, DHA and ALA either in combination or alone in 2008, and the market is predicted to expand to $7 billion by 2011 (Packaged Facts 2009). In 1993, the cash receipts from dairy products accounted for $19.6 billion, ranking third after meat animals and eggs (Stillman 1995). In 2005, fluid milk accounted for $27 billion for cash receipts and the U.S. consumption was estimated at more than 6 billion gallons of milk per year (Anonymous 2006). However, the

addition of omega-3 rich lipids provides a market extension for value-added milk and dairy products. The addition of omega-3 fatty acids into dairy products increases the risk of autooxidation and potential for changes in sensory characteristics. Oxidation of unsaturated fatty acids produces saturated aldehydes and a variety of other minor products, such as vinyl ketones, alkenals, and alkadienals, which mainly contribute to off-flavors in milk (Gudipati and others 2004).

An important factor influencing oxidation flavor in milk is exposure to certain light wavelengths (Webster and others 2009). Wavelengths between 365 and 500 nm cause a significant increase in light oxidation flavors in milk (Herreid and others 1952; Bradfield and Duthie 1956; Sattar and others 1976). Riboflavin is implicated in this oxidation because it acts as a photosensitizer when exposed to specific wavelengths within this range (Bekbolet 1990). Other compounds, such as porphyrin molecules, may also act as photosensitizers (Wold and others 2005). Chlorophyll, which has a porphyrin structure, is naturally occurring in omega-3 fatty acid-rich lipids that come from algal sources and may exist in lipids extracted from fish as well. Photosensitizers accelerate reaction rates of biomolecules, including amino acids and unsaturated fatty acids, such as decosahexanenoic acid (DHA) and eicosapentaenoic acid (EPA) (Choe and Min 2003). Because of the high level of unsaturation in EPA and DHA, oxidation can readily occur in omega-3 fatty acid enriched milk, which leads to reduction of the nutrition level and the formation of small molecular weight volatile compounds that may affect odor and flavor of milk. There is an even greater need to protect the integrity of milk when oxidation sensitive omega-3 fatty acids are added.

Packaging materials can protect against photo-oxidation by blocking or reducing the transmission of certain wavelengths. Commercially, milk products are most frequently packaged in high density polyethylene (HDPE) or polyethylene terephthalate (PET), which transmit between 62% and 85% of light wavelengths between 300-700 nm. TiO_2, ultraviolet (UV) absorbers, can be added to polymer packaging materials that block UV wavelengths without affecting the clarity of the package, but these absorbers do not affect the transmission of visible wavelengths. Milk manufacturers add simple and inexpensive compounds, such as titanium dioxide, when thermoforming HDPE packaging containers, which reduce the transmission of visible wavelengths through the packaging. However, consumers prefer to "see" the food when they are buying (Doyle, 2004); because of this, photo-labile products such as milk are often packaged in transparent containers, even though these containers do not protect against flavor degradation. To develop packaging materials that permit consumers to see the product and also provide a light block or reduce the light wavelengths, polymer materials with unique optical properties, such as iridescent films, may provide an option for packaging value-added milk products, such as omega-3 fatty acid enriched milk.

A price comparison of fluid milk in the local market (Blacksburg, VA) provided a perspective of the cost of packaging and value-added nutrition associated with omega-3 fatty acid addition to milk (Table 3.1). In the local market (Blacksburg, VA), milk products are sold in various packages. Based on addition of omega-3-rich oils, a half gallon Organic Horizon 2% reduced unflavored fat milk (Horizon, Broomfield, CO) is $0.015 cheaper than a half gallon Organic Horizon 2% reduced fat milk with DHA omega-3 (Horizon, Broomfield, CO). Based on the package, milk packaged in HDPE and PET is generally cheaper than milk packaged in

paperboard. The extra cost between milk with DHA and without DHA is probably because of the addition of DHA in the milk.

Table 3.1: Packages and prices for different brands of milk products in the local grocery store (Blacksburg, VA).

Product Name	Description	Packages	Price	Price per ounce	Shelf-life
8 ounce Horizon Organic vanilla reduced fat milk (Horizon, Broomfield, CO)	Flavored reduced fat organic milk	Paperboard	$1.39	$ 0.174	Extended shelf-life (Around 2 months)
Half gallon Organic Horizon 2% reduced fat unflavored milk (Horizon, Broomfield, CO)	Reduced fat organic milk	Paperboard	$3.99	$0.062	Extended shelf-life (Around 1 month)
One gallon Organic Horizon 2% fat reduced fat unflavored milk (Horizon, Broomfield, CO)	Reduced fat organic milk	HDPE	$6.49	$0.051	Standard shelf-life (Around 2 weeks)
Half gallon Organic Horizon 2% reduced fat unflavored milk with DHA omega-3 (Horizon, Broomfield, CO)	Reduced fat organic milk with added omega-3 fatty acids	Paperboard	$4.99	$0.078	Extended shelf-life (Around 1 month)
One pint Kroger 2% reduced fat milk Vitamin A &D added (The Kroger Co. Cincinnati, OH)	Reduced fat vitamin A & D added milk	PET	$0.99	$0.062	Standard shelf-life (Around 2 weeks)

Product Name	Description	Packages	Price	Price per ounce	Shelf-life
One quart Kroger 2% reduced fat milk Vitamin A &D added (The Kroger Co. Cincinnati, OH)	Reduced fat vitamin A & D added milk	PET	$1.49	$0.047	Standard shelf-life (Around 2 weeks)

Therefore, our study can provide suggestions to the dairy processing and packaging industries for protecting against the light oxidation of omega-3 enriched milk, and assist in identifying new packaging materials for protecting milk products.

The overall objectives of this study are:

1. To determine the effectiveness of iridescent films materials, as overwrap on transparent packages, in reducing or preventing light-induced oxidation in extended shelf-life omega-3 fatty acid fortified 2% skim milk. Effectiveness will be determined by measuring changes in volatile compounds and riboflavin destruction over 21 days at 4°C when stored under fluorescent light.
2. To determine sensory intensity of the oxidation flavor of extended shelf-life (21 days at 4 ℃) omega-3 fatty acid fortified 2% skim milk packaged with different light-blocking films.
3. Determine whether the anti-oxidants in fish oil (added as protection of the source oil) can prevent or reduce the oxidation in omega-3 enriched 2% fat milk when stored without any films and foils at 4 °C over 21 days under fluorescent light.

MATERIALS AND METHODS

Milk Product Preparation and Packaging

Milk Manufacture. Fresh raw milk was obtained from the Virginia Tech dairy farm and processed in the Food Science and Technology dairy pilot plant within 24 hrs of collection. Raw milk was stored at 4°C before processing. Before pasteurization, milk was pre-warmed (55 ℃) and separated into cream and skim milk using a pilot plant separator (Model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Canada). Fresh raw cream and fish oil (Ocean Nutrition Canada Ltd., Nova Scotia, Canada) were added to skim milk and standardized to 2.0 ± 0.1% fat (Table 3-2). Milk with fish oil (total milkfat plus fish oil = 2%) was homogenized in a 2-stage homogenizer (10,339 kpa (1500 psi)—first stage; 3,446 kpa (500 psi)—second stage) (Type DX, Cherry Burrel Corp., Delavan, Wisconsin) and ultra high temperature (UHT) pasteurized at 131.1 ºC (268 ºF) for 2 sec (UHT/HTST Lab-25 DH pasteurizer, MicroThermics, Raleigh, NC). Another portion of milk (2L) was processed to 2.0 ± 0.1% milkfat, without adding fish oil, to serve as the control.

Table 3.2: Relative percents[1] of fish oil and milk fat, as percent of total fat, added to achieve targeted[2] EPA and DHA addition in 2% fat milk.

	Fish oil[3] (%)	Milk fat (%)
Milk enriched with fish oil	13.6	86.4
Milk without fish oil	0	100

[1]Detailed calculation is in Appendix G.

[2]Targeted amount of EPA and DHA is 704 mg in 1 L milk.

[3]EPA and DHA in fish oil. The EPA+DHA (mg/g) as free fatty acids in the fish oil is 250 mg/g (Ocean Nutrition Canada Ltd., Nova Scotia, Canada).

UHT processed milk, with and without fish oil, was collected separately in 1 L sterile glass bottles under a laminar flow hood (Atmos-Tech Industries, Ocean, NJ) and capped with sterile

aluminum foil and a screw-on lid. The complete experiment, from milk collection through 21 days of shelf life, was replicated with evaluation of all parameters occurring as described in the following sections. For both replications (n=2) of the experiment, six bottles of milk with fish oil were over-wrapped with different light-interference or light blocking film treatments (n=6), for a total of 12 experimental replications (two replications for each of six film treatments).

Milk Packaging. Milk samples with fish oil were randomly assigned to one of five packaging treatments, designed to block specific wavelengths by using film overwraps (Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ., U.S.A.) (Table 3.3). Four types of films, either singly or combination, were used to wrap glass bottles (1 L Clear Boston Rounds, Fisher Scientific; demission is 8.5 cm and height is 21.5 cm): 4 layers of 9231 Blue-Green film (446nm block); 4 layers of 9231 Red-Green films (570 nm block); the combination of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red (BS 4T), aluminum foil, and no film or foil (Table 3-3). Films were tested for light transmittance by using a Shimadzu UV-2101PC UV-VIS scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Milk without fish oil was not overwrapped to serve as a control for the effect of fish oil on the oxidative process.

Light Treatments. Six bottles per treatment were laid horizontally in a random order under linear fluorescent lighting (2860 lumens; General Electronic CO., Cleveland, OH 44112) in a 4 ℃ walk-in cooler (Tonka, Hopkins, MN) for a 21 days as extended shelf-life study. The average intensity of light exposure at the bottle surface (approx. 182.75 cm^2) was 1210 lux (Table 3.4).

Table 3.3: Film overwrap packaging treatments, evaluated for photo-protection of omega-3 fatty acid enriched[1] fluid milk (2% total fat).

Treatment	**Description[2]**	**# of layers of film in overwrap (film thickness (mm))**	**Major wavelengths blocked**
446 nm block	9231 Blue-Green	4 (0.1)	425-520 nm
570 nm block	9231 Red-Green	4 (0.1)	520-580 nm
BS 4 T	4 layers 9231 Blue-Violet	8 (0.2)	370-460 nm,
	2 layers 9231 Blue-Green		525-580 nm
	2 layers 9231 Red-Red		
Light-Protected	Aluminum Foil	NA	All visible and UV
Light-Exposed	No film or Foil	NA	UV below approximately 300 nm
Milk without fish oil	No film or foil	NA	UV below approximately 300 nm

[1] 704 mg of EPA and DHA was added into 1 L milk.
[2]Films were obtained from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ. BS4T = broad spectrum treatment with less than 5% transmission of all targeted wavelengths (370, 400, 446, and 570 nm). Transmission for 446 nm block (4 layers of 9231 Blue Green films) was approx. 0%, and transmission for 570 nm block (4 layers of 9231 Red Green films) was less than 10%.

The light was measured on the top side of horizontally laid milk bottles. Light intensity was tested using a light meter (Extech Instrument Co.,Walthaun, MA, U.S.A.) in the beginning of each week during experiments, and measured three times. There was no significant difference between two replications.

Table 3.4: Light intensity (lux) delivered to milk (2% total fat; with and without added omega-3 fatty acids[1]) package surface over a 21-day shelf-life (4°C) for two replications.

	Light intensity (lux)	
	Replication 1[1]	Replication 2[1]
Day 1	1250	1310
Day 7	1169	1255
Day 14	1138	1189
Day 21	1200	1165
Average	1189	1229

[1]n=3 measurements per day

Analytical Analyses

To determine the effects of packaging on oxidation of omega-3 enriched milk, milk was analyzed at intervals over a 21-day period for changes in sensory perception of oxidation, riboflavin and tocopherols, volatile compounds, and unsaturated fatty acids. Figure 3.1 illustrates the experimental design and analytical assessments.

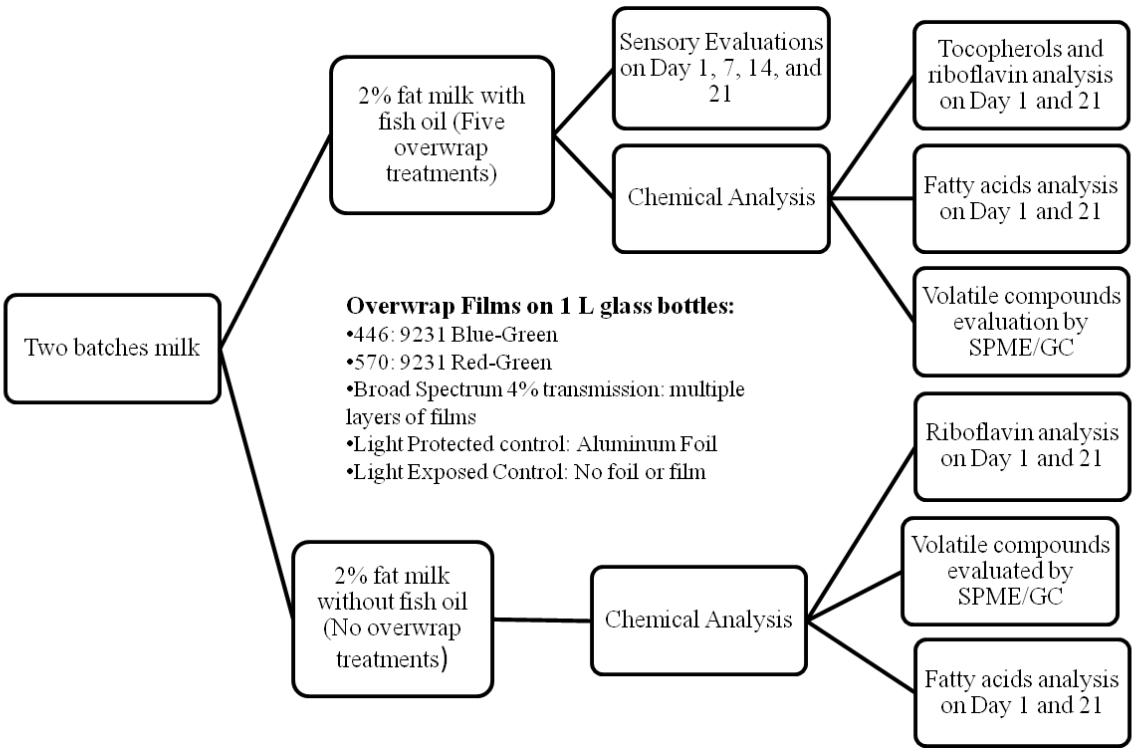


Figure3.1: Overview of project "controlling light oxidation flavor in omega-3 fatty acid enriched 2% milk by packaging films" Design

Compositional Analysis. Water/total solids were measured using AOAC 990.20-Solids (Total) in Milk method (Bradley 2000). Crude protein was measured using AOAC 991.22-Protein Nitrogen Content in Milk (Bradley 2000). Fat was measured using AOAC 995.18 Babcock method-Fat in Cream and AOAC 989.04 Babcock method-Fat in Milk (Bradley 2000). Samples were tested in triplicate for each analysis within 24 hr after processing for each replication.

Microbial Analysis. Standard plate counts of aerobic bacteria and coliforms were conducted based on standard method (Laird and others 2004) at dilutions of 10^{-1}, 10^{-2}, 10^{-3} by using Petrifilm^{TM} (3M, St. Paul, Minn., U.S.A.). Dairy blanks used for dilution were produced using

KH_2PO_4, $MgCl_2$, and deionized water following the standard method (Laird and others 2004). Aerobic count plates and coliform count plates were incubated at 32 °± 1 ℃ for 48 ± 3 hours. Microbial tests were conducted three days before sensory tests (except for day 1 testing).

Antioxidant and Photosensitizer Analyses

Analysis of Tocopherols. Tocopherols were measured directly in fish oil by HPLC (Pocklington and Dieffenbacher, 1988). The flow rate was set at 0.5 ml/min, and run time was 45 min. The solvent was methanol: H_2O (95:5, v/v). Injection volume was 5µl. The UV signal was recorded at 292 nm, and spectrum from 190 to 400 nm was saved. The column (4.6*50 mm) used was an Agilent Poroshell 120 EG-C 18 (Agilent Technologies, Santa Clara, CA.) having a mean particle size of 2.7µM.

The α-tocopherol content of the fish oil is, calculated by:

$$\frac{C*a*D*25}{A*m}\ \mu g/g$$

C = concentratation of the α-tocopherol standard (µg/ml)

A = mean of the peak areas obtained for the α-tocopherol standard

a = mean of the peak areas obtained for the α-tocopherol in test sample

m = mass of test sample taken

D = dilution factor

Riboflavin Analysis. Riboflavin concentrations in milk were analyzed using the fluorometric method, AOAC method 970.65 (Bradley 2000). Riboflavin was measured on a Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instruments, Inc., Columbia, Maryland,

U.S.A.). The fluorescence detection was programmed with an excitation wavelength of 450 nm and an emission wavelength of 520 nm. Standards were made according to AOAC method 970.65 (Bradley 2000). Riboflavin analysis was conducted on day 1 and day 21.

