Protective effects of titanium dioxide packaging modification on sensory and oxidative changes in milk over 35 day shelf-life

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ABSTRACT

Milk is often packaged in translucent containers providing little protection against flavor degradation from light. The effectiveness of TiO_2 modifications of high density polyethylene (HDPE) packaging in affecting light-induced oxidation of extended shelf-life milk (2% total fat) and omega-3 fatty acid enriched milk (2% total fat) was studied. Packaging effectiveness was determined by assessing product quality, including changes in flavor, measuring changes in volatile compounds, thiobarbituric reactive substances and riboflavin concentrations. Products were evaluated over a 35-day shelf-life when stored under fluorescent light (2200 lux) at 4°C. HDPE packaging included clear (no TiO_2) serving as control (light exposed: no light barrier, light protected (foil overwrap) and three different TiO_2 levels (low, medium, high) for the experimental treatments (total of five packaging treatments). TBARS was a good predictor of the perception of changes in sensory characteristics in 2% milk.. Under the experimental conditions used, a TBARS value of 1.3 mg/L could be considered the limiting sensory threshold for oxidized milk. Riboflavin concentration decreased by 10.5% in the light-protected control over 36 days and 28.5% in the high TiO_2 packaged 2% milk, but losses were greater than 40% for all other packages. In omega-3 enriched milk, the high TiO_2-HDPE package provided greater protection of sensory quality and riboflavin than clear, low and medium TiO_2 packaging. However riboflavin decreased by 28% even in the light protected control which is a higher loss than observed in 2% fluid milk without omega-3 lipids. TBARS was greater than 4

mg/L in all products, including the light-protected control within three days, suggesting that oxidative stability was low. Omega-3 milk packaged in clear HDPE package exceeded MDA of 3 mg/L by day 7, suggesting the milk would have changes in sensory quality related to oxidation. The high TiO_2 package protected riboflavin concentration from degradation and controlled MDA concentration the best of the TiO_2 treatments through the test period in both fish oil enriched and non-enriched products.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

There is a growing consumer interest in food products with added components that promote health. Advances in nutrition and food science are moving the food industry toward creating foods that promote optimal health and wellness as well as reduce the risk of chronic disease. These products are given the generic term functional foods or nutraceuticals. These products contain food components that have beneficial effects on human health beyond basic nutritive value (IFT 2006). Functional foods may also improve the quality of life by enhancing the ability to manage chronic diseases. With between $20 billion and $30 billion in sales a year, functional foods comprise about 5 percent of the overall US food market (Anonymous 2009).

Globally, the top functional food markets are beverage products. Beverages are forecast to continue to be the fastest-growing segment and to assume the largest share of the market (56 percent in 2013) (Anonymous 2009). Key drivers are consumer preference for the convenience and versatility of beverages, the relative ease of creating tasty products, and development of innovative packaging. Dairy beverages are widely formulated as functional foods and are a preferred carrier for bioactive ingredients due to convenience, flavor and nutritional value.

Milk is an excellent source of essential nutrients including protein, calcium, phosphorus, riboflavin, and vitamin D, to name a few (Patton 2004). In addition to the basic nutrition, many milk components contribute additional health value. Casein and whey proteins in milk have been related to skeletal muscle repair after endurance exercise (Lemon 2000). Alpha-lactalbumin, an important whey protein in bovine milk, is associated with the prevention of insomnia and may improve next-day alertness (Markus and others 2005). Calcium and vitamin D are critical to maximize bone health. Calcium helps build strong bones and reduces the onset of bone diseases

like osteoporosis, while vitamin D is necessary to absorb calcium. Bovine milk is a rich source for both nutrients, providing an estimated bioavaibility of 30% to 35% of calcium (Anonymous 2010). In the United States, the health and long-term care costs associated with osteoporosis-related fractures are estimated at $18 billion annually (Anonymous 2012).

Milk, a popular functional food in the grocery market, has become an ideal vehicle for delivering additional bioactive components to consumers. Companies such as Smart Balance Inc., Yoplait USA, Inc, and Organic Valley have recently targeted adding the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to milk products. Omega-3 fatty acids are a type of polyunsaturated fatty acid, that include α-linolenic acid (ALA, 18:3ω3), eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3) (Connor 2000). The Mayo Clinic recommends men and women consume 1.6 and 1.1 g per day, respectively, of omega-3 fatty acids (Mayo Clinic 2012). EPA and DHA are acknowledged as the principle omega-3 fatty acids; they have a vital role in a wide range of biological functions linked with increased health benefits.

Omega-3 fatty acids have been associated with to the prevention and reduced risk of cardiovascular disease, arthritis, and are important for brain development and function (Connor 2000; Horrocks and Yeo 1999). The human body cannot synthesize EPA and DHA de novo, therefore adequate amounts of these fatty acids or their precursors must to be provided through the diet. Marine fatty fish, flax and nuts are considered to be the primary dietary sources of omega-3 fatty acids; whereas fatty fish provide EPA and DHA, flax and nuts deliver alpha-linolenic acid (ALA) only. Contemporary diets are seriously deficient in natural sources of omega-3 fatty acids (Sabeena Favin and others 2010). To help supplement this deficit, the

American Heart Association recommends the consumption of fatty fish at least twice a week (Wylie-Rosett 2002). However, diets that include fish may not be widely accepted.

In 2004 the Food and Drug Administration (FDA) announced a qualified health claim on conventional foods that contain EPA and DHA omega-3 fatty acids. Conventional food products that are labeled with a qualified health claim must be supported by credible scientific evidence. Authorization of this claim is based on a systematic evaluation of the available scientific data, as outlined by the FDA's "Interim Procedures for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements" (Anonymous 2008). Retail sales in the U.S. of food and beverage products with a “high omega-3” claim grew 11% in 2010 and approached $4.4 billion. It is predicted in U.S. market that sales of products enhanced with omega 3 fatty acids will approach $7 billion in 2015 (Anonymous 2011). To meet the recommended consumption of PUFAs, especially EPA and DHA, many companies incorporate marine oil into milk products. Unfortunately, light reactive molecules, such as riboflavin, increases the need to protect milk quality from the effects of light induced oxidation. In the case of enriching milk with omega 3 fatty acids, EPA and DHA are highly susceptible to oxidation due to their high levels of unsaturation, increasing the risk of autoxidation (Let and others 2006).

Compounds produced from omega-3 fatty acid oxidation have very low aroma and flavor threshold levels and can negatively impact the quality of omega-3 fatty acid fortified products (Moore and others 2012). Although there are a variety of milk products already on the market fortified with omega-3 fatty acids, a potential problem for milk enriched with omega-3 fatty acids is the increased risk of photooxidation. Oxidation of omega-3 fatty acids decreases their concentrations, negating the value of fortifying the food carrier. Dairy products exposed to light have higher levels of off flavors and degradation of important nutrients. These light induced off

flavors are perceived by consumers and make the products less acceptable or unacceptable as well as shortens the shelf life (Chapman and others 2002). The use of packaging to guard against light energy is a major approach to protecting sensory quality of fluid milk beverages and limit oxidation of sensitive fatty acids, including omega-3 fatty acids.

Purpose of Study

The overall purpose of this research was to quantify performance of TiO_2-dosed high density polyethylene (HDPE), packaging (three different levels; DuPont, Wilmington, DE) on preserving milk sensory quality through prevention or control of photochemically-induced reactions. Emphasis was placed on understanding the control of sensory quality (flavor/aroma) and riboflavin integrity in milk beverages. In addition, the oxidative stability of the beverage products were chemically analyzed to complement the sensory evaluation data. This information will be used to help guide packaging decisions for photo-protection of beverages and foods containing PUFAs and photosensitizers.

This project is designed as two experiments:

1. Protecting 2% extended shelf-life milk using titanium dioxide modifications in packaging over 35 day shelf-life stored in simulated retail conditions.
2. Protecting extended shelf-life milk fortified with omega-3 fatty acids using titanium dioxide modifications in packaging over 35 day shelf-life stored in simulated retail conditions.

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CHAPTER II
REVIEW OF LITERATURE

Potential Health Value of Omega-3 Fatty Acids in Milk and Dairy Foods

Consumption of dietary omega-3 fatty acids (FA) for health benefits has been extensively studied by the medical, scientific, and nonscientific communities. In 2004 the Food and Drug Administration (FDA) announced a qualified health claim on conventional foods that contain EPA and DHA omega-3 fatty acids. Conventional food products that are labeled with a qualified health claim must be supported by credible scientific evidence. Authorization of this claim is based on a systematic evaluation of the available scientific data, as outlined by the FDA's "Interim Procedures for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements" (Anonymous 2008a).

Omega-3 fatty acids have been related to the reduced risk of cardiovascular disease, arthritis, and are important for brain development and function (Connor 2000; Horrocks and Yeo 1999). Carrero and others (2004) showed that omega-3 fortified milk can help reduce risk of heart disease. Volunteers drank omega-3 fortified milk for eight weeks. Following the eight weeks of fortified milk consumption, blood concentrations of total cholesterol, LDL cholesterol and triglycerides, which are markers of heart disease, decreased by 9%, 13%, and 24% respectively. Blood concentrations of omega-3 fatty acids were increased; DHA (docosahexaenoic acid) increased by 20% and EPA (eicosapentaenoic acid) by 33%. The results show that enrichment of milk with omega-3 could be a good way of helping people with an above average risk of heart disease to normalize their cholesterol levels and reduce their risk.

It is more economical to fortify milk with omega-3 FA through formulation and processing then by pre-harvest technologies (Rego and others 2005). Milk and milk-based dairy products that are fortified with omega-3 FA can provide a resource for supplementation of omega-3s into the diet. There are several aseptically paperboard packaged UHT processed milk products on the market in the U.S. that have been fortified with omega-3 FA. One product by Horizon (Horizon, Boulder, CO) claims to provide 32 mg of EPA+DHA per 8 once serving (Moore 2009).

Challenges with Omega-3 Enrichment in Milk

Regulatory Challenges.

Several omega-3 fortified foods currently sold on the market are dairy-based products. While there are low levels of omega-3 FA naturally present in milk (Ellis and others 2006), it is difficult to fortify milk with omega-3 FA and still maintain the descriptor of "milk", due to federal regulations pertaining to milk composition and processing. The Filled Milk Act of 1923 (21 U.S. Code, Section 61-64) states that it is unlawful to participate in the selling of any "filled milk." Filled milk is further defined as any milk, cream, or skimmed milk that has fat or oil, other than milk fat, blended or compounded within it to produce an imitation milk product or product that resembles milk. Another obstacle in promoting omega-3 fortified milk is omega-3 FA are not considered a viable addition to milk under 21 CFR 131.110, which puts forth a standard identity for milk (Anonymous 2003). Reformulating milk to contain foreign fat would require labeling that specifies the product is a dairy or milk based beverage, not milk. To increase the levels of omega-3 FA presently found in milk, research has been done to manipulate the FA composition in milk that cows produce by altering their diet (Rego and others 2005). Donovan and others (2000) found that milk produced through dietary modifications was not

more prone to oxidation due to the higher content of polyunsaturated fatty acid. Therefore, pre-harvest dietary modification to increase omega-3 fatty acids concentration in milk is an optimal natural approach and not restricted by federal regulations for the term "milk".

However, pre-harvest approaches are extremely inefficient in increasing omega-3 fatty acids in milk (Rego and others 2005). As regulated by the Pasteurized Milk Ordinance, any use of synthetic antioxidants and many other ingredients are not permitted in several dairy systems, including milk. However, their uses are permitted for products in formulated food systems, which include formulated dairy-based food systems. The same applies for naturally present antioxidants, such as tocopherols, ascorbic acid, and ascorbyl palmitate are allowed for use in milk but, must be stated on the label (IDFA 2003). This limits the use of antioxidants added for controlling oxidation of omega-3 enriched fatty acids in milk. The dairy industry can overcome these hurdles by marketing a value added dairy-based beverage.

Quality Effects of Omega-3 Fatty Acids. The source quality and type of the omega-3 fatty acid should be considered before incorporation into a dairy product. Dairy foods enriched with omega-3 fatty acid can exhibit sensory characteristics of the source from which the omega-3 FA were derived. Milk has a mild flavor profile that is pleasantly sweet and should exhibit no aftertaste other than the richness imparted by natural components such as milk-fat and other milk solids (Alvarez 2009). Challenges of fortifying milk with omega-3 FA involve impeding any fishy flavor or odor that may be imparted in it as a result of fortification with fish oil. Kolanowski and others (2004) found that EPA and DHA can be added at low levels without any characteristic sensory attributes present in the product. In the same study, Kolanowski and others

found that omega-3 fish oil can contribute a “fishy” notes when added to foods at elevated levels. Omega-3 fatty acids can easily oxidize due to the double bonds present in their molecular structures. Although omega-3 FA are flavorless, oxidation of these lipids, along with other milk lipids, produce volatile oxidation compounds that can contribute undesirable off-flavors and/or aromas.

Other aspects that play an important role on the sensory and nutritional quality of milk and milk-based products enriched with these lipids are the source and processing of the omega-3 oil product. To reduce undesirable volatile compounds that contribute to the negative aroma associated with fish and algal oil, the oil may be deodorized. Microencapsulation of omega-3 rich oils can be used to protect these lipids from oxidation (Hanna 2009). Polyunsaturated lipids can be coated in a protective matrix that helps protect against oxidation and gives added thermo-stability against heat. These microencapsulated lipids then can be spray-dried to produce a potentially oxidatively stable powder that could be added to food products (Kolanowski and others 2004). Another method is to control oxidation of the oils used for fortification with antioxidants.

Hexanal is a volatile oxidation products that can add an unacceptable sensory characteristics to milk. Tocopherol antioxidants have been shown to work synergistically with ascorbic acid to reduce oxidation in vegetable oils (Drinda and Baltes 1999). The combination of the aforementioned antioxidants, added into the appropriate phases at the appropriate levels, may work synergistically together to inhibit or greatly reduce the oxidation of omega-3 FA so that no detectable levels of oxidation components can be determined. However, dairy products, such as cheese, butter, and flavored milks have been shown to be good delivery systems for elevated levels of omega-3 fatty acids. When fortified with long-chain omega-3 polyunsaturated fatty

acids from fish oil, solid high-fat dairy products such as butter, processed cheeses and fresh spreadable cheeses, and flavored milks have the highest ability to mask the sensory characteristics of fish oil (Kolanowski and others 2004).

Oxidation of Milk: Lipid oxidation can occur through several different mechanisms including photooxidation, autoxidation, thermal or enzymatic oxidation (Shahidi and Zhong 2010). Oxidation of milk typically occurs by both photooxidation and autoxidation reactions.

Photo-oxidation. Photooxidation occurs under the presence of light (artificial, sunlight, UV). This process is of particular concern in milk because it occurs quickly due to fluorescent lighting used in retail storage cases. Some compounds, called photosensitizers, absorb light energy and subsequently transfer that energy to other molecules. These compounds are important because they cause the destruction of other milk components usually unaffected by light (Boff and Min 2002). Dairy products contain six types of photosensitizers; riboflavin, protoporphyrin, hematoporphyin, chlorophyll a and b, and two unidentified tetrapyrroles (Wold and Others 2005). However, the most studied photosensitizer is riboflavin (Sattar and deMan 1976; Bekbolet 1990; Skibsted 2000). Riboflavin, also known as vitamin B2, is a water-soluble vitamin found in large amounts in milk, with an average concentration between 1.36 and 1.75 mg/L (Dimick 1982, Zygoura 2004). Riboflavin degradation is also undesirable in foods because it decreases nutritional value (Hoskin 1988).

Riboflavin absorbs light at wavelengths 400, 446, and 570nm (Bekbolet 1990). Upon absorbing light, it then transfers the energy to a reactive form of oxygen known as singlet (Figure 2.1) (Boff and Min 2002). Oxygen dissolved in milk provides a copious source of oxygen, which can potentially react with riboflavin molecules and ultimately causes the degradation of

riboflavin in dairy products (Shahidi and Zhong 2010). Allen and Parks (1979) found that riboflavin in whole milk was reduced by 75% when exposed to 32 hours of fluorescent light (2690 lux). Webster and others (2009) showed a riboflavin loss between 52.6% and 67.5% after 72 hours of exposure in bottles wrapped with films designed to block wavelengths that excite riboflavin. Figure 1 shows how light energy causes the degradation of riboflavin as well as creating singlet oxygen.

Riboflavin excitation: (Frankel 1984).

$$\text{Sens} \xrightarrow{\text{Light}} \text{Sens}^*$$

$$\text{Sens}^* + {}^3O_2 \longrightarrow {}^1O_2 + \text{Sens}$$

Figure 2.1: Shows light energy reacting with riboflavin creating sensitized riboflavin (Sens*), which react with triplet oxygen ($^3O_2$), creating singlet oxygen ($^1O_2$) (modified from Frankel 1984).

Singlet oxygen is the highly reactive form of oxygen. Because of its relatively long lifetime, it plays an important role in the oxidation of foods. The low activation energy of singlet oxygen (0 to 6 kcal/mole) initiates the oxidative reaction regardless of temperature (Lee 2002). This highly sensitive species of oxygen causes oxidation of lipids through autoxidation.

Autoxidation. Autoxidation of unsaturated fatty acids is the most common process of lipid oxidation (Frankel 1980). Autoxidation of lipids is influenced by the presence of dissolved oxygen, metals (copper, iron), salts of fatty acids, and exposure to light, especially direct sunlight or fluorescent lighting (Shahidi and Zhong 2010). In addition to the photo initiator riboflavin, milk contains iron (Fe^{2+}) and copper (Cu^{2+}) at an average of 0.07 and 0.027 mg/8oz respectively

(Anonymous 2008b). These trace amounts of metals may contribute to lipid oxidation by acting as catalysts.

Autoxidation occurs in three steps (Figure 2), initiation, propagation and termination. Initiation results in the production of a free radical (R•) that occurs when an initiator such as heat, light, or a metal extracts a hydrogen from the methylene group adjacent to the double bond in an unsaturated fatty acid (Shahidi and Zhong 2010). This free radical is extremely reactive and attacks other unsaturated sites of nearby molecules, such as EPA and DHA, leading to propagation of the free radical chain reaction, yielding the primary oxidative product, hydroperoxide. During termination, non-reactive secondary products are formed when the free radical species react with themselves. Due their high instability, hydroperoxides easily interact to form secondary products (Choe and Min 2006). These secondary oxidative products, mainly aldehydes and ketones in milk, cause a fishy off-flavor sensed by consumers.

Initiation:

(1) $RH \longrightarrow R\bullet + H\bullet$

(2) $R\bullet + O_2 \longrightarrow ROO\bullet$

Propogation:

(3) $ROO\bullet + RH \longrightarrow ROOH + R\bullet$

(4) $R\bullet + O_2 \longrightarrow ROO\bullet$

Termination:

(5) $R\bullet + R\bullet \longrightarrow R_2$

(6) $R\bullet + ROO\bullet \longrightarrow ROOR$

(7) $nROO\bullet \longrightarrow (ROO)$

Figure 2.2: Above is a representation of the three steps involved in autoxidation (Modified from Webster 2006).

Controlling Oxidation in Milk and Omega-3 Dairy Based Beverages

Besides water, milk is composed of a complex mixture of carbohydrates, proteins, lipids, vitamins and minerals. This composition reflects the fact that this medium is the sole source of food for young mammals. Milk was not intended to be exposed to light and due to its specific composition, it is a highly perishable product with high potential of rapid quality deterioration. Unfortunately, modification of milk composition suggests a need to consider the protective effects of the packaging as milk quality can be readily affected by light. In the case of enriching milk with omega 3 fatty acids, EPA and DHA are highly susceptible to oxidation due to their high levels of unsaturation, increasing the risk of autoxidation (Let and others 2006).

Numerous approaches have been evaluated to control or prevent lipid oxidation. One of the best methods for milk and milk-based products is the use of antioxidants. Raw milk naturally contains α-tocopherol and ascorbic acid at approximately 13-30 mg/kg-1and < 20 mg/kg-1 respectively (Rosenthal and others 1992; van Aardt and others 2003). Antioxidants, including tocopherols, ascorbyl palmitate, and ascorbic acid (Vitamin C) help control oxidation in dairy systems. Tocopherols, which are lipid soluble, exhibit low antioxidant properties when added to many food systems (Huang and others 1994). This may be a result of the natural presence of tocopherols at the maximum level of effectiveness against oxidation. Ascorbyl palmitate and ascorbic acid both have been shown antioxidant properties in dairy products at low levels. Ascorbyl palmitate has been shown to be an effective antioxidant when used at lower concentrations in milk and milk drinks containing 1.5-5% fat (Jacobsen and others 2008). van Aardt and others (2003) found that the addition of alpha-tocopherols and ascorbic acid at levels of 0.025% and 0.025% respectively, limited light-induced flavor in milk after 10 hours of light exposure.

Metal chelators in milk, such as the milk protein lactoferrin, also serve to provide protection against oxidation (Let and others 2006). EDTA is a metal chelating agent that can prevent the contact between transition metals and the unsaturated lipid or hydroperoxide (Let and others 2006).

Packaging for Protection of Milk from Oxidation

Food packaging has evolved from simply a means of storage to an active role in the preservation of food. Packaging is defined as a tool used to ensure delivery of goods to a consumer in the best condition intended for their use (Robertson 2006). Consumer demands for longer shelf life and wider distribution of milk and milk products have resulted in the development of processes and packaging concepts to increase the shelf life of these products. The best way to protect milk and dairy products from oxidation caused by light is with the use of packaging that provides a complete light block (Duncan and Webster 2009). However, the use of packaging that completely blocks light wavelength exposure is unappealing to consumers (Chapman and others 2002). Recently, milk marketing has shifted its focus to packages that are more inviting to consumers.

The usual packaging for milk includes high density polyethylene (HDPE), waxed paperboard, and poly(ethylene) terephthalate (PETE). HDPE and PETE are often pigmented to reduce the transmittance of light wavelengths into milk products. This pigmentation gives plastic milk containers their opaque appearance. van Aardt and others (2001) found that milk packaged in HDPE showed a higher level of oxidative off flavors than milk packaged in PETE with an ultraviolet (UV) light block. However, translucent HDPE proved to be better than PETE without UV block because HDPE blocks approximately 40% of light between 300 and 700nm, whereas

clear PETE only blocks 20% of light in the same range (van Aardt and others 2001). The challenge faced today is designing a package that is translucent or clear, satisfying the consumers' demand to see the product inside, yet reduces the transmission of light wavelengths that initiate oxidative reactions leading to the deterioration of milk quality (Skibsted and Hansen 2000).

van Aardt and others (2001) concluded that amber PETE was most effective in reducing off flavors/aromas of milk, compared to other types of PETE and HDPE. Light wavelengths in the 450-700 nm regions are reduced with the use of amber PETE. Intawiwat and others (2010) suggested that wavelengths higher than 500nm affect milk and dairy products' quality. This region of wavelengths are consistent with wavelengths at which riboflavin absorb light (Borle and others 2001; Hoskin and Dimick 1979), suggesting that this photosensitizer is at least partly responsible for the observed off flavors and aromas in milk exposed to this visible light region. While blocking these wavelengths may prevent the destruction of riboflavin, (Fukumoto and Nakashima 1975; van Aardt and others 2000) found that just blocking UV light did not protect riboflavin from degradation. Various light-blocking filters, applied to the package surface, affected the sensory characteristics of milk as demonstrated by Intawiwat and others (2010). Conversely, they established a decrease in rancid (oxidized) odors when filters that allowed different transmissions of visible light with red (transmission of 570-800 nm) and green (transmission of 500-800 nm) were used.

Light exposure induces two distinctive off-flavors in milk; a burnt feather, sunlight flavor, which predominates for two or three days of storage in milk, and a cardboard or metallic flavor that develops two days later with prolonged light exposure and does not dissipate (Barnard 1973; Skibsted 2000; Alvarez 2009). Dimethyl disulfide and methional, which can be formed from the

oxidation of sulfur containing amino acids such as methionine, are responsible for the sunlight flavor (Jung and others 1998). The cardboard or metallic flavor comes from secondary lipid oxidation products including hexanal, pentanal, ketones, and alcohols (Skibsted 2000).

Volatile compounds hexanal, pentanal, dimethyl disulfide, 1-octene-3-one, acetaldehyde, and 1-hexen-3-one are commonly found in light oxidized milk (Cadwallader and Howard 1998; Cladman and others 1998). van Aardt and others (2001) demonstrated a reduced formation of hexanal and dimethyl disulfide with the use of amber PETE, however acetaldehyde concentrations was not affected. A factor of the formation of these off-flavor producing compounds is the availability of oxygen. Headspace oxygen is also a component of the photooxidation process.

Materials such as PETE/polyethylene naphthalate (PEN) allow a transmittance level of 40% of wavelengths at the 365 nm range and have been shown to reduce hexanal concentrations (Lennersten and Lingnert 2000). Hexanal, a volatile compound that contributes to off-flavors in milk, is commonly regarded as a good measurement of lipid oxidation and related with unfavorable sensory results (Lennersten and Lingnert 2000). High concentrations of aldehydes were found to be the predominant compound in milk exposed to light (Intawiwat and others 2010). Higher concentrations of oxidative products were shown in milk products stored with orange filters (blocking transmittance of 520-800) than red, amber, or green filters blocking the same wavelengths. To determine which volatile compounds were produced in milk as a function of light wavelength, Webster and others (2011) exposed milk to narrow (50 nm) wavelength bands of light. They found wavelengths in the range of 200-400 (UV) produced high amounts of hexanal.