Oxidation Analysis

Fatty Acid Analyses. Fat was extracted from milk and milk containing fish oil on day 1 and 21 by Bligh and Dyer extraction of large sample method (Bligh and Dyer 1959). Extracted fat samples were nitrogen flushed and stored in the -70 ℃ freezer until fatty acid analysis was completed.

Fatty acids were detected by gas chromatography after conversion to fatty acid methyl esters followed by AOCS official method Ce 1b-89 (AOCS 1999). A Shimadzu QP 5050 GC/MS (Shimadzu, Kyoto, Japan) fitted with a SP-2560 (cross-linked and bonded *bis*-cyanopropyl polysiloxane, Supelco Corp., Sigma-Aldrich, St. Louis, MO) capillary column (100m x 0.25mm) was used. Helium carrier gas flow was 21 cm/sec (1 mL/min). A program using a split ratio of 1:20 with an injector temperature of 270 ℃ was used. Run temperature was 140 ℃ to 250 °C at the rate of 2 °C/min with a hold time of 20 min. The total run was 80 min. Fatty acids were identified using a quadrupole mass analyzer, and FAME standard. Solvent (1μL) was directly injected into the GC/MS. The amounts of individual fatty acids were calculated based on method reported in AOCS official method Ce 1b-89 (AOCS 1999).

Volatile Compound Analyses by Solid Phase Micro-Extraction GC/MS. Milk with fish oil and without fish oil samples were analyzed for headspace volatiles to help identify compounds that were present or developed during storage of the dairy-based beverages. Volatile oxidation compounds formed during the storage of the omega-3 fatty acid fortified 2% milk and regular 2% milk were separated and identified using solid phase micro-extraction (SPME) GC/MS. Volatile compounds were extracted and concentrated using a 75 μm carboxenpolydimethyl siloxane (CAR-PDMS) SPME fiber (Supelco, Bellefonte, PA). The CAR-PDMS fiber was conditioned at temperatures recommended by the manufacturer before use. A blank analysis was performed using an empty sealed vial to ensure that extraneous compounds adsorbed from the atmosphere desorbed from the fiber before sample analysis. Sample vials were heated to 50 ℃ while being agitated at 250rpm. The SPME fiber was plunged through a septum and fiber (22 mm) was exposed to the vial headspace for 15min during heating. Volatile oxidation products adsorbed onto the SPME fiber were desorbed and analyzed using an HP5890A (Hewlett Packard, Palo Alto, CA) coupled with a HP5972 series mass selective detector (Hewlett Packard, Palo Alto, CA). Injector temperature was set at 250 ℃, detector temperature was set at 265 ℃, and the program analysis ran in splitless mode with helium carrier gas. External standards of acetaldehyde, propanal, pentanal, 1-penten-3-one, 1-penten-3-ol, hexanal, and 2, 4-heptadienal were used to identify and track oxidation products. Milk samples were tested on Day 1, 7, 14, and 21. Two evaluations for each sample were made on each day. Milk without fish oil samples was set as a control to determine the oxidation products changes as a function of omega-3 fatty acids.

Sensory Analysis

This project involved sensory tests, using human subjects, for changes in omega-3 enriched milk flavor as a result of light exposure. Approval for use of human subjects was received by the Virginia Tech Institutional Review Board (IRB#10-111, Appendix A). Informed consent forms, as approved by the Virginia Tech Institutional Review Board, were completed by each subject in the sensory evaluation laboratory prior to collection of any data.

Selection of Panelists. After consent forms were signed and collected, panelists completed prescreening questionnaires (Appendix B), which were used for selecting future panelists. In addition, two triangle tests were used to screen panelists. Panelists (n=30) tasted commercially processed UHT 2% reduced fat milk (Parmalat, Parma, Italy) purchased from a local grocery store, and the same milk that had been exposed to fluorescent light for 48 hours at 4 ℃. Panelists needed to be correct in both triangle tests in order to be selected for further training; 15 panelists were correct for both samples. A significant difference (α=0.05, β=0.05, p_d= 50%) between UHT 2% reduced fat milk exposed to light for 48 hours and UHT 2% reduced fat milk was observed. Fourteen panelists were selected for participation in the trained panel based on questionnaire responses (no dairy allergies, time availability, and physical condition) and triangle test successes.

***Training Panelists*.** The goal was for panelists to be able to rate differences in oxidation flavor intensity in the omega-3 fatty acid fortified milk over the 21-day shelf-life of the experimental products. Selected panelists were trained to identify aroma and taste differences associated with light-induced oxidation. Training was conducted in a group setting to determine a common vocabulary and attributes desired. Training included practicing the ability to recognize and rate

the light-induced oxidation flavor and aromas in omega-3 fatty acid fortified milk. Panelists' abilities to effectively respond to increasing concentrations of light-induced oxidized flavor were validated on a 10-point category scale, from 0 to 9 (Appendix D) after a minimum of 5 hours training. Panelists were evaluated for the ability to successfully identify the light-induced oxidation flavor in two validation tests before the experimental testing could begin. If they were not successful in initial validation tests, they were asked to complete additional training to improve their abilities before the final sensory evaluation of omega-3 fatty acid fortified milk. Four panelists need additional training based on the validation tests. Training protocols and statics analyis are described in more details in Appendix E and F.

Milk for testing was portioned into 1 oz. plastic cups marked with random 3-digit numbers, and sealed with lids. Samples for training and validation tests were stored in the walk-in cooler (Tonka, Hopkins, MN) at 4 °C and presented to panelists at 4 ℃.

Rating Sensory Perception of Oxidation in Experimental Products. Sensory evaluation of milk in experimental packaging treatments was conducted with panelists stationed in individual sensory booths equipped with white lighting and Sensory Information Management System (Sensory Information Management System, Sensory Computer Systems, LLC, Morristown, NJ) software on touchscreen monitors.

Products were portioned into plastic cups (1 oz.) and sealed with lids. Samples were identified by 3-digit random codes, representative of each treatment, and presented at 4 ℃ in a balanced order. Sensory evaluation used a complete block rating test; 12 panelists each received one sample at a time, and a total of five samples. They rated samples on a 10-point category scale from 0 (no

oxidation flavor) to 9 (extreme oxidation flavor). Due to the intensity of the oxidation flavor, panelists were encouraged to expectorate each sample, cleanse their palate with an unsalted saltine cracker and water, and were required to wait a minimum of one minute between samples, as controlled by the Sensory Information Management System.

Sensory analyses were completed on all lab processed milk with added omega-3 fatty acids on days 1, 7, 14, and 21. Milk without omega-3 fatty acids was not included in the sensory evaluation.

Statistical Analyses

All analytical analyses were conducted in duplicate. Two replications of the experiment were conducted. Data were analyzed by a three-way analysis of variance with the main effects of replication, overwrap treatments, and time. Significant differences were determined by t-test or Tukey-Kramer HSD.

RESULTS AND DISCUSSIONS

The processed products, with and without omega-3 fatty acid enrichment, were similar in gross composition (Table 3.5) and maintained a low microbiological counts (Appendix J) throughout the 21-day shelf life. This allows for interpretation of any differences observed in the milk products to be primarily related to the packaging conditions.

Table 3.5: Gross composition (mean ± sd) for UHT milk enriched with omega-3 fatty acids and UHT milk without omega-3 fatty acids (2 replications).

Formulations	% Fat X (± sd)	% Protein X (± sd)	% Solid X (± sd)
Milk with enriched omega-3 fatty acids	2.0(±0)	3.22(±0.19)	10.80(±0.50)
Milk without enriched omega-3 fatty acids	2.0(±0)	3.46(±0.29)	10.89(±0.31)

Oxidized Flavor Assessment in Milk with Overwrap Treatments

Milk is readily susceptible to off-flavor because of its delicate flavor. Common milk off-flavor criticisms include acid, astringent, barny, bitter, cooked, cowy (acetone), feed, fermented/fruity, flat, foreign, garlic/anion (weedy), stale, malty, oxidized, salty and unclean (psychrotrophic) (Azzara and Campbell, 1992). Oxidized flavor may be associated with two different chemical reactions: autoxidation, which in the extreme case may be classified as metallic, and light-oxidized. The light-oxidized flavor has two classes: one is a light-activated note that comes from protein degradation and is described as sunlight, sunshine, activated, burnt, burnt feather, burnt protein, scorched, cabbage, cooked cabbage, and mushroom (Dunkley and others, 1963). The other light-oxidized note is attributed to photo-oxidation, which described as oxidized, papery, cardboardy, cappy, metallic, tallowy, and oily (Azzara and Campbell, 1992). Higher pasteurization temperature can reduce light-activated and oxidized notes (Bassette and others,

1983). Homogenization can enhance the light-activated note and inhibits the oxidized note (Bassette, 1976).

Milk in the light-protected control treatment, which blocked all visible and UV wavelength, had a very low degree of oxidation off-flavor, as demonstrated by mean sensory ratings at less than 2.2 over the 21-day shelf life (Figure 3.2). All other treatments had significantly higher ratings ($P<0.05$) than light protected milk, even on day 1, and the milk with other overwrapped treatments from day 1 to 21 ($P>0.05$), except milk sample BS4T on day 21, which was not significantly different in sensory rating compared with light-protected control (Table 3.6). Generally, the rating of oxidative flavor increased gradually over time. For milk with complete light block, the ratings were relatively low (from 0 to 3) from day 1 to day 21. For milk with other overwrapped treatments, the ratings were relatively higher (from 3 to 7) from day 1 to day 21.

There were no significant differences among milk with overwrapped treatments 446, 570, BS4T, and LE, and there was no significant difference between light protected milk and milk sample BS4T on day 21. Webster and others (2009) used similar overwrap packaging treatments on milk with 2% milkfat as used in this study and used ranking tests as the sensory tool. Based on ranking tests for sensory evaluation, the light-exposed control treatment, which allowed 75% to 78% transmission of light wavelength from 300 to 340 nm and completely blocked light < 300 nm, was higher in light-oxidized flavor than all other treatments from day 4 to 14. Overall ranking scores also indicated significant differences in oxidized flavor of milk packaged in the different iridescent films (light exposed ^{a}>570 ^{b}>446 ^{c}>BS4T ^{d}>light protected ^{e}). In contrast to

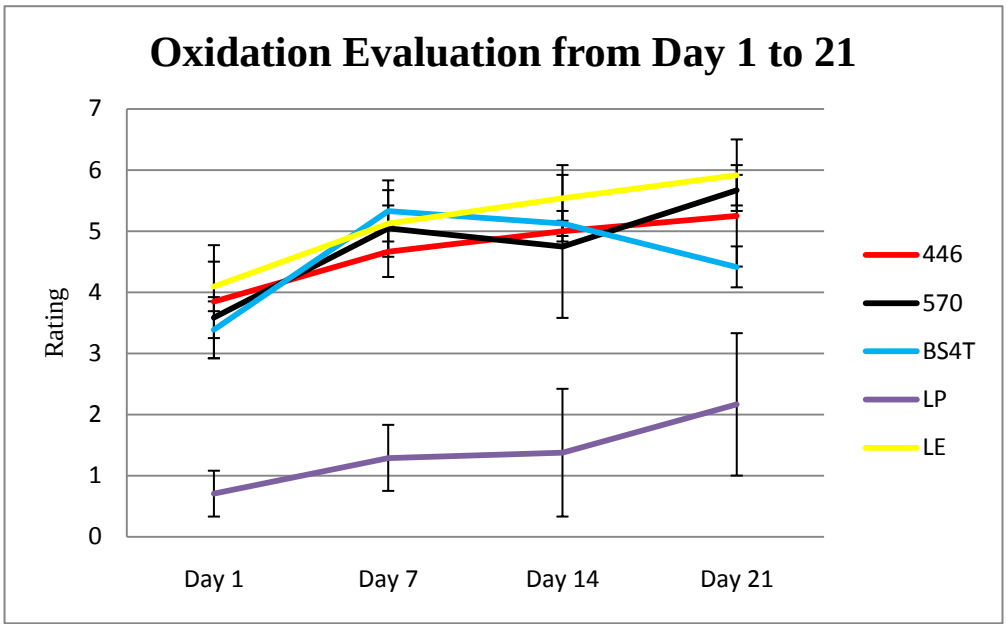


Figure 3.2: Mean sensory ratings (n=12 observations) for oxidized flavor in milk packaged in glass with iridescent or light-blocking overwraps from day 1 to 21. Rating scale is from 0 (none) to 9 (extreme). Legend: 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

the results reported by Webster and others (2009), film overwraps (446, 570, BS4T) did not provide a significant level of protection to oxidized flavor development in this study. This difference may be associated with sensory method selection. Ranking tests can differentiate order of attribute intensity among samples but does not indicate degree of intensity. It is possible that it is harder for panelists to use the rating test than the ranking test. The training evaluation indicated that panelists were trained and obviously could detect products with no oxidation (light-protected treatment); however, sensitivity to very low levels of difference in oxidation may not have been achieved. It is also possible that milk flavor characteristics changed significantly over time, perhaps because of the addition of omega-3-rich oils into the product, causing unique

flavors that confounded the detection of oxidized flavor and increasing the difficulty for panelists to define oxidation flavors in different time blocks (day1, 7, 14 and 21).

Film overwrap treatments, while not highly effective in this current experiment, did illustrate some effects of interest. BS4T overwrapped treatments, which blocked major wavelength 370-446 nm and 525-580 nm and allowed for only 4% light transmission overall, showed no difference existed between three treatments and light-protected milk on day 21. Webster and others (2009) reported that the single wavelength block treatments (446, 570) tended to rank higher in oxidized flavor than the broad spectrum treatment (BS4T). In our research, only on day 1 and 21, BS4T was rated lower than 446 and 570 ($P>0.05$). In addition, although not statistically different ($P>0.05$), Figure 3.2 illustrates that the light-exposed control was generally higher in oxidized flavor compared to the 446 nm film over time. Milk treatments 446 and 570 varied in rating tests from day to day. Previous research in our laboratory and others found that blocking wavelengths between 380 and 500 nm gave better protection for milk than blocking other wavelengths (Bosset and others 1994; Nielon 1999; Hansen and Skibsted 2000; Lennerststen and Lingnert 2000; van Aardt and others 2001; Webster and others 2009). Fanelli and others (1985) pointed out that oxidation reactions and degradation of vitamins, specifically riboflavin, were initiated mostly by wavelengths below 500 nm. Riboflavin has three absorption bands: the first is in the UVB region (280-320 nm, the absorption maxima is 275nm), the second band is in the UVA region (320-380 nm, and the absorption maxima is 370 nm), and the third band in the visible region (blue to green, broad maximum at 430-460 nm), which is the main band responsible for the photo-oxidation of food, especially for the dairy products (Borle and others 2001; Drossler and others 2003). Riboflavin acts as photosensitizer when exposed to

specific wavelengths: 446 nm and 570 nm (Bekbolet 1990). Recent literature (Wold and others 2005, Andersen and others 2008), suggest that other compounds, such as porphyrins, may increase susceptibility of milk to photooxidation at longer visible wavelengths. Such compounds are likely to be in omega-3-rich oils.

Volatile Analyses for Oxidation Products

Solid-phase microextraction (SPME) is an efficient way to isolate and analyze volatile compounds in food, such as fruit, alcohol, coffee (Song and others 1997; Ng and others 1996; Roberts and others 2000). More recently, SPME has been widely used for analyzing odorous volatile compounds including oxidation products in oil-in-water emulsions. Iglesias and others (2007) found that a carboxen/polydimethylsioxane (CAR-PDMS) fiber coating was the most suitable for analysis of fish oil emulsion volatiles. For our project, we also applied CAR-PDMS as the fiber to be used in SPME analysis.

Fish oil contributes unique volatiles when added to milk. 1-Penten-3-one (pungent green odor), 4-*cis*-heptanal (fishy odor), 2, 4-(*trans, trans*)-heptadienal, and 2, 6-(*trans, cis*)-nonadienal (cucumber odor) have been reported as contributing to the unpleasant fishy rancid off-flavor in bulk fish oil and fish oil-enriched milk (Venkateshwarlu and others 2004; Jónsdóttir and others 2005). Jimenez-Alvarez and others (2008) reported that 1-penten-3-ol, 2-penten-3-ol, t-2-butenal, t-2-hexenal, and 2-propenal were in a fish oil emulsion. Toso and others (2002) tested cow milk samples by GC-MS headspace analysis, and reported that the most representative chemical compounds classes in fresh cows' milk were ketones, followed by aldehydes, alcohols, and lower

amount of hydrocarbons, sulphur compounds, esters, and terpenes. Ketones were the most abundant compounds in the milk samples; acetone was the main compounds in the class of ketones. In our study, major peaks were seen at retention times (min) of 1.6, 2.1, 3.3, 6.8, 26.6, and 29.7 (Appendix J). The peak at 2.1 was identified as 2-butanone, which originates from UHT processed milk. Peaks at 1.6, 3.3, 6.8, 26.6, and 29.7 were identified as acetone, pentanal, hexanal, propanoic acid and ethanedioic acid. Most of the volatile compounds adsorbed onto the SMPE fiber eluted from the GC column within 30 minutes. Several compounds:1-penten-3-one, *c*-4-heptanal, *t,t*-2, 4-heptadienal, and *t,c*-2, 6-nonadienal, 1-penten-3-ol, 2-penten-3-ol, *t*-2-butenal, *t*-2-hexnal, and 2-propenal were not detected by SPME/GC. It is possible that the sensitivity of SPME is not enough to detect the low concentration of these compounds.