Webster and others (2009) evaluated the effects of photooxidation by reducing specific wavelength regions that transmitted through packaging. During this study, iridescent films that inhibited the light transmittance of riboflavin excitation wavelengths (400, 446, and 570) were examined. Even with the wavelengths that excite riboflavin blocked to less than 20% transmittance, off flavors, due to light oxidation, were observed by sensory analysis. This find supported the claim previously reported by Wold and others (2005), which stated that there are other compounds in milk acting as photosensitizers at different wavelengths or other means that continue the oxidative process resulting in off flavors and aromas. Packaging designed to protect milk quality and riboflavin from degradation has been pursued since the 1970s. This designed packaging targeted reducing the transmission of wavelengths (400, 446, and 570) absorbed by the photosensitizer riboflavin (Hoskin and Dimick 1979).

One possible packaging innovation that has shown usefulness in protecting against photooxidation is titanium dioxide (TiO_2). Since its commercial production in the early 20th century, titanium dioxide has been widely used as a white pigment in paints, toothpaste and packaging. Titanium dioxide is a photo-responsive material and its importance is steadily increasing in the polymer and plastic industry (Anonymous 2007). Due to its ability to scatter light and absorb UV light energy, titanium dioxide (TiO_2) has been added at different concentrations to HDPE and PETE as a light block (Robertson 2006). A study done by Moysssiadi and others (2004) showed that a multilayer HDPE package pigmented with TiO_2 and carbon black protected milk better than a monolayer HDPE package pigmented with TiO_2, clear PETE and PETE pigmented with TiO_2,when stored under florescent light at 4°C for 7 days. Moyssiadi found the multilayer HDPE package protected milk quality and suffered only a 28% riboflavin loss. Titanium dioxide's ability to scatter light works by its capacity to refract and

diffract light. The particle size of TiO_2 can be altered to affect color of light wavelengths transmitted (Anonymous 2007). Innovation in the use of TiO_2 particle size and refraction properties may improve packaging materials for milk quality protection.

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CHAPTER III
PROTECTING 2% EXTENDED SHELF LIFE MILK USING TITANIUM DIOXIDE MODIFICATIONS IN PACKAGING

ABSTRACT

The effectiveness of innovative TiO_2-loaded high density polyethylene (HDPE) in reducing light-induced oxidation of extended shelf-life milk (2% total fat) was studied. Effectiveness was assessed by sensory evaluation, changes in volatile compounds, thiobarbituric reactive substances (TBARS) and riboflavin concentration. Milk (2%) was stored in HDPE packages loaded with TiO_2 at three levels (low, medium, high) at 2.7°C $\pm$.0.8 for up to 43 days. Light protected (foil wrapped clear HDPE) and light exposed (clear HDPE) served as controls. The high TiO_2-HDPE package provided protection similar to light-protected control package (foil wrapped) through day 22, with less consistent performance by the medium TiO_2 package. Malondialdehyde (MDA) concentration, as measured by TBARS, increased in all treatments during storage. TBARS was a good predictor of the perception of rancidity. Under the experimental conditions used, a TBARS value of 1.3 mg/L could be considered the limiting sensory threshold for differentiating oxidized milk. Riboflavin concentration decreased 10.5% in the light-protected control and 28.5% in the high TiO_2 packaged milk over 36 days, but losses were greater than 40% for all other packages. The high TiO_2 package protected riboflavin concentration from degradation and controlled MDA concentration through the tested period.

Key Words: Oxidation, sensory evaluation, packaging, milk, riboflavin

INTRODUCTION

Milk and milk products are susceptible to light-induced oxidation reactions, which can negatively affect odor and flavor due to volatile compound production and reduce shelf life. Photooxidation of milk occurs under the presence of light (artificial, sunlight) and in both ultraviolet and visible light wavelength regions (Webster and others, 2009). This process is of particular concern in milk because it occurs quickly due to fluorescent lighting used in retail storage cases.

Compounds affected by light are called photosensitizers. These compounds are important because they cause the destruction of other milk components usually unaffected by light (Boff and Min 2002). Dairy products contain six types of photosensitizers: riboflavin, protoporphyrin, hematoporphyin, chlorophyll a and b, and two unidentified tetrapyrroles. However, the most studied photosensitizer is riboflavin (Sattar and deMan 1976; Wold and others 2005; Webster and others 2009). Riboflavin excitation occurs when exposed to light of 250, 270, 370, 400, 446 and 570 nm (Kyte 1995). Detrimental retail case lighting effects can be alleviated by selecting appropriate packaging materials to minimize the transmission of light.

The use of packaging to protect milk from the effect of light is common. However, the best packaging solution for optimal protection of milk quality is to provide a complete light block. Milk is commonly packaged in either high density polyethylene (HDPE) or polyethylene terephthalate (PETE) in the US (Anonymous 2007). HDPE transmits up to 62% of light wavelengths between 300-700 nm. PETE, a packaging material frequently utilized for single-serve milk products, transmits up to 75-85% of visible light. Packaging material can have a protective effect on milk quality through blocking or reducing the transmission of certain light

wavelengths. This protection is based on material thickness, processing conditions and material coloration (Mortenson and others 2004). It is important, then, to develop packaging materials that are consumer friendly yet block the most damaging wavelengths to milk quality.

One possible packaging innovation that has shown usefulness in protecting against photooxidation is titanium dioxide (TiO_2). Since its commercial production in the early 20th century, titanium dioxide has been widely used as a leading white pigment in paints, toothpaste and packaging. Titanium dioxide is a photo-responsive material and its importance is steadily increasing in the polymer and plastic industry (Anonymous 2007). Due to its ability to scatter light and absorb UV light energy, titanium dioxide (TiO_2) has been added at different concentrations to HDPE and PETE (Robertson 2006). Titanium dioxide ability to scatter light works by its capacity to refract and diffract light. Moysssiadi and others (2004) showed that a multilayer HDPE package pigmented with TiO_2 and carbon black protected milk better than a monolayer HDPE package pigmented with TiO_2, clear PET and PET pigmented with TiO_2; when stored under florescent light at 4°C for 7 days. Moyssiadi found the multilayer HDPE package protected milk quality and suffered only a 28% riboflavin loss. The particle size of TiO_2 can be altered to affect color of light wavelengths transmitted (Anonymous 2007). Innovation in the use of TiO_2 particle size and refraction properties may improve packaging materials for milk quality protection.

The overall purpose of this research was to quantify performance of TiO_2-dosed high density polyethylene (HDPE) packaging (three different levels; DuPont™, Wilmington, DE) on preserving milk sensory quality and oxidative stability through prevention or control of photochemically-induced reactions.

Objective. To determine changes in sensory characteristics and volatiles of 2% milk packaged in polyethylene bottles with different levels (3) of TiO_2 modified HDPE packaging (plus controls), up to 43 days of refrigerated (4°C) storage under fluorescent lighting simulating retail storage.

MATERIALS AND METHODS

Packaging. Three HDPE bottle types were evaluated. These bottle types were differentiated using commercially available titanium dioxide pigments (supplied by E.I. DuPont™, Wilmington, DE) contained within the bottle resin yielding bottles with different levels of light protection. In this study, these bottles have been designated as low, medium and high to indicate the level of light protection. The compositional differences of the bottle types were held as confidential and were not revealed to the researchers.

Milk Processing. 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 until processing. Before pasteurization, milk was pre-warmed (55ºC), separated into cream and skim milk using a pilot plant separator (Model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Canada) and standardized. Raw cream was then added to skim milk standardize to 2.0 ± 0.1% fat, and verified by the Babcock method (AOAC 989.04) (Bradley, 2000) . Milk (total milk fat = 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 131.1º C (268º F) for 2 sec (UHT/HTST Lab-25 DH pasteurizer, MicroThermics, Raleigh, NC). Milk was chilled for 24

hrs., then filled into clean 16 ounce HDPE bottles and sealed with a screw cap lid. Approximately 470 ml were dispensed to each bottle using a Wheaton Unispense II (Wheaton Instruments, Millville, NJ). Precautions were taken for filling to be as aseptic as possible under a positive laminar flow hood (Atmos-Tech Industries, Ocean, NJ). During the bottling process, filled bottles were stored in iced coolers to control temperature until transferred to the retail storage case.

High density polyethylene packaging with four levels of TiO_2, including 0% (clear) serving as controls (light exposed: no light barrier); light protected: foil overwrap) and three different TiO_2 modified packaging treatment levels for the experimental treatments (total of five packaging treatments). Clear HDPE packaging (n=140), with no TiO_2, was used as control bottles for light-exposed product as well as overwrapped with foil for light-protected controls (n=140). Five hundred and thirty two bottles was used during the life of the study. Bottle dimensions were 7.16”ht x 3.29”w x 2.1” deep and a volume of 528 ml. Bottles were filled with 470ml of milk, leaving 58ml of headspace above the product.

Storage Conditions. Samples were stored in a refrigerated Friedrich Floating Air (Friedrich 60-10-1056, San Antonio TX) beverage case to simulate retail conditions. The cooler was glass fronted with 5 doors, three shelves in which 2 light bulbs run the length of the dairy case over each shelf at 1/5th and 4/5th the distance from the front of the shelf. Six light bulbs were situated vertically at the front of the dairy case at the ends of the case and in between each door junction. There are poles that hold up the shelves in front of each vertical light bulb. From the top of the bottle to the bulbs at the top of each shelf was a distance of 4.5 inches.

The dairy case was equipped with cool white, 32-watt light bulbs (Alto II, Panasonic, Maple Grove, MN), simulating retail conditions. Bottle placement was randomized so that all treatments were distributed randomly within the dairy case to reduce the effects of different lighting intensity within the case. Temperature and light intensity were measured routinely. The average temperature of the dairy case was 2.7°C +.0.8 Over the 5-week storage study, light intensity measurements were taken in three general locations on each treatment for each day of analysis. Light intensity averaged 2186 lux, with a wide range from 396-3970 lux, depending on sampling location. Because of the broad range of light intensity, multiple bottles of each treatment and each control were randomly selected on each day of analyses for evaluation. For the second replication, samples were randomly placed in a Tonka walk-in cooler (Tonka Inc. Hopkins, MI). The average lux and temperature for replication B was 2421±551 lux and 3.0°C±0.87 respectively. Samples were randomly placed in the dairy case and samples were removed on days 1, 3, 8, 15, 22, 29, 36 for both sensory and chemical testing. Randomization for placing and removing of bottles were performed using JMP 10.0.0 Statistical Discovery Software (SAS Institute, Cary, NC).

Microbial Analysis. Standard plate counts of aerobic organisms were performed based on standard methods to ensure milk was properly pasteurized and maintained satisfactory microbial quality over the storage period (Laird and others 2004). Dairy formulations were plated using Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN). Aerobic count plates were incubated at 32°± 1°C for 48 ± 3 hours. Microbial tests were conducted on day zero and two days prior to sensory tests, (n=10) bottles per test date.

Degradation of Riboflavin by Fluorometric Analysis. Riboflavin concentration in milk was

analyzed using fluorometric method (AOAC method 970.65) (Webster and others 2009; Bradley 2000) and was measured on a Shimadzu RF-1501 Spectroflurophotometer (Shimadzu Scientific Instrument, Inc., Columbia, Md., U.S.A.). Samples were prepared by adding 0.01N hydrochloric acid to milk (10ml) to pH between 5.0-6.0. Next, 0.1 ml of 10N hydrochloric acid was added to the milk sample, then autoclaved under pressure (17.0 psi) for 30 minutes at 121-123°. Samples were cooled and pH adjusted between 6.0-6.5 using 0.025N sodium hydroxide. Hydrochloric acid (0.1 N) was immediately added to stop further precipitation, usually around pH of 4.5. Each sample was then filtered using 25 millimeter syringe 0.2 µm filter (Grace, Deerfield, IL) and 10 ml Norm-Ject syringe (Henke Sass Wolf Inc. Tuttlingen, Germany) before being measured using the spectroflurophotometer. Two bottles per treatment group (n=10) were tested per test date.

Secondary Oxidation By-Products by Thiobarbituric Acid Reactive Substances Analysis. The TBARS method was adapted according to Spanier and Taylor (1991). One milliliter samples from each treatment and control were diluted 1to 4 ratio by adding 4ml of dH_2O. Sample and water was vortexed for approximately 20 seconds, the one ml was pipetted into 15ml disposable centrifuge tube along with 4ml of s odium dodecyl sulfate solution and 0.1 ml of EDTA solution. Tubes were mixed via vortex for 20 seconds, and incubated in a 95°C water bath for 60 min. Tubes were cooled until room temperature in an iced water bath, then mixed with pyridine and butanol solution. Tubes were centrifuged for 20 min at room temperature at 3000rpm. Two and a half milliliters of top organic layer was carefully transferred into a 1.5 ml polystyrene cuvette and absorbance read at 532nm using a spectrophotometer (Milton Roy: Spectronic 21D, Ivyland, PA). TBARS were used to ascertain the development of malondialdehyde, a secondary oxidative product of fatty acids (Caprioli and others 2011). This oxidative product reacts with thiobarbituric acid and creates a reddish/pink color, which can be read spectrophotmetrically.

Two bottles per treatment group (n=10) were tested per test date.

Volatile Chemistry by GC/MS. To help identify volatile compounds developed during storage, milk samples were analyzed for headspace volatiles. Replication 1 samples were analyzed within 24 hrs. of sampling. Replication 2 samples were frozen at -70°C; thawed slowly in the refrigerator and analyzed. Volatile compounds from milk were adsorbed on a solid phase microextraction fiber (SPME) and separated using gas chromatography (GC). An HP 5890 GC with 5972 series mass selective detector HP5MS (Hewlett Packard, Palo Alto, CA) was used to identify volatile head space compounds. Samples (8ml) from each treatment and control were pipetted into 20ml amber vials. Fiber was exposed to the headspace of the vials (4mm) while samples were heated to 45°C on an RCT basic heater with an ETS-D4 Fuzzy Controller (IKA Werke, Wilmington, NC) while being agitated at 250rpm. For 20 min an 85um carboxen-polydimethyl siloxane (PDMS) solid phase microextraction (SPME) fiber (Supelco, Bellefonte, PA) was used to adsorb volatile compounds. Volatiles were desorbed from the fiber onto a DB-5 capillary column (30m x 0.25 mm I.D. x 0.25μm film thickness, J&W Scientific, Folsom, CA) and separated and analyze volatiles, using the following conditions: Helium gas flow: 1.8 ml/min; injector temperature: 280°C; and detector temperature: 280°C. There was an initial run temperature rate of 35°C held for 0.5 min. Then the temperature was increased by 15°C /min to 180°C and held for 0.5 min. The temperature then was increased by 20°C /min to 260°C with the final temperature held for 0.5 min. The total run time was 15.17 min and the program ran in split less mode. The chromatograms were plotted using HP ChemStation software (Hewlett Packard, Palo Alto, CA). To ensure desorption of extraneous compounds adsorbed by the (SPME) fiber's due to atmospheric exposure, before sample analysis, blank analysis were performed using an empty sealed vial. External standards of pentanal and hexanal in distilled water were used to

identify and trace oxidation products. Two bottles per treatment group (n=10) were tested per test date.

Change in Quality by Sensory Analyses. Sensory analysis was performed by triangle tests (Meilgaard and others 2007) on each day of evaluation. Ten milk bottles from each TiO_2-HDPE modified package was compared to the light-protected milk, testing for similarity to determine if the treatment provided equivalent and adequate protection as a light protected product (light-protected control, 100% opacity), using the statistical parameters of proportion of discriminators (p_d) = 30%; α= 0.10; β=0.05. In addition, milk from each TiO_2-HDPE modified package were compared to the light-exposed milk to determine if a difference could be detected between samples based on protection of the product by the packaging material, with the statistical parameters of pd = 30%; α= 0.05; β=0.10. Ten bottles from each treatment and 20 bottles from each control were commingled prior to sample preparation.

Institutional Review Board approval was obtained (IRB 11-477). Informed consent was obtained from a minimum of 98 panelists who were recruited via email. In order to avoid sensory fatigue, an incomplete block design was used; panelists were presented with three three-sample (triangle) sets on each day of evaluation and a minimum of 42 independent responses per comparison were obtained. Each sample set included three numerically coded (3-digit) one-ounce samples (4°C); panelists were told that two samples were identical and one sample is different. Panelists were asked to smell and taste each sample from left to right and record which sample they perceived to be the different sample. Water was provided to rinse between sessions and to limit sensory fatigue; a rest time of one minute was established between each sample set.

Sensory testing was conducted in the FST Sensory Laboratory (room 127). Panelists each worked in a partitioned booth, under white light, and provided responses on a touchscreen monitor. Sensory Information Management Systems (Sensory Computer Management SIMS 2000. Morristown, NJ) software was used to record responses and analyze the data.

Statistical Analysis. Analytical analyses (TBARS and riboflavin) were completed on each day of evaluation on two bottles of each treatment with duplicate samples evaluated from each bottle for TBARS and riboflavin analysis. Volatile analysis was not completed in duplicate on each sample bottle. Sensory analysis was completed on the first replication only. A two-way factorial analysis of variance with repeated measures was used to assess changes in oxidation. These analyses, based on time (n=7 days of testing) and bottle treatment (n=5), were performed using JMP 10.0.0 Statistical Discovery Software (SAS Institute, Cary, NC). Alpha level of 0.05 was used to determine significant differences. The Least Significant Difference contrast tests was used for mean separation when significant differences were found. Sensory data was analyzed based upon statistical parameters mentioned above and equations outlined in Meilgaard and others (2007) by replication as well as for commingled data.

RESULTS AND DISCUSSION

Protection of Sensory Quality. The goal of this research was to determine if the TiO_2 modifications provided better protection than a standard HDPE package (clear control) and was comparable in protection to an optimum light barrier protection (foil control). Therefore, the sensory testing was designed to minimize Type I error (difference testing) when comparing the

TiO_2 packages against the clear control package and to minimize Type II (similarity testing) error in comparison against foil control. Table 3.1 describes the packaging success by providing the p-values for each package comparison over the product shelf-life. As expected, the difference between the foil and clear control packages was noted by day 3 (p=0.0002) and was consistently observed thereafter. High TiO_2 protected better than clear control throughout the shelf-life. The medium and low TiO_2 packages did not provide greater protection than the clear control, but sensory differences were noted by day eight. This suggests the oxidation rate was lower in the milk in the low and medium TiO_2 packaging than that of the milk in the clear control package.

Table 3.1: Summary table of statistical significance (P<.05) of sensory testing for 2% milk by triangle tests, for each packaging treatment (low, medium, high) [1] compared to controls (clear: Cl, foil-wrapped: F) for each day of evaluation.

2% Milk	Day 1	Day 3	Day 8	Day 15	Day 22	Day 29	Day 36[2]	Day 43
Difference test pd = 30%; α= 0.05; β=0.10[3]								
Cl vs low	0.7368	0.2321	0.0018*	0.5151	0.0085*	0.1644	NT	0.7757
Cl vs medium	0.2201	0.5173	<.0001*	0.0029*	0.0000*	0.0075*	NT	NT
Cl vs high	0.0144*	00004*	0.0002*	0.0000*	0.0000*	0.0000*	NT	NT
F vs Cl	0.3141	0.0002*	<.0001*	0.0000*	0.0000*	0.0000*	NT	0.0000*
Similarity Test pd = 30%; α= 0.10; β=0.05[3]								
F vs low	0.1039	0.0790	0.1256	0.0000*	0.0000*	0.0000*	NT	NT
F vs medium	0.1205	0.0040*	0.0143*	0.0109*	0.0006*	0.0000*	NT	NT
F vs high	0.7785	0.2321	0.0085*	0.1644	0.2510	0.0954	NT	0.0009*
Panelists/Test[3]	24-28	27-31	34-36	37-39	35-36	37-38		48

*P< 0.05

[1] Dosage of TiO_2 HDPE modified packaging (low, medium, high,). Controls: F=Foil (HDPE with foil overwrap); Cl=Clear (HDPE bottles with no TiO_2 or foil)

[2]NT= not tested because the difference had already been established; during the Day 36 evaluation period occurred during university spring break and there was not a sufficient local population to complete the testing. Therefore an additional day (Day 43) of testing was included.

[3]Statistical parameters, proportion of discriminators, and power analysis for each day of testing are reported in (Appendix H).

The high TiO_2 package provided the best protection, as indicated by similarity testing against the light-protected control. This package provided protection at a level equivalent to the light-protected control through day 29 and possibly longer, however a difference was observed on day

43. There was an exception on day 8, when the tested population discerned the difference between the high and light-protected package, with 31% correctly identifying the difference, with an estimate of 10-52% of the true population able to find the difference. This marginally surpassed the preset proportion of discriminators (30%) and may have been attributed to a chance disproportionate number of experienced participants in the panel on that day.

With the exception of day 15, the other two TiO_2 treatments, low and medium were statistically different ($p < 0.05$) from light-protected control, indicating that they did not protect from off flavors or aromas as well as full light protection. In addition, milk within the low and medium packages did not change in flavor as extensively as product packaged in the light-exposed control, as there were product differences at days 8 (low and medium). Apparently the light-exposed packaging had significant photooxidation occurring within these bottles that exceeded that of the low and medium TiO_2 packaging.

Distinguishing that the packaging yields a sensory difference from the clear control milk product does not guarantee that milk flavor is sufficiently protected. However, evaluating for similarity to the milk in the light protected (foil) control provided that validation. The low and medium TiO_2 packages provided no significant protection; milk from those packages was different from the light protected milk by the third day. However the high TiO_2 package provided protection equivalent to the light protected milk through day 29 with an anomaly on day eight ($p = 0.0085$).

Chemical Response to Light

Riboflavin. Riboflavin, also known as vitamin B2, is a water-soluble vitamin found in large amounts in milk at an average concentration between 1.36 mg/mL and 1.75 mg/mL (Dimick 1982, Zygoura 2004). Riboflavin degradation is also undesirable in food systems because it

decreases the nutritional value of the food (Hoskin 1988). Although the final sensory evaluation data was collected on day 43, riboflavin analysis was completed on day 36. Riboflavin concentration in milk decreased over the 36 days of evaluation in the TiO_2 and clear control packaging treatments exposed to light with greater than 28% decrease in riboflavin by the end of the storage period. Riboflavin in milk from the light-protected treatment did not change significantly, with only a 10.5% relative loss of riboflavin over the five-week storage period (Figure 3.1). These findings are in accordance with those reported by a number of investigators (Dimick 1973; Hoskin and Dimick 1979; Christy and others 1981; Hoskin 1988; Moyssiadi and others 2004; Webster and others 2009).

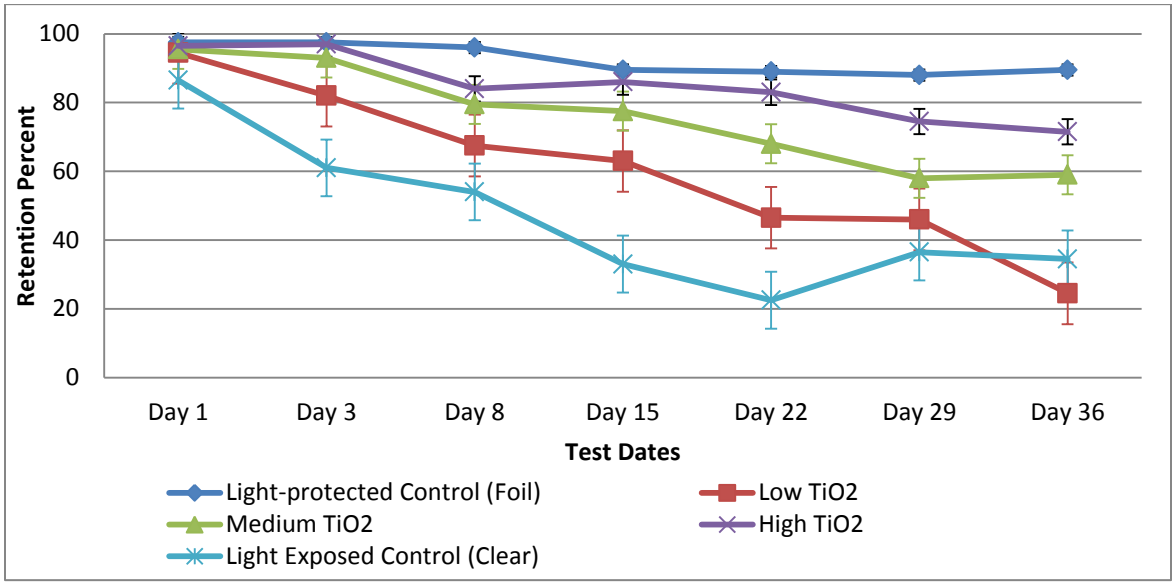

Figure 3.1: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±0.21 (Average of Rep 1 and 2 with standard error).

The high TiO_2 provided protection for riboflavin equivalent to the light-protected (foil) control ($p>0.05$) through 22 days; riboflavin concentration in milk in the high TiO_2-package was significantly higher (<0.05) than in clear bottles. The low and medium TiO_2 packages protected

riboflavin through 15 days of storage ($p>0.05$). All TiO_2 packaging treatments showed significant differences with the clear bottles at Day 22 (Appendix O), indicating that they were able to prevent riboflavin degradation to some degree whereas riboflavin was degraded substantially in the unprotected product. However, in the light exposed control, low, and medium bottles, the degradation of riboflavin had significant losses of 65.5%, 75.5% and 41% respectively. However riboflavin degradation did occur from Day 29 to Day 36 in the high TiO_2 packages. Over the five-week storage period, riboflavin in milk packaged in foil-wrapped control bottles and the high TiO_2 packaging decreased by 10.5% and 28.5% loss, respectively. Webster and others (2009) reported a loss of total riboflavin between 52.6 and 67.5% in 2% milk in light exposed and specialized film packaging treatments within 3 days of refrigerated storage; at the end of the study, 95-98% of the riboflavin was degraded. In the current study, the relationship between packaging protection of riboflavin closely relates to the observed sensory protection with the high TiO_2 providing photo-protection of riboflavin, thus limiting the onset of photo-induced oxidation.