Table 3.6: Volatile compounds found in raw milk, UHT milk, fish oil, and milk enriched with fish oil, and comparison with volatile compounds found in our study by SPME/GC.

	Volatile compounds found in milk or fish oil[1]…				**Observed in this study[2]**						
Volatile Compounds	**Raw**	**UHT**	**FO**	**WFO**	**446**	**570**	**BS4T**	**LE**	**LP**	**NFO**	**References**
Ketones											
Acetone	√	√			√	√	√	√	√	√	Toso and others 2002; Valero and others 2001
2,3-Butanedione	√										Toso and others 2002
2-Butanone	√	√		√	√	√	√	√	√	√	Toso and others 2002; Valero and others 2001; Jónsdóttir and others 2005
2-Pentanone	√										Toso and others 2002
1-Penten-3-one			√	√							Venkateshwarlu and others 2004; Jónsdóttir and others 2005
2-Hexanone	√	√									Toso and others 2002; Valero and others 2001
4-Hydroxy-4-methyl-2-pentanone	√										Toso and others 2002

Volatile Compounds	Volatile compounds found in milk or fish oil[1]…				Observed in this study[2]						References
	Raw	UHT	FO	WFO	446	570	BS4T	LE	LP	NFO	
2-Heptanone	√	√		√							Toso and others 2002; Valero and others 2001; Venkateshwarlu and others 2004
3-Heptanone				√							Venkateshwarlu and others 2004
6-Methyl-5-heptene-2-one	√										Toso and others 2002
Aldehydes											
Acetaldeyde	√	√									Toso and others 2002; Valero and others 2001
Butanal		√		√						√	Valero and others 2001; Venkateshwarlu and others 2004
Isobutanal	√										Toso and others 2002
2-Butanal		√									Valero and others 2001
3-Methylbutanal	√	√									Toso and others 2002; Valero and others 2001
2-Methylbutanal	√	√									Toso and others 2002; Valero and others 2001
2-Butenal		√	√	√							Valero and others 2001; Venkateshwarlu and others 2004; Jónsdóttir and others 2005
Propanal				√	√						Jónsdóttir and others 2005
2-Propanone				√							Jónsdóttir and others 2005
2-Propanol				√							Jónsdóttir and others 2005
2-Propenal			√								Venkateshwarlu and others 2004
Pentanal	√		√	√	√	√	√	√	√	√	Toso and others 2002; Venkateshwarlu and others 2004; Jónsdóttir and others 2005
2-Pentenal			√								Venkateshwarlu and others 2004
2,4-Hexadienal	√		√								Toso and others 2002
Hexanal	√	√	√	√	√	√	√	√	√	√	Toso and others 2002; Valero and others 2001; Venkateshwarlu and others 2004; Jónsdóttir and others 2005
2-Hexenal			√	√							Venkateshwarlu and others 2004;

Volatile Compounds	Volatile compounds found in milk or fish oil[1]…				Observed in this study[2]						References
	Raw	UHT	FO	WFO	446	570	BS4T	LE	LP	NFO	
Heptanal	√		√	√							Jónsdóttir and others 2005 Toso and others 2002; Venkateshwarlu and others 2004;
4-Heptanal			√	√							Jónsdóttir and others 2005 Venkateshwarlu and others 2004;
2,4-Heptadienal			√	√							Jónsdóttir and others 2005 Venkateshwarlu and others 2004;
Nonanal	√										Jónsdóttir and others 2005 Toso and others 2002
2,6-Nonadienal	√										Toso and others 2002
Furfural		√									Valero and others 2001
Alcohols											
Ethanol	√	√					√		√		Toso and others and others 2002
Isobutanaol	√										Toso and others and others 2002
n-butanol	√	√									Toso and others and others 2002
Propanol				√	√						Venkateshwarlu and others 2004
1-penten-3-ol	√		√	√							Toso and others and others 2002; Venkateshwarlu and others 2004;
2-penten-1-ol			√								Jónsdóttir and others 2005 Jónsdóttir and others 2005
1-octen-3-ol			√								Jónsdóttir and others 2005
3-methyl-1-butanol	√										Toso and others and others 2002
1-pentanol	√	√									Toso and others and others 2002; Valero and others 2001
1-hexanol	√										Toso and others and others 2002
2-heptanol	√										Toso and others and others 2002
Hydrocarbons											
Benzene		√			√	√	√	√			Valero and others 2001
3-Methylpentane	√										Toso and others and others 2002

Volatile Compounds	Volatile compounds found in milk or fish oil[1]…				Observed in this study[2]						References
	Raw	UHT	FO	WFO	446	570	BS4T	LE	LP	NFO	
1-Hexene	√										Toso and others and others 2002
Methylfuran		√									Valero and others 2001
3-Methylfuran	√										Toso and others and others 2002
2-Ethylfuran				√							Venkateshwarlu and others 2004
Heptane		√									Valero and others 2001
1-Heptene	√										Toso and others and others 2002
3-Methylenheptane		√									Valero and others 2001
3-Methylheptane		√									Valero and others 2001
Toluene	√	√									Toso and others and others 2002
1-octene	√										Toso and others and others 2002
Sulphur Compounds											
Methylthiomethane	√										Toso and others and others 2002
Dimethyl sulfide		√									Valero and others 2001
Dimethysulphone	√										Toso and others and others 2002
Methyldisulphide	√										Toso and others and others 2002
Dimethyl disulfide		√									Valero and others 2001
Esters											
Methyl acetate	√	√									Toso and others and others 2002; Valero and others 2001
Ethyl acetate	√										Toso and others and others 2002
Ethyl propionate	√										Toso and others and others 2002
Ethyl isovalerate	√										Toso and others and others 2002
Terpene											
α-Pinene	√										Toso and others and others 2002
Limonene	√	√		√	√	√	√	√	√	√	Toso and others and others 2002;

Volatile Compounds	Volatile compounds found in milk or fish oil[1]…				Observed in this study[2]						References
	Raw	UHT	FO	WFO	446	570	BS4T	LE	LP	NFO	
											Valero and others 2001; Venkateshwarlu and others 2004
p-Eymene	√										Toso and others and others 2002

[1]Raw: raw milk; UHT: UHT milk; FO: fish oil; WFO: milk enriched with fish oil.

[2] 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

Comparisons among milk enriched with fish oil samples. The peak areas for acetone and 2-butanone were relatively high in all milk enriched with fish oil. Nursten (1997) pointed out that acetone was a component in fresh raw milk smell. Jeon and others (1978) pointed out that acetaldehyde, acetone, 2-pentanone and 2-heptanone were found in UHT milk when milk was stored at 25 ℃ or 2 ℃ for 24 weeks, and were highly related to stale flavor. Ethanol was detected in sample BS4T, LE, LP, and NFO. Ethanol is probably formed by microbial reduction of the aldehydes. Nursten (1997) also pointed out that ethanol, 2-propanol and 3-methylbutan-1-ol were the most abundant alcohols in fresh raw milk. Jenq and others (1988) reported that n-hexanal and n-pentanal were the compounds in milk most affected by fluorescent light (51 hours exposure). Karatapanis and others (2006) reported that dimethyl disulphide, pentanal, hexanal and heptanal as potential markers of fresh milk quality.

To allow for easier comparison across day and treatment, peak areas were converted to logarithmic scale (Appendix L). Presence/absence of each volatile was noted over time (Table 3.7). Logarithmic values for each compound were compared across time (days 1, 7, 14, 21) and across packaging treatment to determine if unique volatiles or noticeable differences in concentration were observed due to packaging treatment. To better characterize the effect of fish oil on volatile compounds, the light-protected control (with fish oil) was contrasted to the light-exposed control (with fish oil), and the light-exposed controls, with and without fish oil, were compared. Only those differences that were 0.5 log or greater are described (Appendix M).

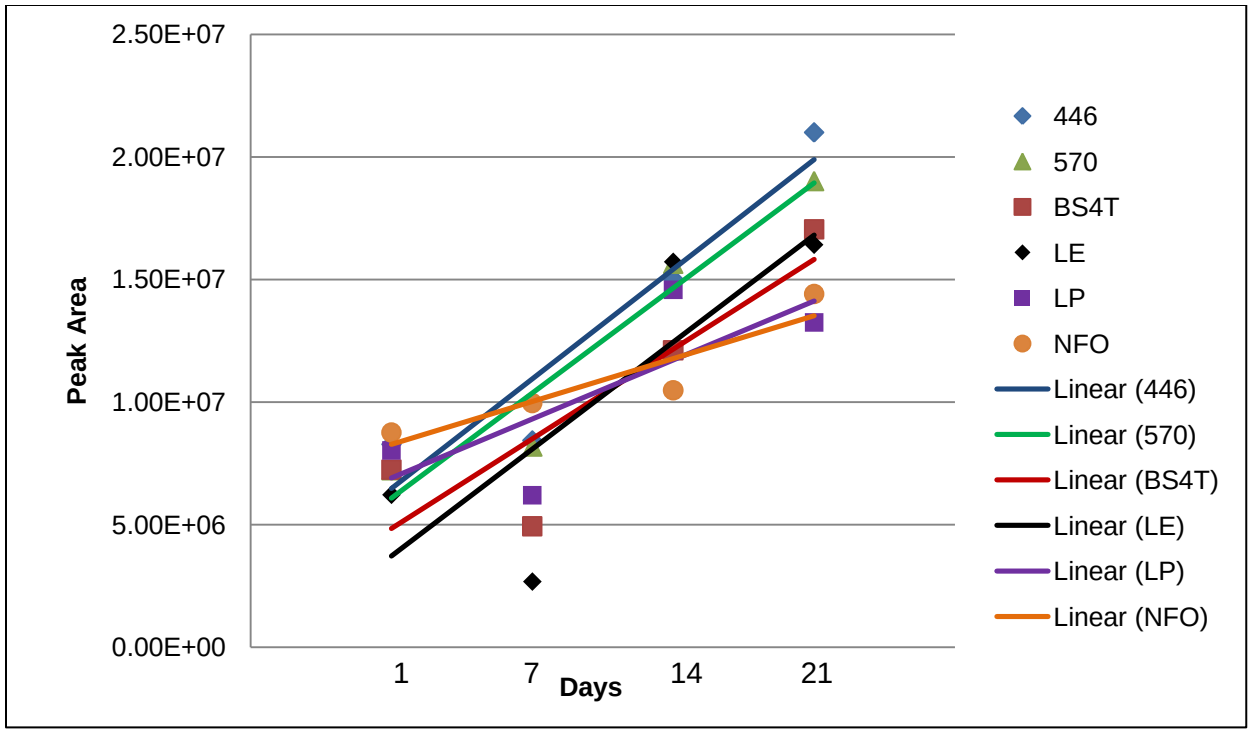


Figure 3.3: Peak area and liner regression for acetone in different milk samples. 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ. Data was based on duplicate analyses from one replication.

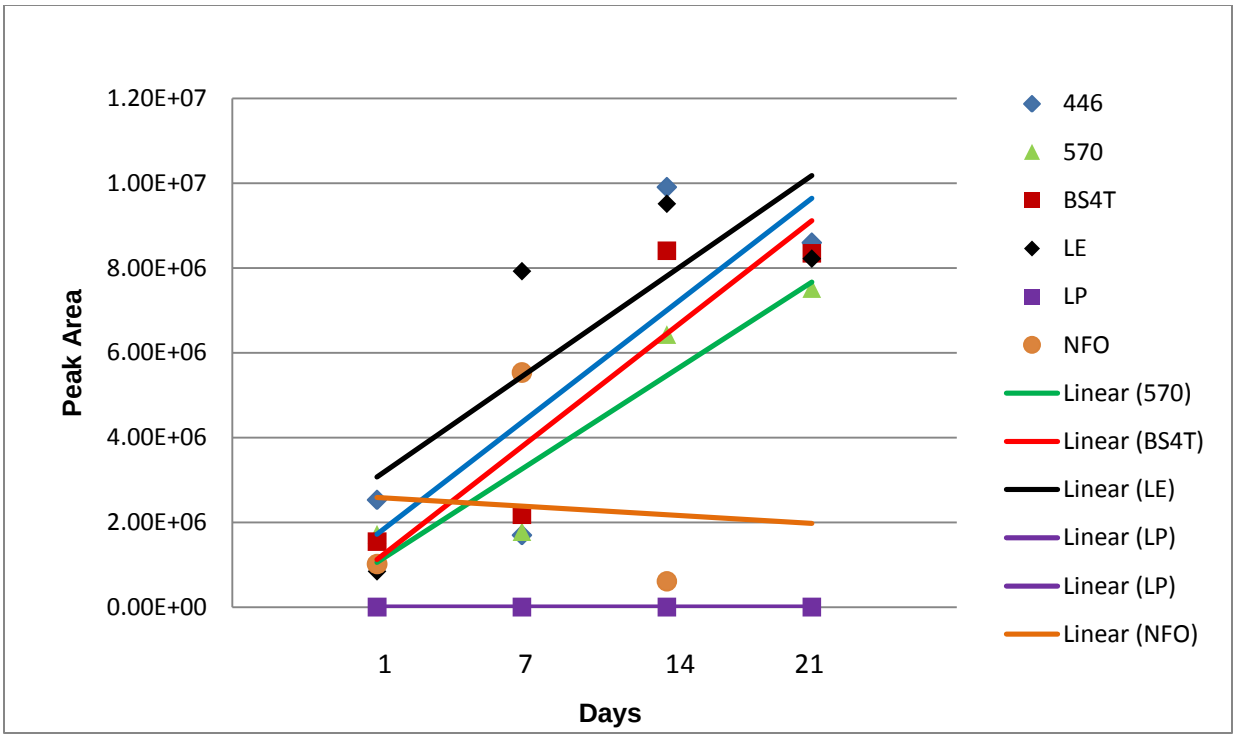


Figure 3.4: Peak area and liner regression for pentanal in different milk samples. 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ. Data was based on duplicate analyses from one replication.

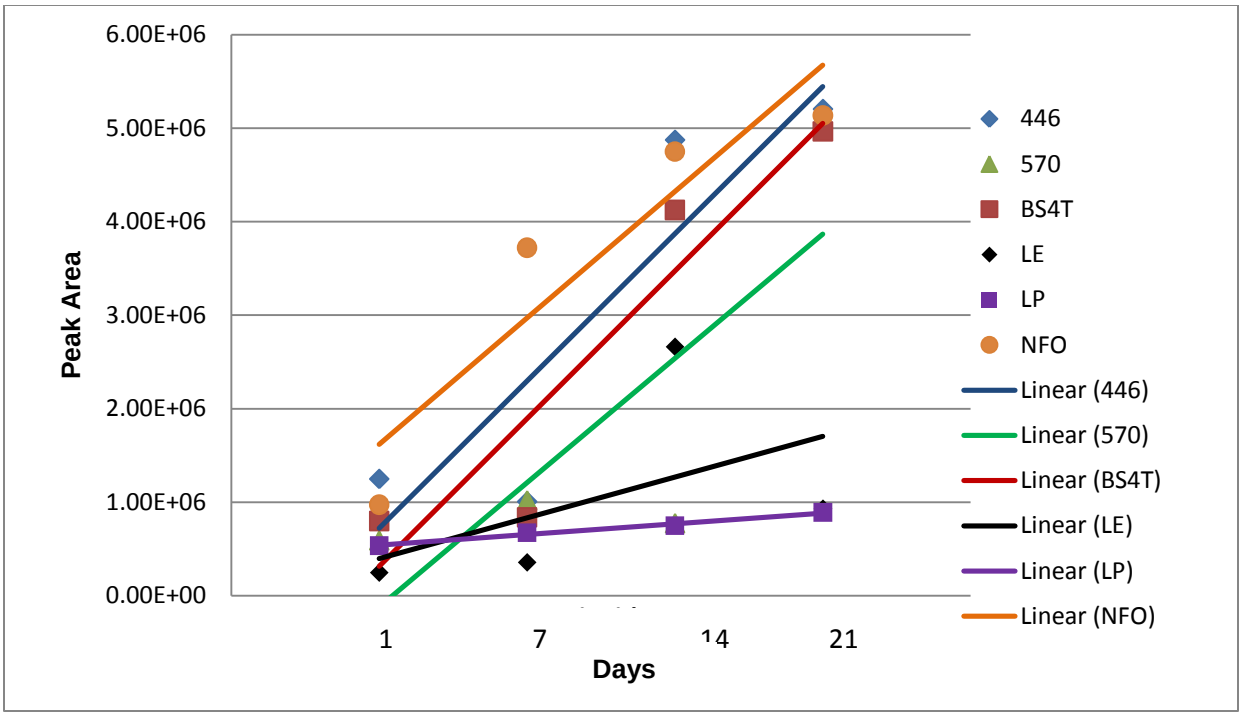


Figure 3.5: Peak area and liner regression for hexanal in different milk samples. 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ. Data was based on duplicate analyses from one replication.