Malondialdehyde (MDA). Malondialdehyde is a secondary end product of the photooxidation of lipids measured by thiobarbituric acid reactive substances (TBARS). It has been studied extensively in meat and milk oxidation and been shown to increase in concentration as photooxidation occurs (Rosenthal 1993; van Aardt and others 2005; Campo and others 2006). It has also been shown to correlate with changes in sensory characteristics of light induced oxidation (Rosenthal 1993). Rosenthal and others (1993) reported levels of TBARS (0.61 mg/L MDA) for whole milk that was stored in the dark for seven days. Milk in this study had initial values in the same range (0.68 mg/L). Campo and others (2006) estimate that a TBARS value

around 2 (mg/L) can be the limiting threshold for the acceptability of oxidized rancidity flavor in beef but no definite value for TBARS has been defined for acceptability of flavor in milk.

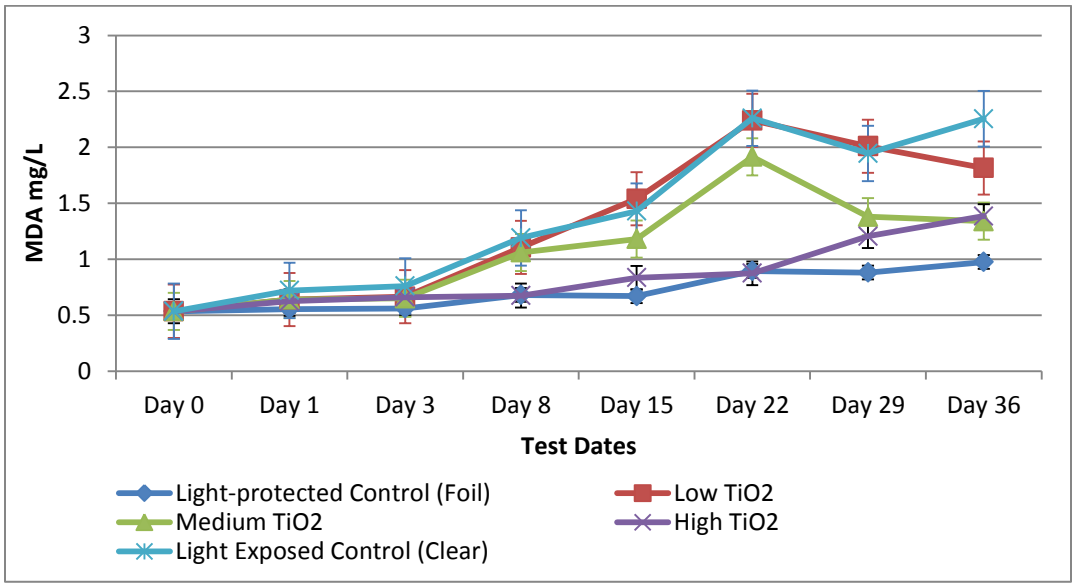

Figure 3.2: Malondialdehyde concentration in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±0.21.

As expected, foil wrapped packages protected the milk optimally as malondialdehyde production was lowest in this group and did not increase significantly ($p > 0.05$) over time (Appendix O). Milk packaged in the high TiO_2 package protected most closely to foil wrapped bottles up to Day 29. However by day 36, malondialdehyde production increased more rapidly than in the milk from the light-protected control package ($p < .05$), although still significantly lower than light-exposed milk (clear control). This closely approximates the trends of riboflavin concentration. An important observation is that the differences in TBARS values closely relates to the observed differences in sensory evaluation. All significant statistical contrasts for sensory comparisons had TBARS values of 1.3 mg/L or greater with two exceptions very early in the shelf-life. On day 36 MDA concentrations in the high TiO_2 package (MDA of 1.43 mg/L) and light-protected

control (MDA of 1.0 mg/L) were significantly different between the two samples. It can be inferred that in milk the limiting threshold is approximately 1.3 mg/L. This does not directly infer a change in sensory acceptability of milk, but may be used to infer a notable change in sensory quality.

Volatile Composition. Several compounds (pentanal, hexanal, dimethyl disulfide and 2-propanone) increased in peak area over time. Hexanal, pentanal are commonly used as an indicator of lipid oxidation (Min and Schweizer 1983, Kim and others 2003, Moore and others 2012, Li 2011). Hexanal area count were significantly higher in the light-exposed control and the packaging treatments than the light-protected treatment by day 15 (Appendix O) and this finding remained true through day 36 (Fig. 3.3). The TiO_2 packaging treatments were not significantly different from one another in hexanal area count through day 29 of the experiment. However, several treatments were significantly lower in hexanal area counts than the light-exposed control on several days of the experiment. The medium and high TiO_2 treatments appeared to control conditions to limit hexanal production, relative to the light-protected control. These finding are in agreement with Mortensen and others (2003) who found that hexanal increased significantly in Havarti cheese when stored under lighting that emits wavelengths of 366, 405, and 436 nm, with the highest increase when cheese was exposed to 405 nm. This is also in agreement with work in our laboratory from Webster and others (2009).

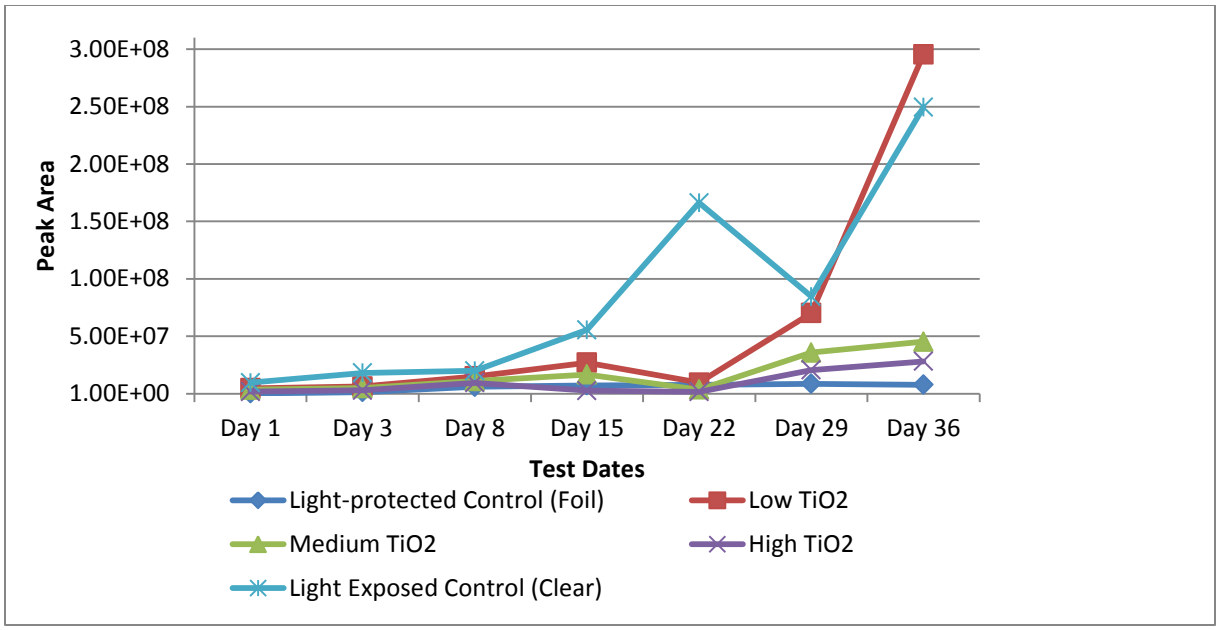

Figure 3.3: Hexanal concentration in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±0.21.

Pentanal was detected in all treatments, starting on days 3. The light-exposed treatment had a significantly higher area counts of pentanal than the light-protected treatment (Figure 3.4). The TiO_2 packaging treatments were significantly different from each other in pentanal area counts, but not from the light-exposed treatment or the light-protected treatment (Appendix O). Previous work from our laboratory found that exposure of regular milk to light of 610 nm produced higher amounts, although not statistically significant, of pentanal than exposure to full light and 395, 463, 516, and 567nm (Webster and other 2009).

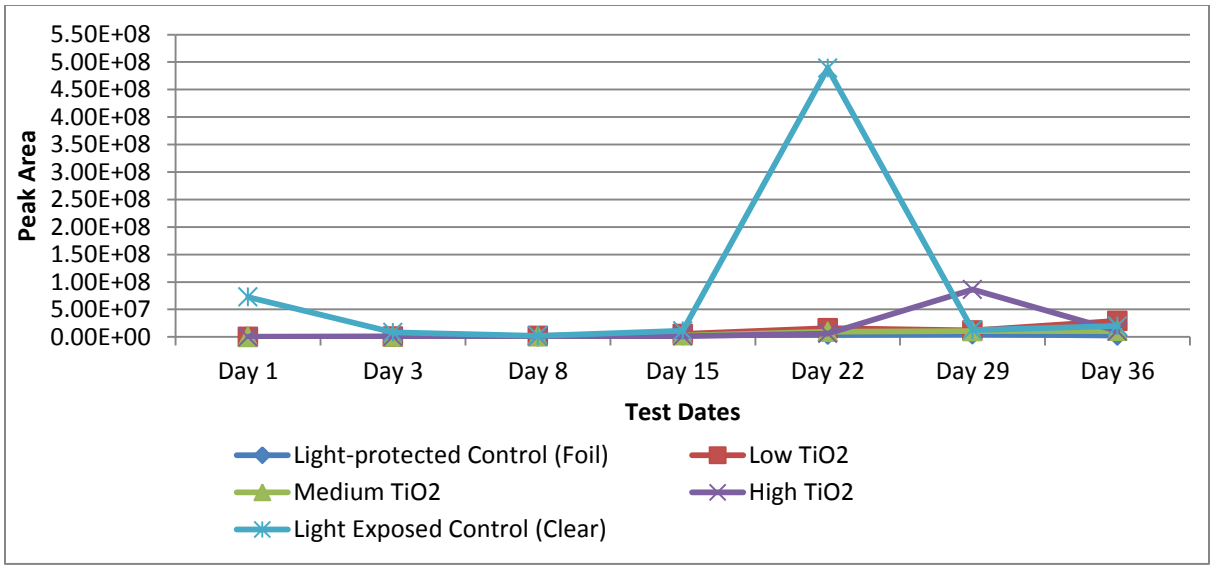

Figure 3.4: Pentanal concentration in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±0.21.

Pentanal and hexanal are common secondary lipid oxidative compounds found in milk (Mehta and Bassett 1978; Farrer 1983; Bekbolet 1990; Cadwallader and Howard 1998; Rysstad and others 1998; van Aardt and others 2005; Mestdagh and others 2005; Webster and others 2009). The statistical contrasts for hexanal and pentanal are similar to the results of the riboflavin analysis (Appendix O). Hexanal in milk in different packaging treatments as early as Day 1 are not significantly different from one another, and over time, light-protected control and high TiO_2 packaging protect well as there are no significant differences between those treatments over a 4 week period. However, by week 5 (day 36), milk in the high TiO_2 packages had higher hexanal production compared to light-protected milk. Furthermore, the light-exposed control, low and medium TiO_2 were unable to prohibit hexanal production.

CONCLUSION

Packaging can be modified to protect milk sensory quality and control the photo-oxidation of riboflavin by manipulating photo-responsive molecules in the packaging. Titanium dioxide addition, with appropriate configuration and density, can be used in HDPE packaging to protect milk quality over an extended shelf-life (four weeks) very effectively. TBARS of 1.3 mg/L or higher is indicated as a marker of sensory quality changes due to light exposure. The dairy industry can use this information to improve packaging and fluid milk quality for extended shelf life fluid milk.

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CHAPTER IV
PROTECTING EXTENDED SHELF-LIFE MILK FORTIFIED WITH OMEGA-3 FATTY ACIDS USING TITANIUM DIOXIDE MODIFICATIONS IN PACKAGING

ABSTRACT

The effectiveness of innovative TiO_2-loaded high density polyethylene materials in reducing light-induced oxidation of extended shelf-life omega-3 fatty acid enriched milk (2% total fat) was studied (100mg EPA and DHA/8 oz. serving). Effectiveness was assessed by sensory evaluation, changes in volatile compounds, thiobarbituric reactive substances (TBARS) and riboflavin concentration. Milk (2% milkfat) was stored in high density polyethylene bottles loaded with TiO_2 at four levels (none, low, medium, high) at 2.7°C +.0.8 for up to 35 days. High TiO_2-HDPE package provided greater protection of sensory quality and riboflavin than clear, low and medium TiO_2 packaging. However riboflavin decreased by 28% even in the light protected control, which is a higher loss than observed in 2% fluid milk without omega-3 lipids. Malondialdehyde (MDA), as measured by TBARS, was greater than 1.4 (mg/L) in all products, including the light protected control within three days, suggesting that oxidative stability was low. Omega-3 enriched milk packaged in clear HDPE package exceeded MDA of 3.0 (mg/L) by day 7, suggesting the milk would not be of sufficient sensory quality. However the high TiO_2 package and the light protected control (foil overwrap) controlled oxidation to < 3.0 (mg/L MDA) through 21 days and 35 days, respectively.

Key Words: Oxidation, Sensory Evaluation, Package, Milk, Omega-3, Photosensitizer

INTRODUCTION

Growing medical evidence supports the association of omega-3 fatty acids with improved health benefits, such as cardiovascular health and aiding in brain development, with their regular consumption (Ruxton and others 2004; Siddiqui and others 2004; Horrocks and Yeo 1999). Increased consumer awareness and low consumption of omega-3 FA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), from their natural food sources (Kris-Etherton and others 2000), has prompted the food industry to design more omega-3 fatty acid fortified food products. In 2004 the Food and Drug Administration (FDA) announced a qualified health claim on conventional foods that contain EPA and DHA omega-3 fatty acids. Conventional food products that are labeled with a qualified health claim must be supported by credible scientific evidence. Authorization of this claim is based on a systematic evaluation of the available scientific data, as outlined by the FDA's "Interim Procedures for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements" (Anonymous 2008a). Current studies indicate the current intake of EPA+DHA in the typical western diet is 100 to 200 mg/day (Kris-Etherton and others 2000); however the World Health Organization and the North Atlantic Treaty Organization recommend 300 to 500 mg EPA+DHA/day and The American Heart Association recommends 500 to 1800 mg EPA+DHA/day (Anonymous 2008b), which corresponds to two fatty fish meals per week. Foods that meet the suggested standards for fortification of omega-3 fatty acids include frequent consumption, processing, storage and packaging conditions that protect omega-3 fatty acids from oxidation (O'Donnell 2008). Dairy products serve as good vehicles for increasing consumption of the healthful omega-3 fatty acids. Extended shelf-life dairy products, which are recognized for

excellent nutritional value, are stored at refrigeration temperatures and should be protected from light in order to protect the inherent dairy nutrient quality.

The addition of omega-3 fatty acids into dairy products increases the risk of oxidation and potential for changes in sensory characteristics. Oxidation of unsaturated fatty acids produces aldehydes and a variety of other minor products, such as ketones and alkenals, which mainly contribute to off-flavors in milk (Gudipati and others 2004). Incorporating omega-3 rich oil sources into milk can cause sensory issues based on the source oil and oxidative deterioration of these polyunsaturated fatty acids. Marine fish oils and algae oils, are considered the primary sources of long chain omega-3 fatty acids. Fish oil-fortified food products are susceptible to aromas indicative of the oil origin as well as from omega-3 fatty acid oxidation, which decreases the nutritional impact of the product and produces volatile oxidation end-compounds that negatively impact the aromas and/or flavors of the product.

An important factor influencing oxidation flavor in milk is exposure to certain light wavelengths (Webster and others 2009). There is a significant increase in photo-oxidation flavors in milk when exposed to wavelengths between 365 and 500 nm (Herreid and others 1952; Bradfield and Duthie 1956; Sattar and others 1976). Riboflavin acts as a photosensitizer when exposed to specific wavelengths within this range (Bekbolet 1990). Other compounds have been identified to act as photosensitizers (Wold and others 2005). Chlorophyll, an identified photosentizer, is naturally occurring at low levels in milk; chlorophyll is also in omega-3 fatty acid-rich lipids derived from algal sources and extracted from fish as well (Wold and others 2005). Photosensitizers advance oxidative reaction rates of biomolecules, including amino acids and unsaturated fatty acids, such as DHA and EPA (Choe and Min 2003). Due to 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 volatile compounds that may have a negative effect on sensory characteristics of milk, including aroma and flavor. Moore and others (2012) found an increase of the volatile compound 1-pentene-3-ol in omega-3 fortified dairy-based beverage system during 35 days of storage. They also showed decreases in EPA and DHA over the same 35 day storage period in the formulated dairy-based beverage systems. There is a great need to develop packaging that protects the integrity of milk when oxidation sensitive omega-3 fatty acids are added.

MATERIALS AND METHODS

Fresh raw milk was obtained from the Virginia Tech dairy farm and processed in the Food Science and Technology dairy pilot plant. of collection. Fresh raw cream and fish oil OmegaPure® E (Houston, Texas) (10% wt./wt.) was then added to skim milk and standardized to 2.0 ± 0.1% fat (Table 1.0). Milk with fish oil (total milk fat 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 131.1º C (268º F) for 2 sec (UHT/HTST Lab-25 DH pasteurizer, MicroThermics, Raleigh, NC) (Li 2011).

Table 4.1: Relative percent's of fish oil and milk fat, as a percent of total fat, added to achieve targeted EPA and DHA (100mg/8 oz.) addition in 2% fat milk.

	Fish oil (%)	Milk fat (%)
Milk enriched with fish oil	10.0	90
Milk without fish oil	0	100

These calculations were made based on the recommended intake of (400mg) EPA and DHA (Anonymous 2008b) over 4 cups/day of milk, using OmegaPure® E (Houston, Texas) fish oil to reach this targeted amount (Appendix D).

Sensory evaluations, TBARS, volatile chemistry by gas chromatography, riboflavin concentration were assessed using the same experimental conditions as described in Chapter 3.

RESULTS AND DISCUSSION

Protection of Sensory Quality. The goal of this research was to determine if the TiO_2 modifications provided better protection than a standard HDPE (TiO_2) package (clear control) and was comparable in protection to be optimum light barrier protection (foil control). Therefore, the sensory testing was designed to minimize Type I error (difference testing) when comparing the TiO_2 packages against the clear control package and to minimize Type II (similarity testing) error in comparison against foil control. Table 4.2 describes the functionality by providing the p-values for each package comparison over the product shelf-life. As expected, the difference between the foil and clear control packages was noted within 24 hrs. of storage ($p=0.0002$) and was consistently observed thereafter. High TiO_2 packaging protected milk sensory quality better than clear control, creating a noticeable difference in sensory quality within 7 days and the remainder of refrigerated 35-day shelf-life, as evident from the low starting p-values ($p<0.05$).

The medium and low TiO_2 packages did not provide greater protection than the clear control, but sensory differences were noted between the milk in the clear control package and that in the low and medium TiO_2 packages for about a 2 week period in the middle of the shelf-life (Days 14 and 21 for low TiO_2; Days 7 and 14 for medium TiO_2). This suggests the oxidation rate was lower than that of the milk in the clear control package, perhaps because the packaging influenced the amount of light energy that was transmitted into the product and the effects on the photosensitizers and subsequent autoxidation.

Table 4.2: Summary table of statistical significance (P<.05) of sensory testing for omega-3 enriched milk by triangle tests, for each packaging treatment (low, medium, high) [1] compared to controls (clear: Cl, foil-wrapped: F) for each day of evaluation.

Difference Test pd[3] = 30%; α= 0.05; β=0.10							
Omega-3 Milk	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35
Cl vs low[1]	0.4624	0.2201	0.0867	0.0246*	0.0327*	0.6117	NT[2]
Cl vs medium	0.178	0.9281	0*	0.0016*	0.6117	0.2852	0.1423
Cl vs high	0.8618	0.5182	0.0003*	<0.0001*	<0.0001*	<0.0001*	NT
F vs Cl	0.0056*	0.0008*	0*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Similarity Test pd = 30%; α= 0.10; β=0.05							
F vs low	0.0918	0.4108	0.0011*	0.0044*	<0.0001*	<0.0001*	NT
F vs medium	0.0284*	0.1537	0.0125*	0.0006*	<0.0001*	<0.0001*	NT
F vs high	0.0002*	0.6793	0.6967	0.0044*	0.1256	0.002*	0.0003*
Panelists/Test[3]	24-26	26-28	34-36	36-39	34-35	33-35	48

*P< 0.05

[1] Dosage of TiO2 HDPE modified packaging (low, medium, high,). Controls: F=Foil (HDPE with foil overwrap); Cl=Clear (HDPE bottles with no TiO_2 or foil)

[2]NT= not tested because the difference had already been established

[3]Statistical parameters, proportion of discriminators, and power analysis for each day of testing are reported in Appendix I.

The sensory quality of omega-3 milk packaged in the high TiO_2 modified HDPE package was not protected as long as observed in similarity testing for the 2% milk (Chapter 3), with equivalent quality only up to Day 14; the protection for 2% milk was observed through Day 29. On day 14, tested population discerned the difference between the high and light-protected package, with 33% correctly identifying the difference. This marginally surpassed the preset

proportion of discriminators (30%) and may have been attributed to a chance disproportionate number of experienced participants in the panel on that day. However, the high TiO_2 modified HDPE package provided the best protection against sensory characteristics of oxidation. The high TiO_2 modified HDPE package provided greater protection of sensory quality, compared to the clear package as within 24 hrs.

The high TiO_2 modified HDPE package was as effective as the light-protected control (foil wrapped) in protecting milk flavor for 7 days but was differentiated on Day 14; however, this treatment package still afforded some protection as the milk was still differentiated from the milk packaged in the light-exposed control (clear) during the latter part of the storage study. With the exception of day 1, the other two treatments, low and medium were statistically different ($p<.05$) from light-protected control (F), indicating that they did not protect from off flavors or aromas as well as full light protection. In addition, they were not statistically different from clear packaging for at least the first two weeks also supporting that they did not provide protection any better than the clear bottles during the early exposure.

Day 1 appears to be an anomaly but this may be partially explained by the participants on that day of evaluation. When the portion of discriminators (Appendix M) were assessed for each sensory day, the number of discriminators was higher on day 1 of sensory testing indicating that many panelists were able to discriminate oxidized aroma/flavor of the milk. Distinguishing that the packaging yields a sensory difference from the clear control milk product does not guarantee that milk flavor is sufficiently protected. However, evaluating for similarity to the milk in the light protected (foil) control provided that validation. The low and medium TiO_2 packages provided no significant protection; milk from those packages was different from the light

protected milk by the third day. However the high TiO_2 package provided protection equivalent to the light protected milk up to day 14.

Chemical Response to Light

Riboflavin. Riboflavin, also known as vitamin B2, is a water-soluble vitamin found in large amounts in milk at an average concentration between 1.36 mg/mL and 1.75 mg/mL (Dimick 1982, Zygoura 2004). Riboflavin degradation is also undesirable in food systems because it decreases the nutritional value of the food (Hoskin 1988). Data for riboflavin analysis is shown in Figure 4.1. Statistical analysis of milk indicates that the high TiO_2 modified HDPE package provided protection that was not significantly different from foil wrapped, but was significantly different from clear bottles. In fact, the high TiO_2 modified HDPE package protected as well as foil wrapped for as long as 21 days as there are no statistical significant differences between those two treatments up to that point (Appendix P). All treatment bottles show significant differences with the light-exposed bottles at Day 28 indicating that they are able to prevent riboflavin degradation to some degree whereas riboflavin was degraded substantially in the unprotected product. Wavelengths that are primarily responsible for the photo-oxidation of food, especially for milk, are those between 430 to 460 nm (Borle and others 2001). Borle and others (2001) found that riboflavin has maximum absorption at 370 and 436nm.

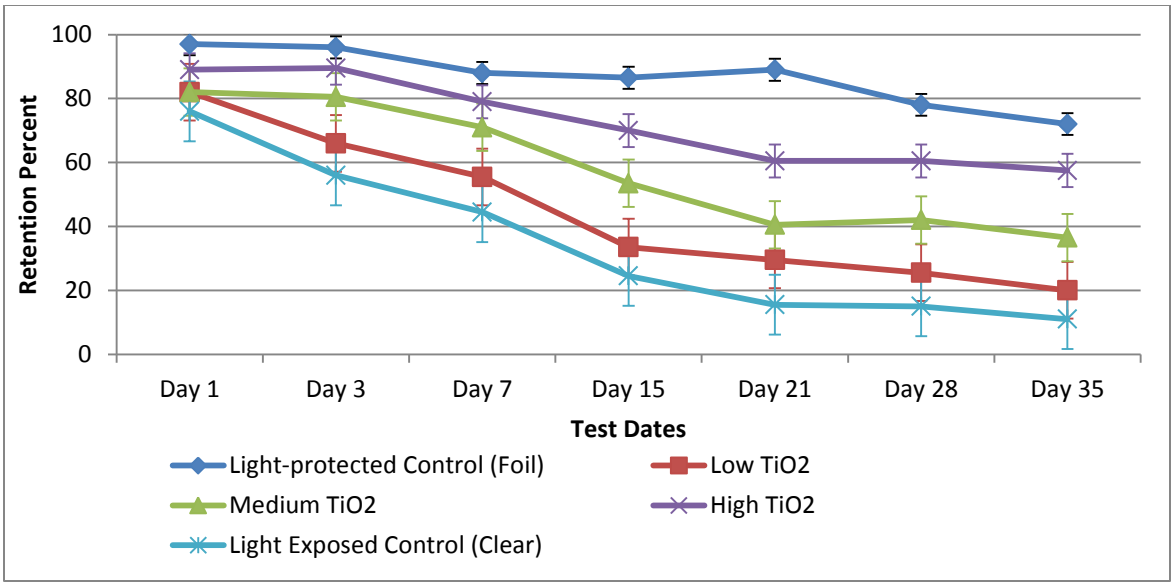

Figure 4.1: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 milk exposed to 2286±141 lux intensity at 2.85°C±21 (Average of Rep 1 and 2 with standard error).