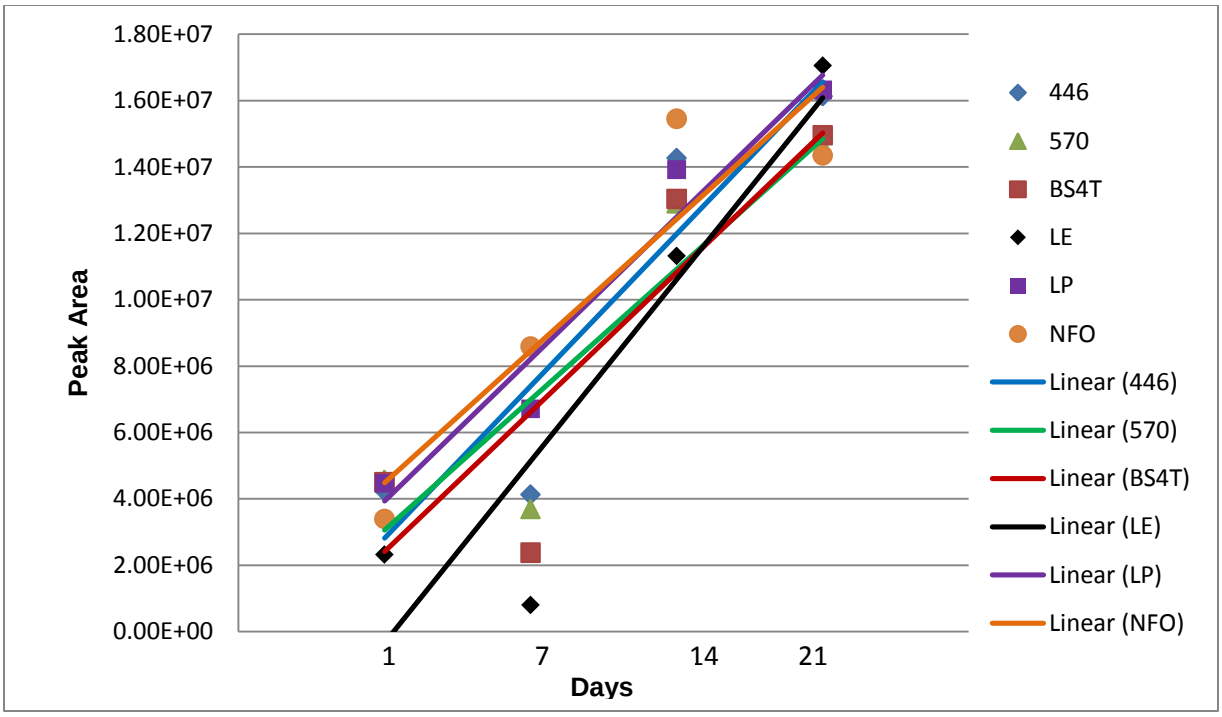


Figure 3.6: Peak area and liner regression for 2-butanone in different milk samples. 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

Table 3.7: Detection of volatile compounds in fish-oil enriched UHT milk with different light-interference packaging overwraps over the 21-day refrigerated (4°C) shelf life (days 1, 7, 14, and 21).

Compounds	Treatments[1]					
	446	570	BS4T	LE	LP	NFO
Ethanol	NA	NA	7	7	7	1
Acetone	1,7,14, 21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21
2-butanone	1,7 14, 21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21
Pentanal	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	NA	1,7,14
Hexanal	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21
Benzene	7, 14	7,14,21	14	1,14,21	NA	NA
Butanal	NA	NA	NA	7,14	7	NA
Propanol	1	NA	NA	NA	NA	NA
Propanoic acid	1,7, 14,21	1,7	1	14,21	1,7, 14,21	7,14,21
Ethanedi-oic acid	1,7,14,21	7,14,21	1,14	1,14,21	1,7,14,21	1,7,14,21
Limonene	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21	1,7,14,21

[1] Treatments: 446: overwrapped with 4 layers of 9231 Blue-Green; 570: overwrapped with 4 layers of 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

Ethanol was only detected on milk treatments of BS4T (day7), LE (day 7), LP (day7), NFO (day 1). Acetone, 2-butanone, hexanal, and limonene were found in all milk treatments through day 1 to day 21. Pentanal was detected in all light-induced milk samples from day 1 to 21, except milk

sample NFO on day 21; and was not detected in LE. Benzene was detected on 446, 570, BS4T, and LE. Propanol was only detected in 446 on day 1. Butanal was only detected in LE (day 7, 14) and LP (day 7).

Acetone was reported as a volatile compound in UHT milk (Valero and others 2001). 2-Butanone was found in both UHT milk and milk enriched with fish oil (Valero and others 2001; Venkateshwarlu and others 2004). Hexanal was found in UHT milk, fish oil, milk enriched with fish oil (Valero and others 2001; Venkateshwarlu and others 2004; Jónsdóttir and other 2005). Limonene was found in UHT milk and milk enriched with fish oil (Valero and others 2001; Jónsdóttir and other 2005). We all found of these compounds in our study.

Butantal was detected by other researchers in UHT milk and milk enriched with fish oil (Valero and others 2001; Venkateshwarlu and others 2004). We didn't detect butanal in NFO probably because SPME/GC is not sensitive enough to detect low amounts of butanal. Propanol was detected by other researchers in milk enriched with fish oil (Venkateshwarlu and others 2004), and it was only detected in 446 on day 1 in our study.

We didn't found benzene, butanal, and propanol in NFO. Benzene was found in UHT milk in another study (Valero and others 2001), and we also detected it in milk enriched with fish oil. It is possible that the amount of benzene in NFO is too little to be detected by SPME/GC, and both fish oil in milk samples and light (446, 570, BS4T, and LE) promote increasing the amount of benzene, and was detected by SPME/GC for the high amounts. The peak areas of hexanal for NFO were generally higher than LE from day 1 to 21, especially on day 7 (difference in $\log_{10}$ (area)>1) and 14(difference in $\log_{10}$ (area)>0.5). The peak areas of 2-butanone for NFO were generally bigger than LE from day 1 to 21, especially on day 7 (difference in lg (area)> 1.5) and

21(difference in lg (area)>0.5). It seems that fish oil can reduce the productions of hexanal and 2-butanone.

LE had significantly higher hexanal than the LP treatment on day 14 (difference in $\log_{10}$ (area)>0.5). The areas for hexanal for 570 and LP were similar from day 1 to 14, but the area of hexanal for 570 was relatively higher than LP on day 21(difference in $\log_{10}$ (area)> 0.5). There was no significant different in areas for oxidation products between LP and 446. The areas for hexanal for BS4T and LP were similar from day 1 to 7, but the area of hexanal for BS4T was relatively bigger than LP on day 14 and 21(difference in $\log_{10}$ (area)> 0.5). The areas for acetone for BS4T and LP were similar on day 1, 7, and 21, but the area of acetone for BS4T was jumped on day 14, which had a relatively larger area than LP on day 14(difference in $\log_{10}$ (area)> 0.5). Compared to 446 and BS4T, hexanal, which is in milk sample 570, delayed increasing on day 14.

Hexanal is a volatile compound that contributes to off-flavors in the milk, and is commonly regarded as a good measurement of lipid oxidation and related with sensory results (Robards and others 1988; Anderssen and Lingnert 1998; Lennersten and Lingnert 2000). Hexanal is an important dairy flavor. It can be present in various dairy products, such as UHT milk, pasteurized milk, light-oxidation milk, and sour cream butter (Chapman and others 2002). The flavor for hexanal can be described as cut-grass, green/pea pod flavor (Vara-Ubol and others 2004), but the flavor can changed from green when is combined with other compounds (Bott and Chamber 2006). The thresholds for hexanal range from 0.03-0.6 ppm depend on solvents and methods of analysis (Kocher 1996).

The peak area of hexanal is generally related to our sensory evaluation results. Overwrapped treatments all have higher peak areas for hexanal, which also have significant higher ratings than light-protected treatment from day 1 to day 21.Webster and others reported (2009) that changes in peak areas of hexanal was not enough to explain the difference in light oxidation flavor of overwrapped treatment. Some scholars also found that the concentration of hexanal does not relate perfectly to the sensory characteristics (Hedegaard and others 2006).

Benzene, which was detected in 446, 570, BS4T, and LE, is toxic for human beings. It can affect blood cell formation and development, and also can cause myelofibrosis, which is a disease in which the bone marrow is replaced with fibrous tissue (Gist and Burg 1997). Fabietti and others (2000) reported that 0.88 μg kg^{-1}, 0.82 μg kg^{-1}, 0.12 μg kg^{-1}, 0.70 μg kg^{-1}, 0.35 μg kg^{-1} were detected in pasteurized whole milk, pasteurized semi-skimmed milk, pasteurized fully-skimmed milk, whole UHT milk, semi-skimmed UHT milk, respectively.

Wavelengths and volatile chemicals

Exposure to different light wavelengths can result changes in the volatile compound observed. Webster and others (2011) found that hexanal concentration was significantly higher in UHT 2% milk when exposed to 200-400 nm than in UHT 2% milk exposed to 516, 567, and 610 nm. They also reported that pentanal concentration was significant greater when UHT 2% milk was exposed to 200-400 nm than milk exposed to 516 nm. Intawiwat and others (2010) reported that wavelengths longer than 500 nm can affect the quality of the milk, and protoporphyrin and the chlorophyllic compounds were most likely the effective photosensitizers when the wavelength of light was longer than 500 nm. They also reported that the color of the filters affected the content of the oxidation products. The concentration of propanol, 2-hexanal, 2-heptenal, and 2-nonenal

were higher in milk packed with noncolored filters than that packed with colored filters (red, green, amber and orange). Milk packed in orange (light transmission from 450-800 nm) filter had higher concentration of oxidation products than those packed with amber (light transmission from 500-800 nm), green (light transmission from 500-800 nm), and red (light transmission from 570-800 nm) filters.

In our study, milk sample 446 blocked wavelengths between 425-520 nm, 570 blocked wavelengths between 520 -580 nm, and BS4T blocked wavelengths between 370-446 nm, and 525-580 nm. Milk sample 446, 570, and BS4T all had acetone, 2-butanone, hexanal, pentanal, benzene, propanoic acid, and ethanedioic acid. Our research did not show big changes of volatile compounds when wavelengths were partially blocked. Compared to 446, 570, and BS4T, pentanal and benzene were not detected in LP samples. Blocking all visible light and UV light can prevent light oxidation and produce lower concentrations of some oxidation products.

Overall, many compounds, such as pentanal, hexanal, 2-butanone increased in concentration, as reflected in increase peak areas, over time. Pentanal showed a delay in production in milk packaged in films 446, 570, and BS4T, compared to LE products. Peak areas of hexanal in milk packaged in films 446, 570, and BS4T were generally higher than that in LE products. Compared to 446, 570, and BS4T, pentanal and benzene were not detected in LP, but butanal, which was not detected in milk overwrapped treatments, was found in LP. Comparing volatiles of NFO and LP to light exposed milk with fish oil, peak areas of hexanal and 2-butanone in light-exposed NFO were generally larger. NFO showed relatively larger peak areas for 2-butanone on day 14, compared to other milk samples, suggesting antioxidants in the fish oil can reduce the production of some light oxidation volatiles. The tocopherol content in the fish oil used in this study was 12.97 μg/g. Therefore, the tocopherol content in 1 L milk enriched with fish oil was 36.45μg/L.

Riboflavin Destruction

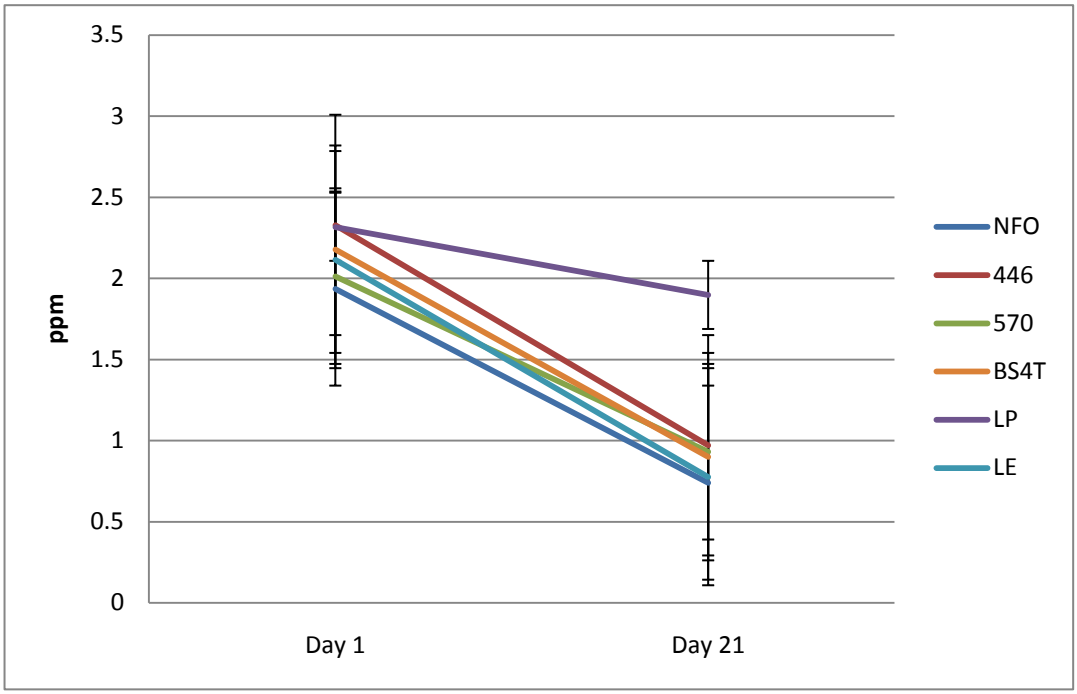


Figure 3.7: Riboflavin reduction for milk treatments from day 1 to day 21 with standard deviation. Milk samples were stored at 4 ℃ under the fluorescent light with film overwrap treatments. Film overwrap treatments-- 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

There was no significant difference in the amount of riboflavin among the five products exposed to light (446, 570, BS4T, LE, and NFO) on day 1. On day 1, after 24 hours light exposure, riboflavin concentration in LP was significantly ($P<0.05$) higher than that in NFO, which did not have the benefit of additional antioxidants associated with the addition of fish oil. There was no significant difference among LP and other overwrapped treatments: 446, 570, BS4T, and LE.

On day 21, riboflavin concentration in LP was significantly ($P<0.05$) higher than that in all other samples (NFO, 446, 570, BS4T, and LE). This finding is in accordance with a number of researchers. Webster and others (2009) also found the light-protected treatment was significant higher in riboflavin concentration than other partially wavelengths blocked treatments. Mestdagh and others (2005) found that PET bottle protected from light and 3-layered (white-black-white) PET bottle prevented riboflavin destruction much better than transparent 3-layered PET with an active oxygen-binding inner layer and transparent monolayer PET provided with a UV-absorbing additive.

There was no significant difference in riboflavin concentration among light exposed samples (NFO, 446, 570, BS4T, and LE) on day 21. LE and other overwrapped treatments had significant decrease in riboflavin concentration (>50%) over time. This finding is similar to that reported by Webster and others (2009). Webster and others (2009) found that there was no significant difference when certain wavelengths were blocked, except for blocking the 400 nm wavelength, in regard to preventing riboflavin destruction. Borle and others (2001) reviewed that wavelengths between 430 to 460 nm are primarily responsible for the photo-oxidation of food, especially for milk. However, for milk sample 446, even though the film blocked wavelengths between 425-520 nm, it was not very efficient in preventing riboflavin destruction.

The addition of antioxidants (α-tocopherol) in fish oil in milk overwrapped treatments (446, 570, BS4T, and LE) did not show significant difference in preventing riboflavin compared to milk without fish oil NFO, which didn't contain antioxidants α-tocopherol. This result corresponds to van Aardt and others (2005) who found that more aroma-active flavor compounds were detected in light-exposed milk added with α-tocopherol than light-exposed milk without α-tocopherol when milk was exposed to the light for 10 hours, which did not limit oxidation flavors in milk.