In the light-protected control package, riboflavin remained relatively unchanged over time (Figure 4.1), with light-protected control package having only 28% loss of riboflavin over the 5 week period. However, in the light-exposed low, and medium TiO_2 packages, the degradation of riboflavin in the milk showed significant differences from the light-protected control package, 89%, 80% and 63.5% respectively. These numbers indicate much greater degradation of riboflavin than in 2% milk reported in chapter 3; there was only 10.5% loss of riboflavin in the light protected control milk and a 28.5% loss for the high TiO_2 package. This can be due to the addition of the omega-3 fatty acids. Their high degree of unsaturation makes them highly reactive with excited riboflavin, and possibly chlorophyll, causing degradation and nutritional quality loss. These finding are similar to Li (2011) who found a 74% reduction in riboflavin with omega-3 milk fully exposed to light.

Malondialdehyde (MDA). Malondialdehyde is a secondary product of the photooxidation of lipids measured by thiobarbituric acid reactive substances (TBARS). It has been studied extensively in meat and milk oxidation and been shown to increase in concentration as photooxidation occurs (Rosenthal 1993; van Aardt and others 2005; Campo and others 2006). It has also been shown to correlate with changes in sensory characteristics of light induced oxidation (Rosenthal 1993). Rosenthal and others (1993) reported levels of TBARS (0.61 mg/L) for whole milk that was stored in the dark for seven days. Milk in this study had initial values in the same range (0.68 mg/L). Campo and others (2006) estimate that a TBARS value around 2 (mg/L) can be the limiting threshold for the acceptability of oxidized in beef but no definite value for TBARS has defined acceptability in milk. Based on TBARS results from chapter 3, it can be inferred that in milk the limiting threshold is approximately 1.3 (mg/L).

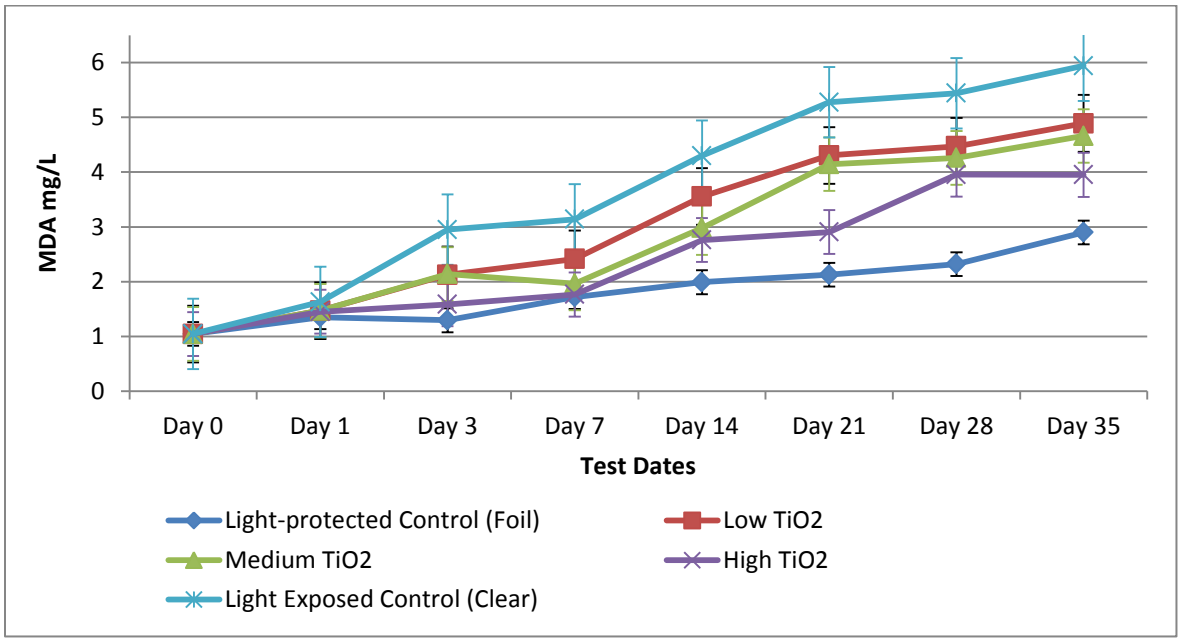

Figure 4.2 : Malondialdehyde concentration in TiO2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 milk exposed to 2286±141 lux intensity at 2.85°C±21.

Light-protected control packages protected the omega-3 enriched milk the best as malondialdehyde production was lowest in this group and did not have any significant differences over time; however, MDA did increase to levels much greater than observed in milk without omega-3 oils added. Milk packaged in the high TiO_2 protected most closely to the light-protected control package up to Day 21 and there was no significant between the two packaging groups through day 7 (Appendix P). However between day 28 and 36, malondialdehyde production was higher in this group, although still significantly lower than the light-exposed package. This closely approximates the trends of riboflavin concentration.

All MDA values are > 1.3 mg/L in the omega-3 milk by day 1, indicating that the fish oil increased the risk of oxidation in the milk, even without light exposure. As noted in chapter 3 a MDA greater than 1.3 (mg/L) suggest the milk will taste oxidized. However, comparisons against milk without fish oil were not evaluated so this cannot be verified. The higher MDA values, due to fish oil, suggest that the protective effect of the packaging may be less obvious since even the control milk was undergoing some oxidation. Light protected control package had a TBARS value of 2.75 (mg/L) at five weeks compared to 1.10 (mg/L) in regular milk. The difference in TBARS at day 21 of 2.39 (mg/L) in the high TiO_2 packaged milk compared to 1.80 (mg/L) in the light protected milk was not sufficient to create a difference in sensory perception. It is possible that the quality of omega-3 milk is likely to be less acceptable than regular milk. Acceptability was not tested, however.

MDA increased to levels greater than 2.0 (mg/L) within 3 days and 5.0 (mg/L) by 21 days in omega-3 milk exposed to light. Light exposed control is not a sufficient package for protecting omega-3 fatty acid from oxidation due to her photooxidation and subsequent autoxidation. Even

low to moderate TiO_2 levels in packaging did not provide sufficient protection of omega-3 fatty acids for even short periods of time.

Volatile Composition. Hexanal and pentanal are commonly used as an indicator of lipid oxidation (Min and Schweizer 1983; Kim and others 2003; Moore and others 2012; Li 2011). Karatapanis and others (2006) reported that dimethyl disulphide, pentanal, hexanal and heptanal as potential markers of fresh milk quality. Volatile compounds such as 1-penten-3-ol, 2-penten-3-ol, t-2-butenal, t-2-hexenal, and 2-propenal were found in a fish oil emulsion (Jimenez-Alvarez and others 2008).

Hexanal was found in UHT milk, and milk enriched with fish oil (Valero and others 2001; Jónsdóttir and other 2005; Li 2011). Butanal was detected by other researchers in UHT milk and milk enriched with fish oil (Valero and others 2001; Venkateshwarlu and others 2004). However in this study, butanal was not detected; probably due to the SPME/GC not being sensitive enough to detect low amounts.

The high TiO_2 package appeared to control conditions to limit hexanal production, secondary to the light-protected treatment. These finding are in agreement with Mortensen and others (2003) who found that hexanal increased significantly in Havarti cheese when stored under lighting that emits wavelengths of 366, 405, and 436 nm, with the highest increase when cheese was exposed to 405 nm. This is also in agreement with work in our laboratory from Webster and others (2009).

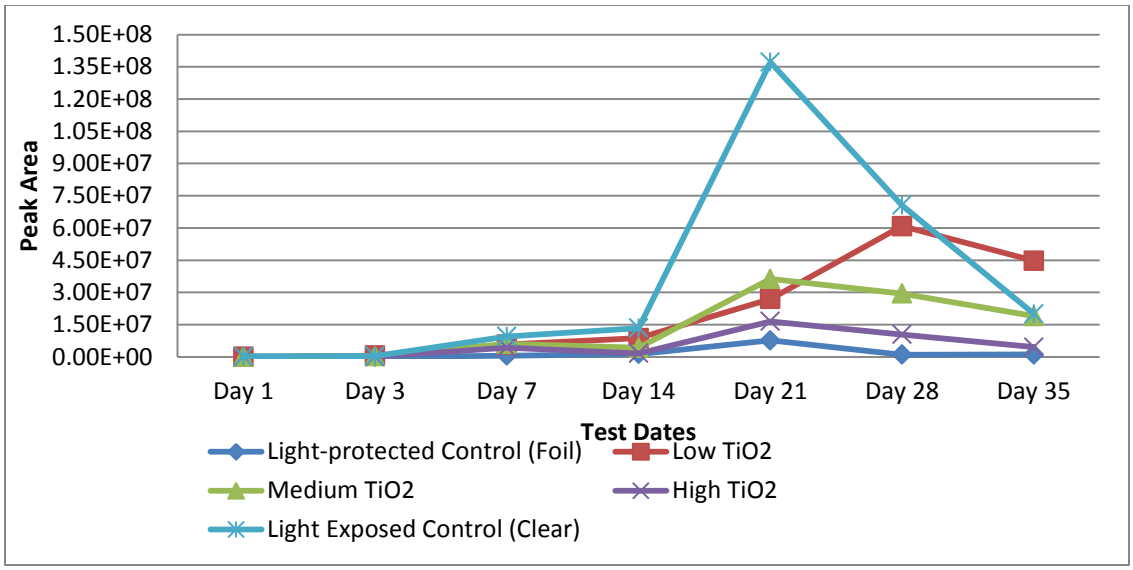

Figure 4.3: Hexanal peak areas in TiO2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 milk exposed to 2286±141 lux intensity at 2.85°C±21.

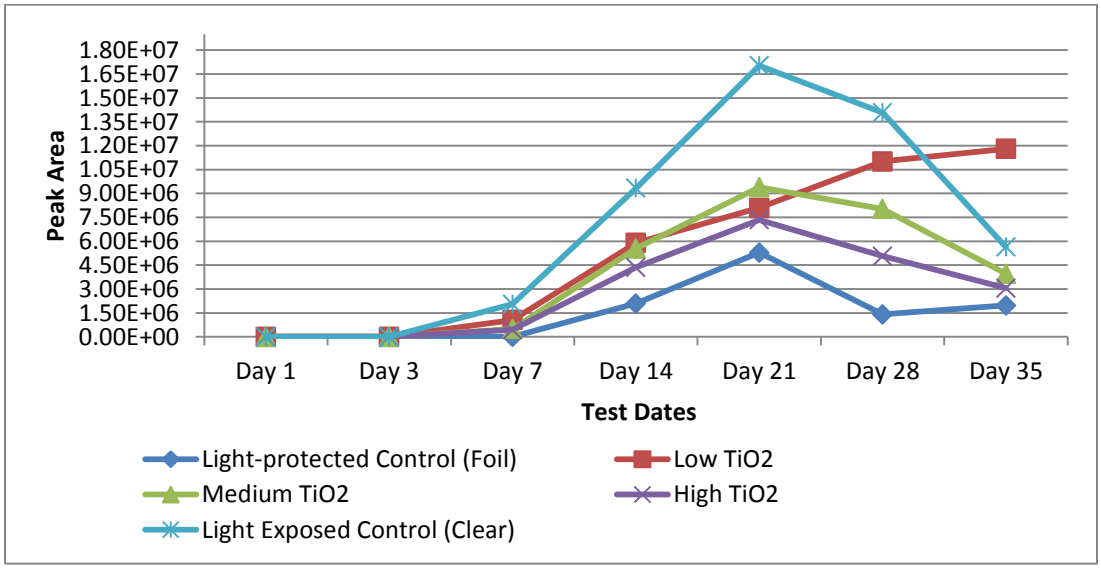

Figure 4.4: Pentanal peak areas in TiO2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 milk exposed to 2286±141 lux intensity at 2.85°C±21.

The contrasts for hexanal and pentanal are identical to the results of the riboflavin analysis (Appendix P). Hexanal in milk in different packaging treatments as early as Day 1 are not significantly different from one another, and over time, the light protected control and the high

TiO_2 package protected well as there are no significant differences in these bottles over a 4 week period. However, by week 5, the high TiO_2 package increased hexanal production compared to foil wrapped bottles. Furthermore, the light exposed, low and medium TiO_2 packages were unable to prohibit hexanal production. Pentanal was detected in all treatments, beginning on day 7. The light-exposed control had a significantly higher area counts of pentanal than the light-protected control package. These finding are similar to the volatile composition found in chapter 3. However the results differ from what Moore and others (2012) reported. They found hexanal levels to decrease during storage, when investigating omega-3 acid fortified beverages. Moore and others (2012) and Li (2011) also found 1-penten-3-ol in their omega-3 fortified dairy product, a compound not found in this study.

CONCLUSION

Selecting appropriate packaging for omega-3 enriched milk is critical for protecting product sensory quality and retaining nutrient and health value. The use of high TiO_2 packaging provides greater protection of sensory and nutritional quality than does clear or low to medium TiO_2 HDPE packaging. Even a complete light protected package does not completely protect omega-3 enriched milk from the risk of oxidation. Antioxidants may assist in providing additional protection.