Fatty acid analysis

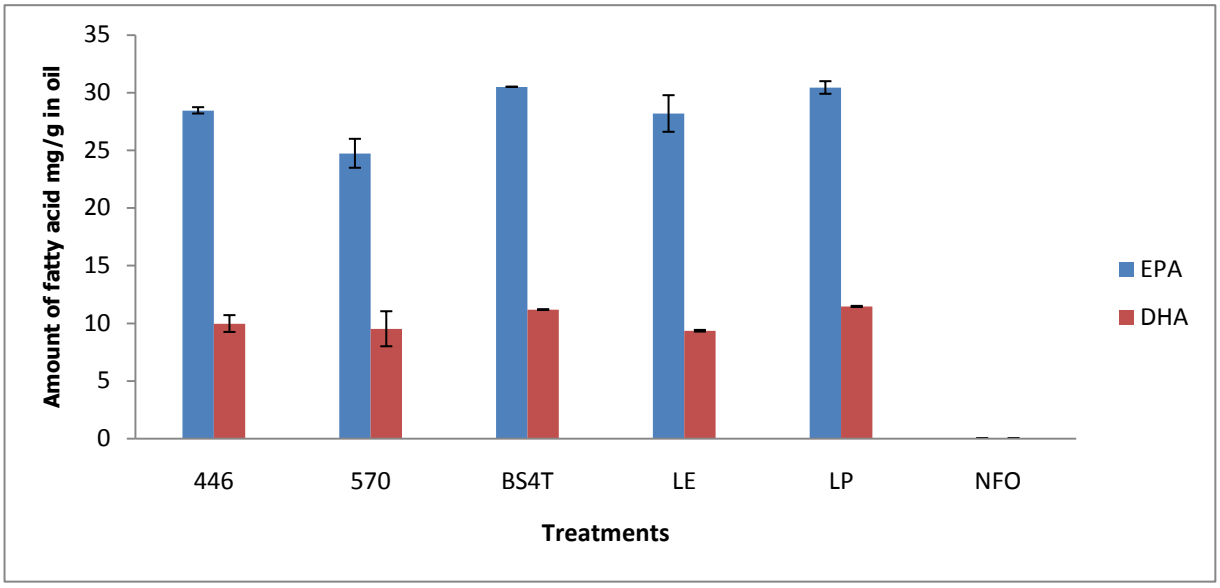


Figure 3.8: Amounts of EPA and DHA (mg/g) in oil extracted from overwrapped milk samples on day 21. Milk samples were stored at 4 ℃ under the fluorescent light with film overwrap treatments-- 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

The amount of EPA and DHA in fish oil is expected to be 35.9 mg/g. There was no significant differences for EPA and DHA in fish oil on day 0 and EPA and DHA in overwrapped treatments on day 21 ($p<0.05$). NFO, as we expected, did not contain any EPA and DHA.

A full fatty acid profile of oils extracted from overwrapped milk on day 21 and fish oil (Ocean Nutrition Canada Ltd., Nova Scotia, and Canada) is given in Table 3.8. Due to the large number of fatty acids in the products, peak areas less than 5% were not examined. The fish oil used for

this study was reported by the manufacturer (Ocean Nutrition Canada Ltd., Nova Scotia, Canada.) to contain a minimum 250 mg/g DHA+EPA.

The amount of DHA and EPA was detected as 263.9 mg/g. On day 21, the amount EPA and DHA was no significantly different in overwrapped milk treatments compared to that in fresh fish oil. That's means after 21 days fluorescent light (1210 lux), the amount of DHA and EPA can provide recommended nutrients to consumers. On day 21, the amounts of EPA and DHA did meet the recommended consumption, which was 500 mg/day set by the American Heart Association (Anonymous 2008).

There was no significant difference in EPA and DHA concentrations among overwrapped milk treatments at the end of shelf-life. EPA and DHA undergo photooxidation under light during the storage time, resulting in the formation of hydroperoxides, which are unstable. Hydroperoxides are tasteless, and continue in a chain reaction into secondary oxidation products, such as acids, alcohols, hydrocarbon, and etc. The ratio of unsaturated fatty acids to saturated fatty acids in fresh fish oil was higher than that in overwrapped milk treatments.

Table 3.8: Area (%) of major fatty acids identified in oil extracted from overwrapped treatments milk samples (446, 570, BS4T, LE, LP, and NFO) on 21 days of storage at 4 ℃ under the fluorescent light.

Area % of lipids ($\bar{x} \pm sd$)						
	Treatments on day 21[1]					
Fatty acids	446	570	BS4T	LE	LP	NFO
4:0	1.4(±0.02)	1.0(±0.50)	0.2(±0.002)	0.2(±0.03)	0.0(±0.00)	0.2(±0.20)
6:0	1.1(±0.04)	1.4(±0.25)	0.9(±0.04)	1.2(±0.09)	1.1(±0.13)	1.7(±0.27)
8:0	0.8(±0.10)	1.2(±0.05)	0.8(±0.25)	1.1(±0.01)	1.1(±0.15)	1.1(±0.14)
10:0	2.2(±0.04)	2.5(±0.43)	2.4(±0.07)	2.6(±0.13)	2.3(±0.26)	2.9(±0.18)
12:0	2.3(±0.04)	2.5(±0.55)	2.9(±0.04)	3.1(±0.01)	2.7(±0.27)	3.2(±0.18)
14:0	10.9(±0.58)	10.8(±1.48)	11.5(±0.47)	11.9(±0.67)	11.6(±0.63)	12.1(±0.71)
16:0	38.5(±2.30)	27.2(±0.40)	33.7(±0.94)	36.1(±5.25)	35.5(±0.93)	35.3(±0.58)
16:1ω7	1.3(±0.91)	1.3(±1.65)	3.5(±0.44)	2.7(±0.01)	2.6(±0.04)	0.9(±1.22)
17:0	0.5(±0.02)	0.4(±0.05)	0.5(±0.00)	0.4(±0.00)	0.5(±0.03)	0.3(±0.35)
18:0	13.7(±0.70)	12.5(±0.53)	11.9(±3.25)	12.4(±0.66)	11.8(±0.07)	13.1(±0.23)
18: 1ω9	24.9(±0.07)	25.2(±2.11)	22.5(±0.24)	21.9(±0.93)	25.0(±2.20)	24.6(±3.56)
18: 2ω6	3.09(±0.16)	2.8(±0.42)	2.5(±0.00)	2.4(±0.00)	2.4(±0.20)	1.4(±1.98)
20:5ω3	3.1(±0.19)	2.7(±0.83)	2.8(±0.00)	3.1(±1.12)	2.4(±0.18)	NA
22:6ω3	1.1(±0.08)	0.9(±0.45)	1.0(±0.03)	0.8(±0.10)	1.0(±0.07)	NA
Saturated	66.5	67.1	67.7	69.0	66.6	73.2
Mono+	33.5	32.9	32.3	31.0	33.4	26.8

Poly unsaturated						
EPA+DHA	4.20[a]	3.64[a]	3.79[a]	3.98[a]	3.37[a]	0

[1]n=2 replications

[a] means within columns with different super script are significantly different ($p<0.05$)

Table 3.9: Area (%) of major fatty acids identified in fresh fish oil (Ocean Nutrition Canada Ltd., Nova Scotia, Canada.)

Fatty acids	Area % of lipids ($\bar{x} \pm sd$)
4:0	NA
6:0	NA
8:0	NA
10:0	NA
12:0	NA
14:0	11.8(±0.49)
16:0	27.2(±0.41)
16:1ω7	11.4(±0.05)
17:0	NA
18:0	4.3(±0.33)
18: 1ω9	11.7(±0.05)
18: 2ω6	0.8(±0.08)
20:5ω3	23.5(±0.47)
22:6ω3	9.2(±0.33)
Saturated	43.4
Mono+ Poly unsaturated	56.6
EPA+DHA	32.7

CONCLUSIONS

Our tests shows that milk enriched with fish oil is susceptible to fluorescent light and can cause light-oxidized flavor in milk and loss of nutrients. In our study, milk enriched with fish oil overwrapped with iridescent films, which block certain wavelengths, had more intense oxidation flavors compared to that milk completely blocked light through day 1 to day 21. The SPME/GC analysis showed that blocking certain wavelengths can somehow decrease or delay the production of volatile compounds in milk enriched with fish oil, but not significantly. The antioxidant α-tocopherol, which was in fish oil, did not show significantly prevent the deduction of riboflavin and EPA and DHA. Packaging that provides a complete light block is still the best way to prevent light-oxidized flavor in milk enriched with fish oil.

REFERENCES

Anonymous. 2006. Senator Feinstein Co-Chairs Newly Formed Congressional Dairy Farmer Caucus. [cited 2011, June 17] Available from: http://feinstein.senate.gov/06releases/r-dairy-caucus.htm

Anonymous. 2008. Fish and omega-3 fatty acids. [cited 2011, June 17] Available from: http://www.americanheart.org/presenter.jhtml?identifier=4632.

Anonymous. 2011. How much food from the dairy group is needed daily? [cited 2011, June 17] Available from http://www.mypyramid.gov/pyramid/milk_amount_table.html

AOCS Official Method Ce 1b-89 Fatty Acid Composition by GLC Marine Oils. 1999.

Anderssen K, Lingnert H. 1998. Influence of oxygen concentration on the flavour and chemical stability of cream powder. Lebensmittel-Wissenschaft und-Technologie 31(3):245–51.

Azzara DC, Campbell LB. 1992. Off-flavors of dairy products. Off-Flavors in Foods and Beverages. Charalambous G, ed. Amsterdam: Elsevier. 329-374p.

Bassette R, Fung DYC, Roberts H. 1983. Effect of pasteurization temperature on susceptibility of milk to light-induced flavor. Journal of Food Protection 46:416-419.

Bassette R. 1976. Effects of added copper on concentrations of volatile materials produced in milk. Journal of Milk and Food Technology 39:117.

Bekbolet M. 1990. Light effects on food. Journal of Food Protection 53(5): 430-40.

Benjamins L, Amafilter BV. 1986. Modern filtration processes in vegetable oil industry. World Conference on Emerging Technologies in the Fats and Oils Industry. American Oil Chemists' Society. Champaign, IL .p72.

Bligh EC, Dyer WJ, Bligh E. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37(8): 911-917.

Bott LL, Chambers E. 2006. Sensory characteristics of combinations of chemicals potentially associated with beany aroma in foods. Journal of Sensory Studies 21(3): 308-321.

Borle F, Sieber R, Bosset JO. 2001. Photo-oxidation and photoprotection of foods, with particular reference to dairy products: An update of a review article (1993–2000). Sciences des Aliments 21:571–590.

Bradfield A, Duthie AH. 1965. Protecting milk from fluorescent light. American Dairy Review. 27: 110-114 In: Bradley R. 1983. Eliminating light-activated flavor. Dairy Record 84: 168-70.

Bradley RL. 2000. Dairy products. In: William Horwitz W, editor. Official methods of analysis of AOAC Intl. Vol. II. 17th ed. Gaithersburg,Md.: AOAC Int. 1–83p.

Chapman KW, Whited LJ, Boor KJ. 2002. Sensory threshold of light-oxidized flavor defects in milk. Journal of Food Science 67(7):2770-2773.

Choe EO, Min DB. 2003. Chemistry and reactive oxygen species in foods. Journal of Food Science 70:142-59.

Cleland LG, James MJ, Proudman SM. 2003. The role of fish oils in the treatment of rheumatoid arthritis. Drugs 63: 845–853.

Doyle, M. 2004. Consumers have long list of packaging wishes and pet peeves. Food & Drug Packaging 68(8): 24-8.

Drossler P, Holzer W, Penzkofer A, Hagamann P. 2003. Fluorescence quenching of riboflavin in aqueous solution by methionin and cystein. Chemical Physics 286:409–20.

Dunkley WL, Pangburn RM, Franklin JD. 1963. Fluorescent light influences flavor and vitamins in milk. Milk Dealer. 52: 52.in Jeng WW, Bassette RR, Crang RE. 1988. Effects of light and copper ions on volatile aldehydes of milk and milk fractions. Journal of Dairy Science 71(9): 2366-2372.

Fabietti FF, Delise MM, Piccioli Bocca AA. 2000. Aromatic hydrocarbon residues in milk: preliminary investigation. Food Control 11(4): 313-317.

Fanelli AJ, Burlew JV, Gabriel MK. 1985. Protection of milk packaged in light density polyethylene against photodegradation by fluorescent light. Journal of Food Protection 55:112–117.

Gist GL, Burg JR. 1997. Benzene: A review of the literature from a health effects perspective. Toxicology and Industrial Health 13(6): 661-714.

Gudipati V, Let MB, Meyer AS, Jacobsen CC. 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. Journal of Agricultural and Food Chemistry 52(2): 311-317.

Herreid EO, Ruskin B, Clark GL, Parks TB. 1952. Ascorbic acid and riboflavin destruction and flavor development in milk exposed to the sun in amber, clear paper and ruby bottles. Journal of Dairy Science 35:772–8. In: Bradley R. 1983. Eliminating light-activated flavor. Dairy Record 84:168–70

Hedegaard RV, Kristensen D, Nielsen JH, Frost MB, Ostdal H, Hermansen JE, Kroger-Ohlsen M, Skibsted LH. 2006. Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk. Journal of Dairy Science 89:495–504.

Iglesias J, Lois S, Medina I. 2007. Development of a solid-phase microextraction method for determination of volatile oxidation compounds in fish oil emulsions. Journal of Chromatography 1163(1/2): 277-287.

Jeng WW, Bassette RR, Crang RE. 1988. Effects of light and copper ions on volatile aldehydes of milk and milk fractions. Journal of Dairy Science 71(9): 2366-2372.

Jimenez-Alvarez D, Giuffrida F, Golay P, Cotting C, Destaillats F, Dionisi F, Keely B. 2008. Profiles of volatile compounds in milk containing fish oil analyzed by HSSPME-GC/MS. European Journal of Lipid Science and Technology 110: 277-283.

Jónsdóttir R, Bragadóttir M, Gudmundur AO. 2005. Oxidatively Derived Volatile Compounds in Microencapsulated Fish Oil Monitored by Solid-phase Microextraction (SPME). Journal of Food Science 70(7): 433-440.

Jimenez-Alvarez DD, Giuffrida FF, Golay PA, Cotting CC, Destaillats FF, Dionisi FF, Keely BB. 2008. Profiles of volatile compounds in milk containing fish oil analyzed by HS-SPME-GC/MS. European Journal of Lipid Science and Technology 110(3), 277-283.

Karatapanis AE, Badeka AV, Riganakos KA, Savvaidis IN, Kontominas MG. 2006. Changes in flavour volatiles of whole pasteurized milk as affected by packaging material and storage time. International Dairy Journal 16(7):750-761.

Kochar SP. 1996. Oxidative pathways to the formation of off-flavours. Food Taints and Off-Flavours. 2nd Ed. Blackie Academic & Professional: Glasgow. p.168–225.

Laird DT, Gambrel-Lenarz A, Scher FM, Graham TE, Reddy R, Microbiological count methods, in Standard Methods For The Examination Of Dairy Products, 17th Edition, H.M. Wehr, Frank, J.F., Editor. 2004, American Public Health Association: Washington, DC. p. 153.

Mestdagh FF, Meulenaer B, Clippeleer J, Devlieghere FF, Huyghebaert AA. 2005. Protective influence of several packaging materials on light oxidation of milk. Journal of Dairy Science 88(2): 499-510.

Miraglio AM. 2006. Omega-3s: Focus on the Future. Food Product Design 17-21.

Ng LK, Hupe M, Harnois J, Moccia D. 1996. Characterization of commercial Vodkas by solid phase micro-extraction and gas chromatography/mass spectrometry analysis. Journal of Agriculture and Food Chemistry 70, 380–388.

Nursten HE. 1997. The flavour of milk and dairy products: I. Milk of different kinds, milk powder, butter and cream. International Journal of Dairy Technology 50: 48-56

Jeon IJ, Thomas EJ, Reineccius GA.1978. Production of volatile flavor compounds in ultrahigh temperature processed milk during storage. Journal of Agricultural and Food Chemistry 26:1183-1188.

Packaged Facts. 2009. Functional foods and beverages in the US, 4th edition. Rockville, MD.

Pocklington WD, Dieffenbacher AA. 1988. Determination of tocopherols and tocotrienols in vegetable oils and fats by high performance liquid chromatography: results of a collaborative study and the standardised method. Pure and Applied Chemistry 60(6): 877-892.

Roberts DD, Pollien P, Milo C. 2000. Solid-phase microextaction method development for headspace analysis of volatile flavor compounds. Journal of Agriculture and Food Chemistry 48: 2430–2437.

Robards K, Kerr AF, Patsalides E, Korth J. 1988. Headspace gas analysis as a measure of rancidity in corn chips. Journal of the American Oil Chemists' Society 65:1621–6.

Sattar A, deMan JM, Alexander UC. 1976. Stability of edible oils and fats to fluorescent light irradiation. Journal of the American Oil Chemists' Society. 53: 473-477.

Song J, Gardner BD, Holland JF, Beaudry RM. 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of-fight mass spectrometry. Journal of Agriculture and Food Chemistry 45: 1801–1807.

Stillman R, Blayney D, Miller J, Crawford T. 1995. The U.S. Dairy Industry. [cited 2011, June 17] Available from: http://pdic.tamu.edu/black/stillman.pdf

Sunatree V, Chambers E, Chambers DH. 2004. Sensory characteristics of chemical compounds potentially associated with beany aroma in foods. Journal of Sensory Studies 19(1):15-26

Toso and others B, Procida G, Stefanon BB. 2002. Determination of volatile compounds in cows' milk using headspace GC-MS. Journal of Dairy Research 69(4): 569-577.

Venkateshwarlu G, Let M, Meyer A, Jacobsen C. 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. Journal of Agricultural and Food Chemistry 52(2): 311-317.

van Aardt M, Duncan SE, Marcy JE, Long TE, Hackney CR. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. Journal of Dairy Science 84(6):1341–7.

van Aardt M, Duncan SE, Marcy JE, Long TE, O'Keefe SF, Nielsen-Sims SR. 2005. Effect of antioxidant (α-tocopherol and ascorbic acid) fortification on light-induced flavor of milk. Journal of Dairy Science 88(3): 872-880.

Webster JB, Duncan SE, Marcy JE, O'Keefe SF. 2009. Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelengths by interference. Journal of Food Science 74(9): 390-398.

Webster JB, Duncan SE, Marcy JE, O'Keefe SF. 2011. Effect of narrow wavelength bands of light on the production of volatile and aroma-active compounds in ultra high temperature treated milk. International Dairy Journal 21(5): 305-311.

Wold JP, Veberg AA, Nilsen AA, Iani VV, Juzenas PP, Moan JJ. 2005. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. International Dairy Journal 15(4): 343-353.

APPENDICES

Appendix A: Institutional Review Board Approval Letter

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24061
540/231-4991 Fax 540/231-0959
e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572 (expires 6/13/2011)
IRB # is IRB00000667

DATE: February 24, 2010

MEMORANDUM

TO: Susan E. Duncan
Qin Li

FROM: David M. Moore

SUBJECT: **IRB Exempt Approval:** "Sensory Evaluation and Analysis about Omega-3 Fatty Acid Added Milk Products" , IRB # 10-111

I have reviewed your request to the IRB for exemption for the above referenced project. The research falls within the exempt status, CFR 46.101(b) category(ies) 2,6.

Approval is granted effective as of February 23, 2010.

As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in the research protocol. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.

2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

cc: File

Appendix B
Questionnaires for selecting sensory panelists

Date: _____ **Panelist #** (from consent form) _____

Please fill out the form, return through the window, and then start the sensory evaluation.

The results of your performance as a panelist will be kept strictly confidential except to the investigators. There are no more than minimal risks for participating in this survey; completion of this survey is voluntary and you are free to withdraw from this study at any time without penalty.

1. Indicate your age group:
   - 18-25
   - 26-35
   - 36-45
   - 46-55
   - 56-65
   - over 65
     a. **You must be at least 18 years old to participate in this study. If you are under 18, please do not continue. Come to the sensory kitchen to receive a token of appreciation for your interest in the study.**

2. Indicate your gender:
   - Male
   - Female

3. How would you classify yourself?
   - American Indian or Alaska Native
   - Asian
   - Black or African American
   - Caucasian/White
   - Hispanics of any race
   - Native Hawaiian or Pacific Islander
   - Multiracial
   - Would rather not say
   - Other: _____

4. How often (# times per week) do you consume milk?
   - Never or up to several times per month
   - 1-3 servings per week
   - 4-6 servings per week
   - 7-9 servings per week
   - 10-12 servings per week
   - more than 12 servings per week
5. Do you have any of the following conditions or use any of the following products?

   Yes No

   a. Lactose intolerance
   b. Food allergies to dairy or fish
   c. Diabetes
   d. Hypoglycemia
   e. Oral or gum disease
   f. Tobacco products
   g. Take medication that could affect your taste and/or smell

6. Are you interested in participating in additional sensory trainings if you are selected from this sensory test? The sensory trainings may require extra time (Training: 1 to 1.5 hours per session, 3 or 4 sessions total. Sensory Test: approximately 2 tests, each test may take 20 to 30 minutes.)

   Yes No

7. If you are interested in participating in the training and subsequent product evaluation, please check all times you are generally available for training during March and April.

Time	Monday	Tuesday	Wednesday	Thursday	Friday
8am to 9am					
9am to 10 am					
10am to 11am					
11am to 12pm					
12pm to 1pm					

1pm to 2pm					
2pm to 3pm					
3pm to 4pm					
4pm to 5pm					

Please return the survey through the window, and then start the sensory evaluation. Thanks.

Appendix C: Demographic information for selecting panelists

Selecting panelists based on the questionnaires and two triangle tests. Based on the questionnaire, 19 (63.3%) females and 11 (36.7%) males, total 30 panelists, participated in this study. The majority for participants were Caucasian/White (20, 66.7%), followed by Asians (5, 16.7%), then Hispanics of any race (3, 10%) and Native Hawaiian or Pacific Islander (2, 6.7%).

Table C.1: Demographic information for participants' ages

Age Range	Female	Male
18-25	13(43.3%)	7 (23.3%)
26-35	3 (10%)	3 (10%)
36-45	2 (6.67%)	0
46-55	1 (3.33%)	1 (3.33%)
Total	19(63.3%)	11(36.7%)

The majority of participants (50%) drink milk 1-3 servings per week, followed by 4-6 servings per week (33.3%), 7-9 servings per week (6.7%) and never or up to several times per month (6.7%), and more than 12 servings per week. Based on mypyramid.gov, the recommended consumption for milk group is daily needed for women and men from 9 to 51+ years old is 3 cup daily (Anonymous 2011). The questionnaire showed the amount of the consumption of milk for our panelists were much lower than the recommended dairy milk drink.

Table C.2: Information for participants consuming milk

Servings	Never or up to several times per month	1-3 servings per week	4-6 servings per week	7-9 servings per week	10-12 servings per week	More than 12 servings per week
Male (18-25)	2 (6.7%)	3(10%)	2 (6.7%)	0	0	0
Male (26-35)	0	2 (6.7%)	1 (3.3%)	0	0	0
Male (36-45)	0	0	0	0	0	0
Male (46-55)	0	1 (3.3%)	0	0	0	0
Female (18-25)	0	6 (20%)	4 (13.3%)	2 (6.7%)	0	1 (3.3%)
Female (26-35)	0	2 (6.7%)	1 (3.3%)	0	0	0
Female (36-45)	0	1 (3.3%)	1 (3.3%)	0	0	0
Female (46-55)	0	0	1 (3.3%)	0	0	0
Total	2 (6.7%)	15(50%)	10 (33.3%)	2 (6.7%)	0	1 (3.3%)

Appendix D: Reference rating for training sensory panelists

Table D.1: Reference rating for training sensory panelists and the rating was made based on the discussion among sensory panelists after they tasted milk samples (see appendix E for sample preparation) at different levels of oxidation.

Days of light exposure	Expected Rating
0	0
3	3
4	5
5	6
6	7
14	9

Appendix E

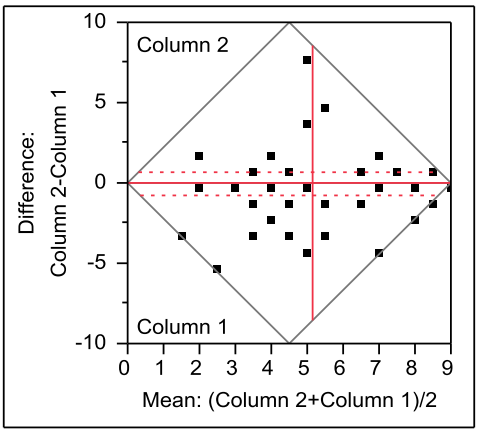
Statistical analysis and results for sensory training validation tests

1. Objective: determine whether significant difference exists between two validation tests.
Hypothesis: there is no difference between the mean values of the rating of the validation tests.

Paired t test

Because P-Value = 0.8983 >0.05, there is no significant difference.

Difference: Column 2-Column 1



Column 2	5.14286	t-Ratio	-0.12866
Column 1	5.19048	DF	41
Mean Difference	-0.0476		
		Prob > |t|	0.8983
Std Error	0.37013	Prob > t	0.5509
Upper 95%	0.69987	Prob < t	0.4491
Lower 95%	-0.7951		
N	42		
Correlation	0.51065		

2. Objective: determine whether significant difference exists between first rating test in the two validation tests.

Hypothesis: there is no difference between the mean values of the first rating of the validation tests.

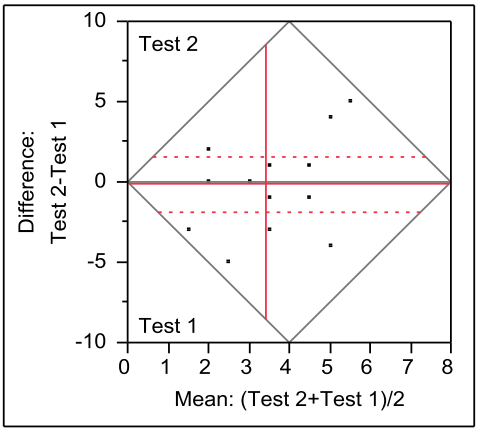
Paired t test

P-value= 0.8581

There is no difference between the mean values of the first rating of the validation tests.

Matched Pairs

Difference: Test 2-Test 1



Test 2	3.35714	t-Ratio	-0.18234
Test 1	3.5	DF	13
Mean Difference	-0.1429	Prob > |t|	0.8581
Std Error	0.78346	Prob > t	0.5709
Upper 95%	1.54971	Prob < t	0.4291
Lower 95%	-1.8354		
N	14		
Correlation	-0.1143		

3. Objective: determine whether significant difference exists between second rating test in the two validation tests.

Hypothesis: there is no difference between the mean values of the second rating of the validation tests.

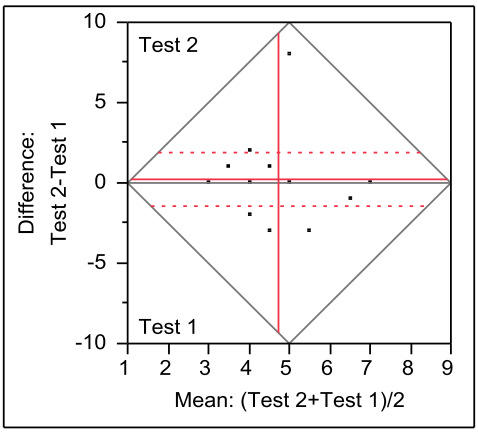
Paired t test

P-value= 0.7693

There is no difference between the mean values of the second rating of the validation tests.

Matched Pairs

Difference: Test 2-Test 1



Test 2	4.84615	t-Ratio	0.3
Test 1	4.61538	DF	12
Mean Difference	0.23077	Prob > |t|	0.7693
Std Error	0.76923	Prob > t	0.3847
Upper 95%	1.90678	Prob < t	0.6153
Lower 95%	-1.4452		
N	13		
Correlation	-0.2029		

4. Objective: determine whether significant difference exists between third rating test in the two

validation tests.

Hypothesis: there is no difference between the mean values of the third rating of the validation tests.

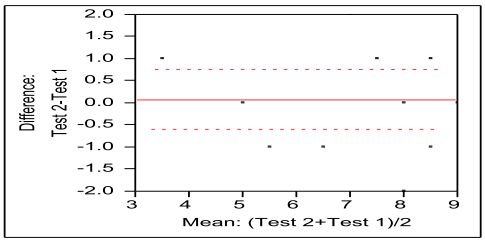
Paired t test

P-value= 0.8078

There is no difference between the mean values of the second rating of the validation tests.

Matched Pairs

Difference: Test 2-Test 1



Test 2	7.30769	t-Ratio	0.248708
Test 1	7.23077	DF	12
Mean Difference	0.07692	Prob > |t|	0.8078
Std Error	0.30929	Prob > t	0.4039
Upper 95%	0.75081	Prob < t	0.5961
Lower 95%	-0.597		
N	13		
Correlation	0.80724		

5. N= The rating of the sample in the first test- the rating of the sample in the second test

When N>2, the variation is higher than expected.
If more than one of the tests have higher expected variation, the panelist need to be retrained.

The rating for the first sample should be in the range of 0-3,
The rating for the second sample should be in the range of 4-6,
The rating for the third sample should be in the range of 7-9.
If more than three of the tests have rating number not located in this range, the panelist need to be retrained.

Based on these criteria, four people need to be retrained.

Appendix F

Training guide for oxidized-flavor omega-3 fatty acid added milk

In the training, I used the milk from the dairy farm, homogenized with omega-3 fatty acid, and then pasteurize them.

Training

Day 1 (1 hour)

Materials:

Consent form for training

Water cups and spit cups

Regular milk samples homogenized with omega-3 fatty acid and oxidized under light for 6 days.

Regular milk with omega-3 fatty acid added without light treatment

Brita water

Non-salt crackers

Triangle test score sheets

Plan:

- Establish how to taste samples: (10 min)
    - ✧ Taste without smelling before hand, just bring sample to mouth, and expectorate.
    - ✧ Water for rinsing mouth
    - ✧ The right way to taste samples: Take a generous sip, roll the milk around in the mouth, and then expectorate. After taste, draw a breath of air slowly through the mouth and then exhale slowly though the nose.
- Explain why the light-oxidized flavor is important. (5 min)
    - ✧ Light-oxidized flavor defects and nutrient losses in dairy products are a consequence of product exposure to fluorescent lighting in the retail display cases.
    - ✧ Light-oxidized flavor defects result from two types of photodegradative reactions:

- ✧ One type involves proteins and amino acids and occurs rapidly, sometime within minutes. Resulting flavors are characterized as burnt protein (i.e. burnt feather or hair), burnt cabbage, mushroom, or plastic-like.
- ✧ Another type involves oxidation of unsaturated fatty acid, and occurs more slowly. Resulting flavor are typically perceived as similar to old vegetable oils, cardboard, or metallic.

- Sign the consent form (5 min)
- Taste light-protected milk, and tell panelists that this is reference sample. (5 min)
- Give panelists a set of triangle test, using regular milk and 6 days oxidized regular milk. Tell them that they can taste reference samples during the training session. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 6 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- The trainees who get both two questions right, can leave. The trainees who get wrong need to stay do one more triangle test.

Day 2 (1hour)

Materials:

Water cups and spit cups

Milk samples

Oxidized milk samples

Brita water

10 point scale paper

Non-salt crackers

Triangle test scoresheet

Establish sample tasting

- How to taste samples? (5 min)
  - ✧ Spit before raise.
  - ✧ How big a bite to take?
  - ✧ How much cracker to eat?

- Give panelists reference samples
- Give panelists a set of triangle test, using regular milk and 5 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Define vocabulary: (5 min)

Light-oxidized

- Give panelists a set of triangle test, using regular milk and 5 days oxidized regular milk. (10 min)

After this, tell them that which is oxidized milk.

- Check how many people right
- Give panelists a set of triangle test, using regular milk and 4 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 4 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 4 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- The trainees who get all questions right, can leave. The trainees who get wrong need to stay do one more triangle test.

Day 3 (1 hour)

Materials:

Water cups and spit cups

Milk and oxidized milk

Brita water

10 point scale paper

Non-salt crackers

Triangle test scoresheet

- Give panelists a set of triangle test, using regular milk and 3 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 3 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 3 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.

Summarize again the characteristics of the oxidized milk.

- Give panelists a set of triangle test, using regular milk and 2 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.
- Give panelists a set of triangle test, using regular milk and 2 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.

- The trainees who get all questions right, can leave. The trainees who get wrong need to stay do one more triangle test.

- Give panelists a set of triangle test, using regular milk and 2 days oxidized regular milk. (10 min)
  After this, tell them that which is oxidized milk.

Day 4 (1 hour)

Materials

Water cups and spit cups

Milk and oxidized milk

Brita water

10 point scale paper

Non-salt crackers

Triangle test scoresheet

- Give panelists milk samples: regular, 2 days, 4 days, 6 days, and 14 days oxidized samples as reference. (20 min)

Ask them to rank 8 samples, and see their correctness.

- Tell panelists to use 10 points scale to mark samples.

0: regular milk

1: the oxidized flavor between 0 and 2.

2: 2 days milk

3: the oxidized flavor between 2 and 4

4: 4 days milk

5: the oxidized flavor between 4 and 6

6: 6 days milk

7: the oxidized flavor between 6 and 8

8: 14 days

9: higher than 14 days

- Give them a paper that shows the scores and let them remain the reference samples.

- Give them samples: 2 days, 6 days, and 14 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

- Give them samples: 0 days, 4 days, and 7 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

- Give them samples: 1 days, 3 days, and 5 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

Validation test

Give them samples: 2 days, 4days, and 7 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

Day 5 (1 hour)

Materials:

Water cups and spit cups

Brita water

Milk and oxidized milk

Non-salt crackers

Triangle test scoresheet

10 points scoresheet

- Give them a paper that shows the scores and let them remain the reference samples.

- Give them samples: 3 days, 7 days, and 14 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

- Give them samples: 0 days, 3days, and 6days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

- Give them samples: 8 days, 2 days, and 8 days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

Validation test

- Give them samples: 2 days, 4 days, and 7days. Let panelists score them, and they can use reference samples during scoring, and give them right answers. (10 min)

Appendix G

Calculations for the amounts of the fish oil and dairy cream added in two batches of 2% fat milk.

Recommended consumption of milk for adults is 3 cups/day (USDA).

I use Ocean Nutrition fish oil and the EPA+DHA (mg/g) as free fatty acid in the fish oil is 250 mg/g. In 1 Liter 2% skim milk, the amount of fat is 1000ml*1.03g/ml*2%=20.6g

The dietary recommendation for EPA+ DHA is 500 mg/day, the ocean nutrition fish oil we need to add: 500mg/250mg*1g=2 g for 3 cups

The fish oil we need to add is: 2.81g/L

Then, we can calculate back how much cream we need to add back into milk to make 2% skim milk.

Appendix H: Training scoresheets

SCORECARD

"A"-"Not A" Test

Name: Date:

Type of sample: Milk

Instructions: Smell the sample for 10 seconds. Take a generous sip, roll the milk around in the mouth, and then expectorate. After each sample, record your response below, rinse you palate with water, and eat crackers.

Sample	Oxidized	Not Oxidized
543		
671		
312		
289		
372		

SCORECARD

PAIRED COMPARISION TEST

Name: Date:

Type of sample: Milk

Instructions: Taste sample 578 first, and then taste sample 199, and enter your verdict below.

If no difference is apparent, enter your best guess.

Which sample is more oxidized?

578 199

Appendix I. Oxidation evaluations from day 1 to 21

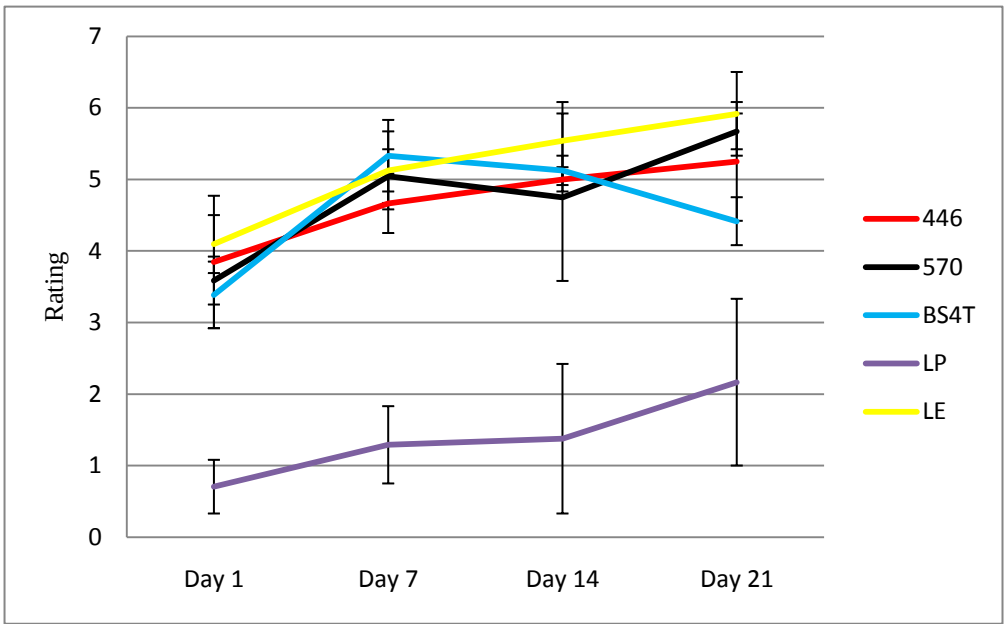


Figure I.1: Mean ratings (n=12 observations) for oxidized flavor in milk packaged in glass with iridescent or light-blocking overwraps from day 1 to 21. Rating scale is from 0 (none) to 9 (extreme). Legend: 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light. All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

Appendix J: Retention Times of SPME/GC External Reference Compounds

Table J.1: Retention Times of SPME/GC External Reference Compounds

Standard Name	Retention Time (min)
Acetaldehyde	1.435
Butanal	2.069
1-penten-3-ol	3.122
1-penten-3-one	3.187
Propanal	3.42
Pentanal	3.6
Hexanal	6.690
2,4-heptadienal	16.679

Appendix K: Microbial counts (colony forming units) on aerobic and coliform, Petrifilm™ count plates for milk enriched with fish oil during 21 days storage at 4 ℃.

Table K.1: Microbial counts (colony forming units) on aerobic and coliform, Petrifilm™ count plates for milk enriched with fish oil during 21 days storage at 4 ℃.

Day 1						
Plates	Aerobic			Coliform		
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Samples						
BG	0,0 [1]	0,0	0,0	0,0	0,0	0,0
RG	0,0	0,0	0,0	0,0	0,0	0,0
BV	0,0	0,0	0,0	0,0	0,0	0,0
LE	0,0	0,0	0,0	0,0	0,0	0,0
LP	0,0	0,0	0,0	0,0	0,0	0,0
Day 7						
Plates	Aerobic			Coliform		
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Samples						
BG	0,0	0,0	0,0	0,0	0,0	0,0
RG	0,0	0,0	0,0	0,0	0,0	0,0
BV	0,0	0,0	0,0	0,0	0,0	0,0
LE	0,0	0,0	0,0	0,0	0,0	0,0
LP	0,0	0,0	0,0	0,0	0,0	0,0
Day 14						

Plates	Aerobic			Coliform		
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Samples						
BG	0,0	0,0	0,0	0,0	0,0	0,0
RG	0,0	0,0	0,0	0,0	0,0	0,0
BV	0,0	0,0	0,0	0,0	0,0	0,0
LE	0,0	0,0	0,0	0,0	0,0	0,0
LP	0,0	0,0	0,0	0,0	0,0	0,0

Day 21

Plates	Aerobic			Coliform		
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Samples						
BG	0,0	0,0	0,0	0,0	0,0	0,0
RG	0,1	0,0	0,0	0,0	0,0	0,0
BV	1,1	0,0	0,0	0,0	0,0	0,0
LE	0,0	0,0	0,0	0,0	0,0	0,0
LP	0,1	0,0	0,0	0,0	0,0	0,0

10^{-1}= 1:10 dilution, 10^{-2}= 1:100 dilution, 10^{-3}= 1:1000 dilution
[1] colony forming units in replication 1, colony forming units in replication 2

Appendix L: Peak areas for various compounds in different milk samples from day 1 to 21.

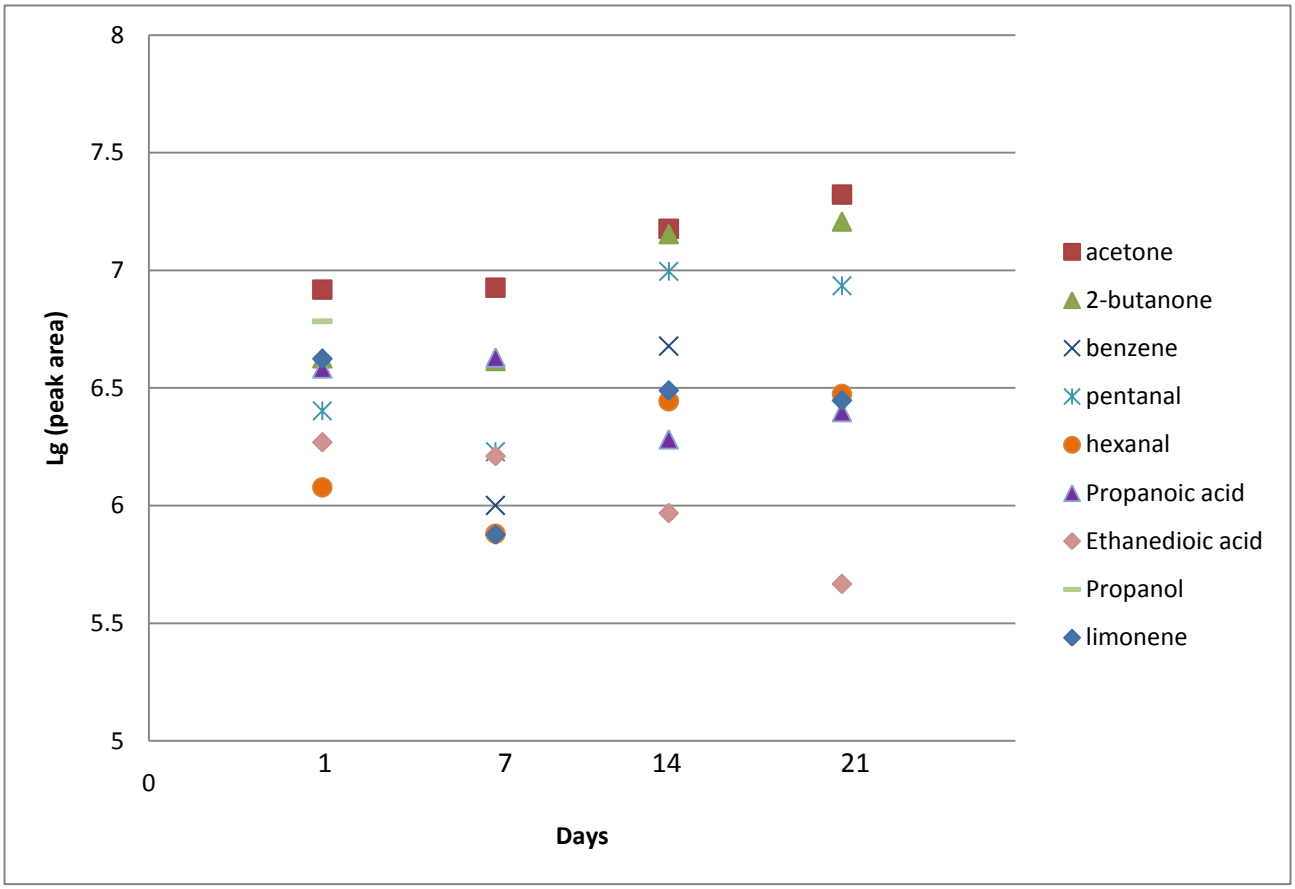


Figure L.1: Log_{10} (Peak area) for various compounds in omega-3 enriched milk packaged in glass bottles with 4 layers of 9231 Blue-Green iridescent film (446 nm block), which blocks riboflavin excitation wavelengths from 425-520 nm, from day 1 to 21 at 4°C. Transmission for 446 nm block (4 layers of 9231 Blue Green films) was approx. 0%. Films were obtained from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

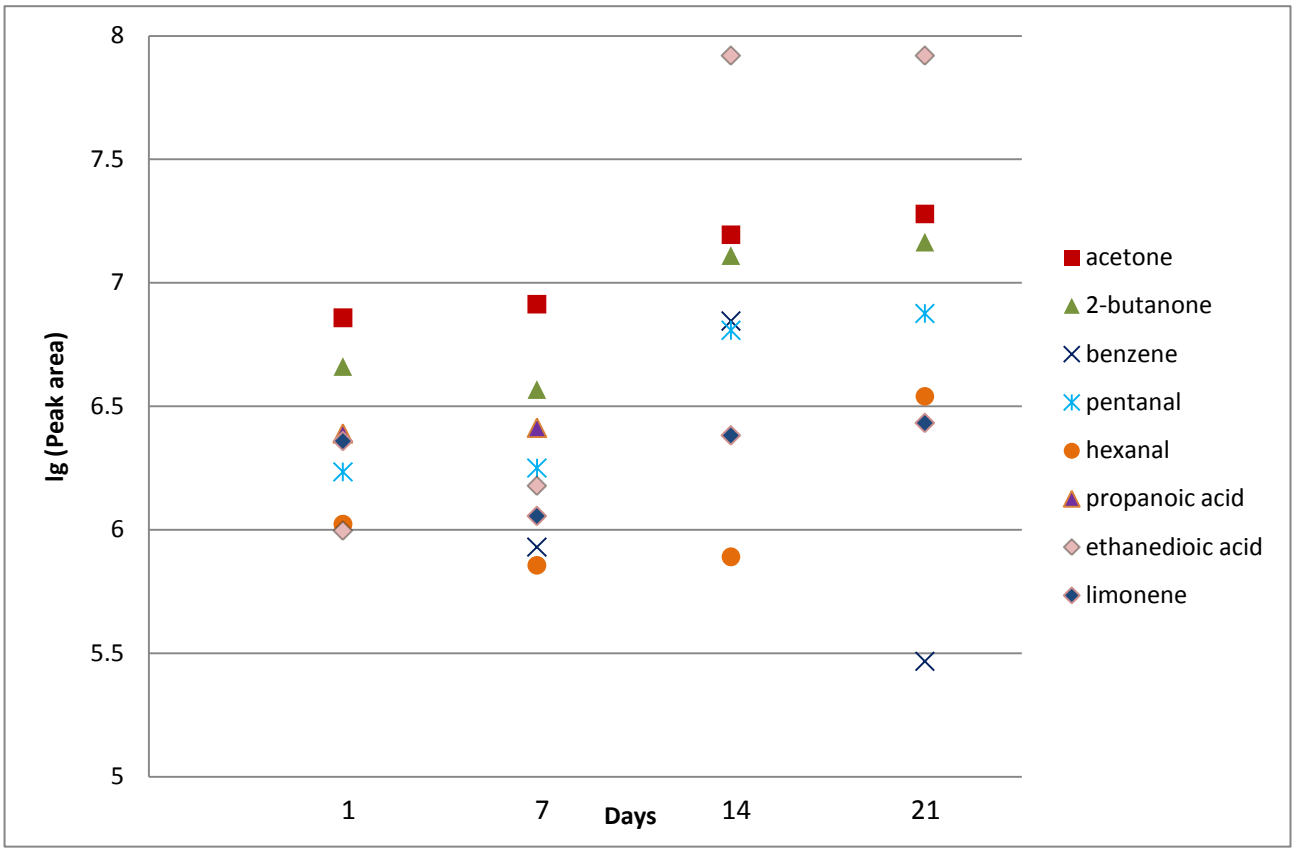


Figure L.2: Log_{10} (Peak area) for various compounds in omega-3 enriched milk packaged in glass bottles with 4 layers of 9231 Red-Green iridescent film (570 nm block), which blocks riboflavin excitation wavelengths from 520-580 nm, from day 1 to 21 at 4°C. Transmission for 570 nm block (4 layers of 9231 Red- Green films) was less than 10%. Films were obtained from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

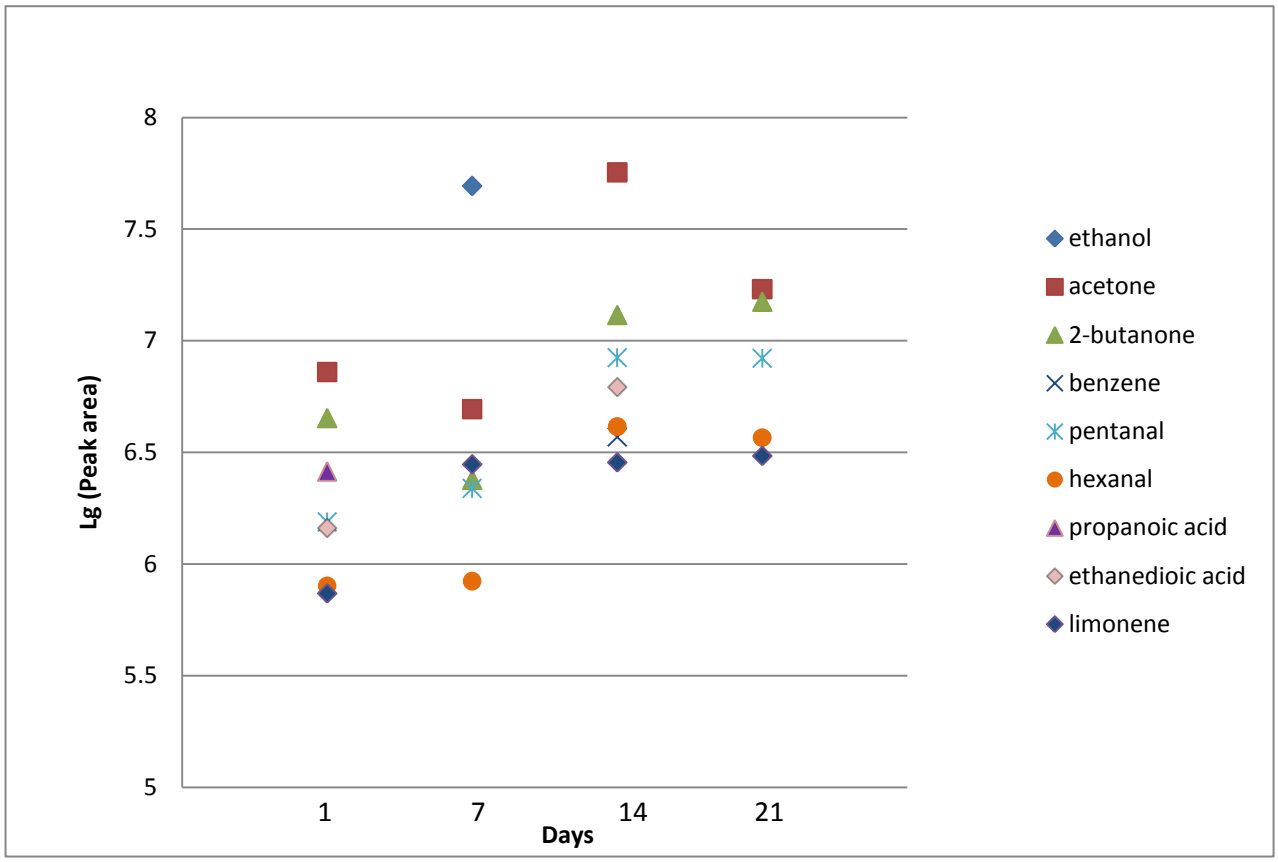


Figure L.3: Log_{10} (Peak area) for various compounds in omega-3 enriched milk packaged in glass bottles with the combination of 4 layers of 9231 Blue-Violet, 2 layers of 9231 Blue-Green, and 2 layers of Red-Red films (BS4T), which blocks wavelengths from 370-446 nm and 525-580 nm, from day 1 to 21 at 4°C. Transmission for BS4T was less than 5%. Films were obtained from Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

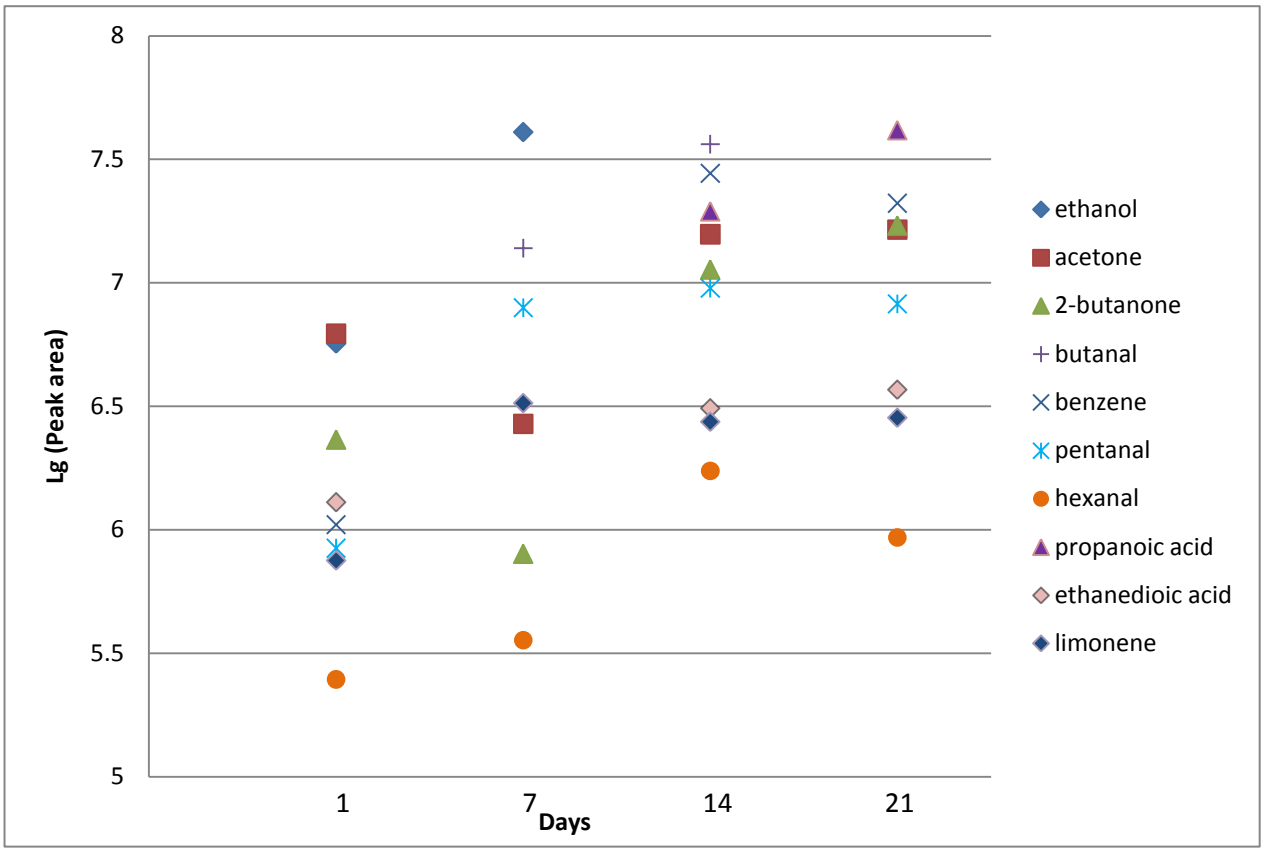


Figure L.4: Log_{10} (Peak area) for various compounds in the milk exposed (LE) directly to the fluorescent light, from day 1 to 21 at 4 °C. The glass of milk bottle can block wavelengths below approx. 300 nm.

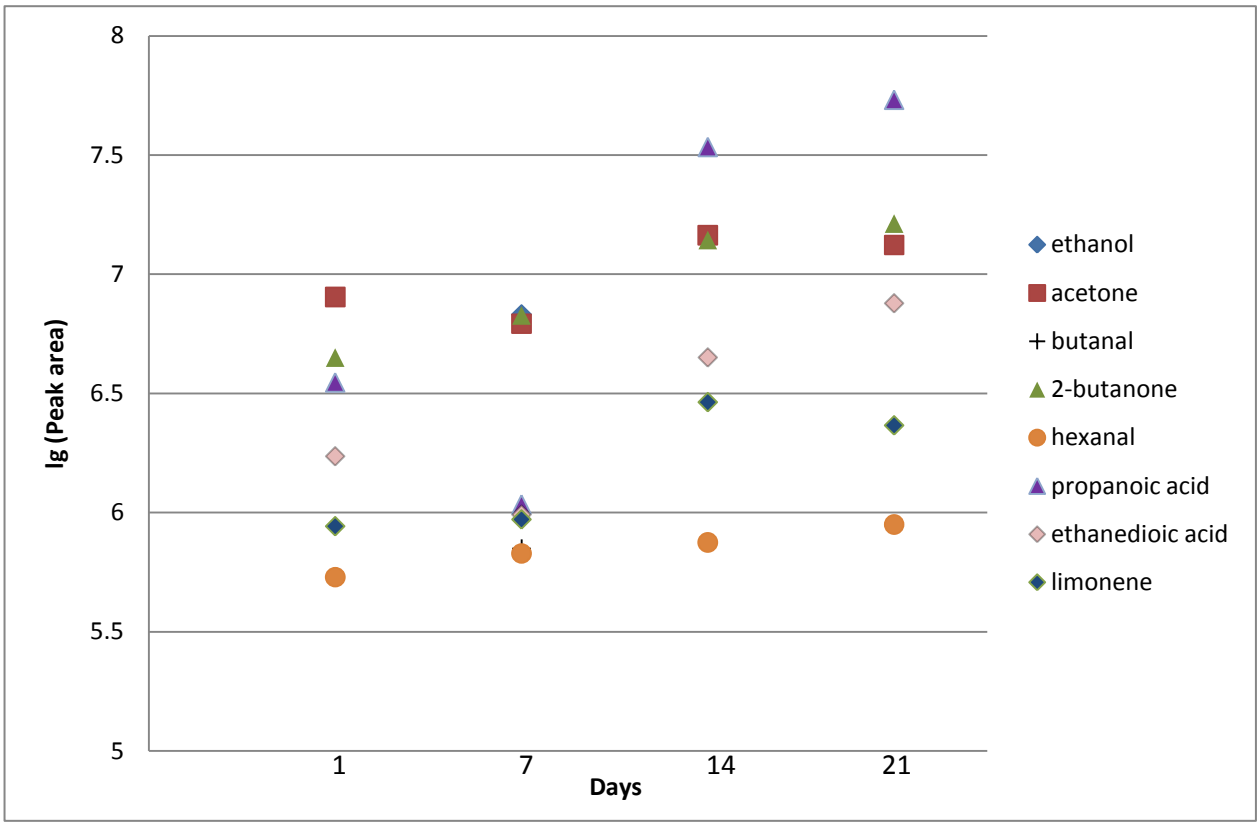


Figure L.5: Log_{10}(Peak area) for various compounds in the milk packaged with foil (LP) to prevent light, from day 1 to 21 at 4 °C. This treatment completely blocked all visible and UV light.

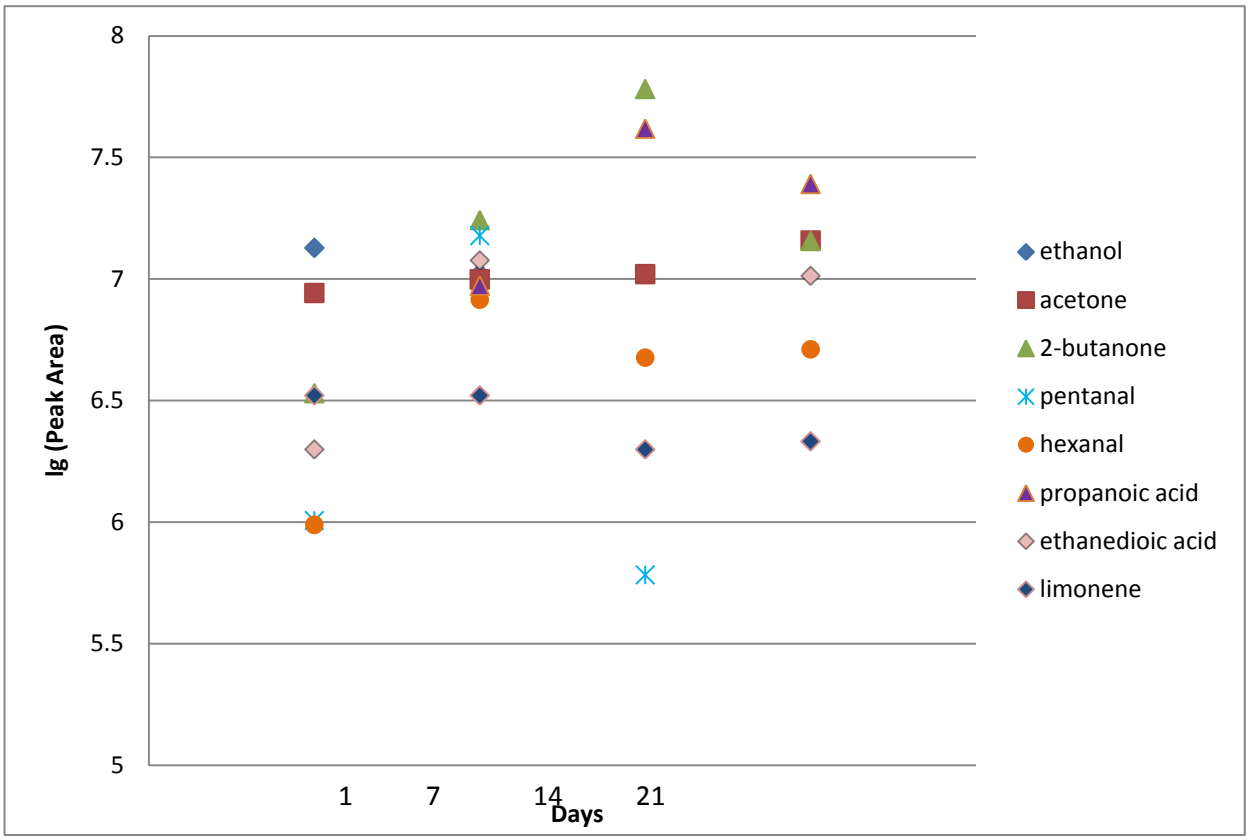


Figure L.6: Log_{10} (Peak area) for various compounds in the milk without fish oil (NFO) exposed directly to the fluorescent light, from day 1 to 21 at 4 °C. The glass of milk bottle can block wavelengths below approx. 300 nm.

Appendix M: Comparison of log (area) for hexanal, acetone, 2-butanone, limonene, and pentanal, from day1 to 21 in different milk treatments.

Table M.1: Log_{10} (Hexanal Area) from day 1 to 21 in different milk treatments.

Treatments[1]	Days			
	1	7	14	21
446	6.08[a]	5.88[a]	6.44[a]	6.47[a]
570	6.02[a]	5.86[a]	5.89[b]	6.54[a]
BS4T	5.90[a]	5.92[a]	6.62[a]	6.57[a]
LE	5.39[b]	5.55[a]	6.24[ab]	5.97[b]
LP	5.73[ab]	5.83[a]	5.88[b]	5.95[b]
NFO	5.99[a]	6.92[b]	6.68[ac]	6.71[a]

[1]446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a b c d] mean within columns with different super script are different based on the difference of Log_{10}(Hexanal Area) >0.5.

Table M.2: Log_{10} (Acetone Area) from day 1 to 21 in different milk treatments.

Treatments[1]	Days			
	1	7	14	21
446	6.92[a]	6.93[a]	7.18[a]	7.32[a]
570	6.86[a]	6.91[a]	7.19[a]	7.28[a]

BS4T	6.86[a]	6.69[a]	7.75[b]	7.23[a]
LE	6.79[a]	6.43[b]	7.20[a]	7.22[a]
LP	6.90[a]	6.79[a]	7.16[a]	7.12[a]
NFO	6.94[a]	7.00[a]	7.02[a]	7.16[a]

[1]446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a b] mean within columns with different super script are different based on the difference of Log_{10}(Acetone l Area) >0.5.

Table M.3: Log_{10} (2-butanone Area) from day 1 to 21 in different milk treatments.

Treatments[1]	**Days**			
	1	**7**	**14**	**21**
446	6.63[a]	6.62[a]	7.15[a]	7.21[a]
570	6.66[a]	6.57[a]	7.11[a]	7.17[a]
BS4T	6.65[a]	6.38[a]	7.12[a]	7.17[a]
LE	6.37[a]	5.90[b]	7.05[a]	7.23[a]
LP	6.65[a]	6.83[ac]	7.14[a]	7.21[a]
NFO	6.53[a]	7.24[c]	7.78[b]	7.16[a]

[1]446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a b c] mean within columns with different super script are different based on the difference of Log_{10} (2-butanone Area) >0.5.

Table M.4: Log_{10} (Limonene Area) from day 1 to 21 in different milk treatments.

Treatments[1]	Days			
	1	7	14	21
446	6.62[a]	5.88[a]	6.49[a]	6.45[a]
570	6.36[ab]	6.06[ab]	6.38[a]	6.43[a]
BS4T	5.87[b]	6.45[b]	6.45[a]	6.48[a]
LE	5.88[bc]	6.51[b]	6.44[a]	6.45[a]
LP	5.94[bc]	5.97[a]	6.46[a]	6.37[a]
NFO	6.52[a]	6.52[b]	6.30[a]	6.33[a]

[1]446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a b c] mean within columns with different super script are different based on the difference of Log_{10} (Limonene Area) >0.5.

Table M.5: Log_{10} (Pentanal Area) from day 1 to 21 in different milk treatments.

Treatments[1]	Days			
	1	7	14	21
446	6.40[a]	6.23[a]	7.00[a]	6.93[a]

570	6.24[a]	6.25[a]	6.81[a]	6.88[a]
BS4T	6.19[a]	6.34[a]	6.92[a]	6.92[a]
LE	5.93[a]	6.90[b]	6.98[a]	6.91[a]
LP	NA	NA	NA	NA
NFO	6.01[a]	7.18[b]	5.78[b]	NA

[1]446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red; LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora® Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a b] mean within columns with different super script are different based on the difference of Log_{10} (Pentanal Area) >0.5.

Appendix N: Concentration (mg/g) of EPA and DHA in extracted oil from milk samples stored for 21 days at 4°C under the fluorescent light with 1210 lux.

Table N.1: Mean[1] concentration (mg/g) of EPA[2] and DHA[2] in extracted oil from milk samples stored for 21 days at 4°C under the fluorescent light with 1210 lux.

Treatments	mg EPA/g oil (Mean ± std)	mg DHA/g oil (Mean ± std)	mg EPA+DHA/g oil
446[3] on day 21	29.9 ±2.1[b]	11.3 ± 0.17[b]	38.4
570 on day 21	23.5 ±1.8[b]	8.0 ± 2.1[b]	34.2
BS4T on day 21	30.1 ±0.6[b]	10.6 ±0.82[b]	41.7
LE on day 21	31.4 ± 4.6[b]	8.7 ±0.85[b]	37.5
LP on day 21	29.9 ±0.77[b]	12.5 ±1.5[b]	42.0
NFO on day 21	NA	NA	NA

[1] n=2 replications

[2]EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; calculated based on tricosanoic acid internal standard

[3] 446: overwrapped with 9231 Blue-Green; 570: overwrapped with 9231 Red-Green; BS4T (Broad Spectrum, 4% transmission): overwrapped with combinations of 4 layers 9231 Blue-Violet, 2 layers 9231 Blue-Green, and 2 layers 9231 Red-Red: LP (Light Protected): overwrapped with foil; LE (Light Exposed): no overwrap, exposed directly to light; NFO: milk without fish oil exposed directly to the fluorescent light, All films were provided by Aurora Standard Films, 9231 series, Engelhard Corp., Iselin, NJ.

[a] means within columns with different super script are significantly different ($p<0.05$)