ACKNOWLEDGMENT

Funding for this project was provided by DuPont, Wilmington, DE.

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Appendices

Appendix A: Institutional Review Board Approval Letter

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24060
540/231-4606 Fax 540/231-0959
e-mail irb@vt.edu
Website: www.irb.vt.edu

MEMORANDUM

DATE: October 14, 2011

TO: Susan E. Duncan, Virginia Fernandez-Plotka, Laurie Bianchi, Daryan Johnson

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: Effect of TiO2 on the Stability of Riboflavin in Food Systems. Phase II: Protecting Sensory Quality and Riboflavin Integrity in Value-Added Milk and Soy Products Based on TiO2 Opacity of Polyethylene Films

IRB NUMBER: 11-477

Effective October 14, 2011, the Virginia Tech IRB Administrator, Carmen T. Green, approved the amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at http://www.irb.vt.edu/pages/responsibilities.htm (please review before the commencement of your research).

PROTOCOL INFORMATION:
Approved as: **Exempt, under 45 CFR 46.101(b) category(ies) 2, 6**
Protocol Approval Date: **7/7/2011**
Protocol Expiration Date: **NA**
Continuing Review Due Date*: **NA**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:
Per federally regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Appendix B: Human Subject Consent Form

Informed Consent for Participants in Research Projects Involving Human Subjects (Sensory Evaluation)

Title Project: Protective Effect of Packaging Materials on Sensory Quality of Milk

Investigators: Susan E. Duncan, PhD, RD, Laurie Bianchi, Daryan Johnson Virginia Fernandez-Plotka,

I. Purpose of this Research/Project

You are invited to participate in a study to determine whether consumers can detect a difference in the aroma and flavor of milk protected with different packaging.

II. Procedures

You will be evaluating three sample sets of milk for sensory quality. Each set will have three samples. You will identify the sample that is different, based on aroma and flavor, within each set and respond using a touchscreen computer monitor. There are seven sensory sessions over five weeks. You are encouraged to participate in all sessions, if possible.

III. Risks

There are only minimal risks associated with this study. Individuals with allergies to certain food components, particularly milk, may be at risk.

IV. Benefits

The goal of this research is to determine if an alternative packaging product may provide protection for milk flavor and nutrient quality. Your participation in this study will provide valuable information to the packaging industry about the best way to protect flavor and nutritional quality of milk.

V. Extent of Anonymity and Confidentiality

The results of your performance as a panelist will be kept strictly confidential except to the investigator. Individual panelists will be referred to by a code number for data analyses and for any publication of the results.

VI. Compensation

You will be compensated with a small edible treat at the end of every session in which you choose to participate. Your name will be entered into a drawing for a $100 Kroger gift card each time you participate. Chances of winning are based on the number of times you participate

Appendix B: Con't

(maximum number is 7). The anticipated number of entries is estimated at 756. The drawing will occur at the end of the study.

VII. Freedom to Withdraw

If you agree to participate in this study, you are free to withdraw from the study at any time without penalty.

VIII. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

Evaluate 3 sets (3 samples each) of milk, as presented, and provide answers using a touch screen computer.

IX. Subject's Permission

I have read the consent form and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

Date: _____

Subject Signature: _____

Subject Printed Name: _____

Appendix C: Table of exposure time in hours.

Appendix Table C-1: Exposure (hrs.) to light (2186 ± 869 Rep. 1, 2421 ± 551 Rep. 2) in dairy case (2.7°C ± 0.8 Rep. 1; 3.0°C ± 0.87 Rep. 2) for 2% milk packaged in five different light-barrier packaging treatments over a 5-week storage period

Day	1	3	8	15	22	29	36
Hours Block A	21	72	191	358	527	696	864
Hours[1] Block B	23	72	192	360	528	696	864

[1]Time from when bottles were placed in dairy case until withdrawn for sensory and chemical evaluations.

Appendix Table C-2: Exposure (hrs.) to light (2186 ± 869 Rep. 1, 2385 ± 187 Rep. 2) in dairy case (2.7°C ± 0.8 Rep. 1; 3.0°C ± 0.87 Rep. 2) for Omega-3 enriched milk packaged in five different light-barrier packaging treatments over a 5-week storage period

Day	1	3	7	14	21	28	35
Hours Block A	21	72	166	335	502	670	838
Hours[1] Block B	23	72	168	336	504	672	840

[1]Time from when bottles were placed in dairy case until withdrawn for sensory and chemical evaluations.

Appendix D: Calculations for the amounts of the fish oil added in 2% milk.

Based on the proximate composition sheet provided with the oil by OmegaPure® E, EPA+DHA (mg/g) as free fatty acid in the fish oil was estimated at 215 mg/g (400mg/215mg*1g=2g for 4 cups). In 303 liter (80 gallons) of 2% milk, the amount of fat is 303,000ml*1.03g/ml*2%=6241.8g (Total fat). The amount of fish oil that was added is 598.4g/l, and then the amount of cream needed to be added back into the skim to make 2% milk was calculated.

Appendix E 1-3 : Table E: Mean and standard deviation of riboflavin concentration (µg/ml) of 2% milk (rep. 1, 2% milk (rep. 2), and average of 2% milk (rep. 1 & 2) treated over 36 days in 5 different treatment bottles; light-protected, light-exposed, low, medium, high TiO_2 levels.

2% MILK (Rep. 2) Sensory & Analytical Testing [2]																				
PACKAGING TREATMENT	**DAY**																			
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.64	±	.02	0.41	±	.01	0.56	±	.02	0.15	±	.04	0.13	±	.02	0.41	±	.03	0.37	±	.04
Low[1]	0.71	±	.00	0.64	±	.01	0.50	±	.04	0.43	±	.03	0.37	±	.00	0.43	±	.00	0.21	±	.05
Medium	0.71	±	.00	0.70	±	.03	0.57	±	.03	0.57	±	.10	0.53	±	.09	0.51	±	.01	0.62	±	.01
High	0.71	±	.02	0.72	±	.02	0.59	±	.05	0.64	±	.02	0.67	±	.14	0.68	±	.01	0.55	±	.13
Light-Protected Control (Foil)	0.72	±	.01	0.73	±	.01	0.71	±	.01	0.67	±	.02	0.67	±	.00	0.70	±	.01	0.71	±	.01
2% MILK (Rep. 2) Light intensity 2421 ± 551 (Lux) Analytical Testing Only [2]																				
PACKAGING TREATMENT	**DAY**																			
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.62	±	.01	0.46	±	.01	0.46	±	.02	0.44	±	.03	0.31	±	.01	0.08	±	.03	0.13	±	.02
Low	0.67	±	.00	0.54	±	.01	0.64	±	.02	0.65	±	.02	0.41	±	.02	0.21	±	.02	0.15	±	.00
Medium	0.68	±	.00	0.65	±	.01	0.78	±	.01	0.77	±	.05	0.56	±	.01	0.33	±	.02	0.26	±	.00
High	0.69	±	.02	0.69	±	.01	0.84	±	.03	0.82	±	.01	0.65	±	.06	0.41	±	.02	0.55	±	.07
Light-Protected Control (Foil)	0.71	±	.01	0.69	±	.01	0.81	±	.13	0.86	±	.03	0.71	±	.03	0.71	±	.01	0.71	±	.01
2% Milk (Average of Rep. 1 & 2) Light Intensity 2304 ± 166 (Lux)[3]																				
PACKAGING TREATMENT	**DAY**																			
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	S.E.	x	±	S.E.	x	±	S.E.	x	±	S.E.	X	±	S.E.	x	±	S.E.	x	±	S.E.
Light-Exposed Control (Clear)	0.63	±	.01	0.44	±	.04	0.51	±	.10	0.30	±	.21	0.22	±	.13	0.25	±	.23	0.25	±	.17
Low	0.69	±	.03	0.59	±	.07	0.57	±	.10	0.54	±	.16	0.39	±	.03	0.32	±	.16	0.18	±	.04
Medium	0.70	±	.02	0.68	±	.04	0.68	±	.15	0.67	±	.14	0.55	±	.02	0.42	±	.13	0.44	±	.25
High	0.7	±	.01	0.71	±	.02	0.72	±	.18	0.73	±	.13	0.66	±	.01	0.55	±	.19	0.55	±	0
Light-Protected Control (Foil)	0.72	±	.01	0.71	±	.03	0.76	±	.10	0.77	±	.13	0.69	±	.03	0.71	±	.01	0.71	±	0

[1]Titanium Dioxide Modified High Density Polyethylene Packaging Treatment (Low, Medium, High)

[2]Mean based on 2 bottles/day/block.

[3]Overall mean based on 4 observations.

[4]Day 0 Riboflavin Concentration 0.73 µg/ml (Block A) 0.73 µg/ml (Block B)

Appendix F 1-3: Table F: Mean and standard deviation of **malondialdehyde** concentration (mg/L) of 2% milk (Rep. 1), 2% milk (Rep. 2), and Average of 2% milk (Rep. 1 & 2) treated over 36 days in 5 different treatment bottles; light-protected (foil wrapped), light-exposed (clear), low, medium, high TiO_2 levels.

2% MILK (Rep. 2) Sensory & Analytical Testing																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.76	±	.02	0.78	±	.01	1.4	±	.32	1.49	±	.13	2.59	±	.26	1.67	±	.25	2.24	±	.33
Low	0.69	±	.01	0.71	±	.01	1.52	±	.24	1.88	±	.33	2.85	±	.49	1.94	±	.44	1.76	±	.41
Medium	0.73	±	.01	0.71	±	.01	1.45	±	.19	1.31	±	.21	2.38	±	.41	1.3	±	.39	1.23	±	.34
High	0.69	±	.01	0.72	±	.11	0.71	±	.02	1.03	±	.05	1.00	±	.09	1.11	±	.01	1.43	±	.07
Light-Protected Control (Foil)	0.62	±	.01	0.6	±	.01	0.73	±	.04	0.76	±	.00	1.11	±	.05	1.06	±	.08	1.0	±	.12
2% MILK (Rep. 2) Light intensity 2421 ± 551 (Lux) Analytical Testing Only																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.68	±	.03	.74	±	.08	0.98	±	.22	1.37	±	.00	1.93	±	.31	2.22	±	.08	2.27	±	.08
Low	0.59	±	.02	0.62	±	.05	0.69	±	.05	1.2	±	.03	1.63	±	.04	2.08	±	.08	1.87	±	.34
Medium	0.55	±	.02	0.59	±	.04	0.67	±	.05	1.45	±	.01	1.45	±	.09	1.46	±	.17	1.45	±	.17
High	0.56	±	.13	0.52	±	.02	0.64	±	.02	0.64	±	.04	0.75	±	.14	1.30	±	.00	1.34	±	.11
Light-Protected Control (Foil)	0.49	±	.03	0.52	±	.08	0.63	±	.03	0.58	±	.03	0.68	±	.11	0.70	±	.08	0.95	±	.09
2% Milk (Average of Rep. 1 & 2) Light Intensity 2304 ± 166 (Lux) [3]																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**8**			**15**			**22**			**29**			**36**		
	x	±	S.E.	x	±	S.E.	x	±	S.E.	x	±	S.E.	X	±	S.E.	x	±	S.E.	x	±	S.E.
Light-Exposed Control (Clear)	0.72	±	.05	0.76	±	.02	1.19	±	.19	1.43	±	.21	2.26	±	.67	1.95	±	.10	2.26	±	.11
Low	0.65	±	.08	0.67	±	.06	1.10	±	.13	1.54	±	.07	2.24	±	.71	2.01	±	.57	1.82	±	.57
Medium	0.64	±	.12	0.65	±	.08	1.06	±	.13	1.18	±	.18	1.92	±	.54	1.38	±	.10	1.34	±	.16
High	0.62	±	.08	0.66	±	.14	0.68	±	.30	0.84	±	.06	0.88	±	.35	1.21	±	.28	1.39	±	.18
Light-Protected Control (Foil)	0.55	±	.08	0.56	±	.28	0.68	±	.28	0.67	±	.20	0.90	±	.42	0.88	±	.18	0.98	±	.16

[1]Titanium Dioxide Modified High Density Polyethylene Packaging Treatment (Low, Medium, High)

[2]Mean based on 2 bottles/day/block.

[3]Overall mean based on 4 observations.

Appendix G 1-3: Table G: Mean and standard deviation of **riboflavin** concentration (µg/ml) of Omega-3 milk (Rep. 1), 2% milk (Rep. 2), and Average of 2% milk (Rep 1 & 2) treated over 35 days in 5 different treatment bottles; light-protected (foil wrapped), light-exposed (clear), low, medium, high TiO_2 levels.

Omega-3 MILK (Rep. 1) Light Intensity 2186 ± 869 (Lux)[2] Sensory and Analytical Testing																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.56	±	.00	0.16	±	.11	0.15	±	.03	0.06	±	.00	0.05	±	.01	0.05	±	.01	0.06	±	.00
Low	0.63	±	.00	0.37	±	.01	0.29	±	.01	0.15	±	.00	0.13	±	.02	0.08	±	.01	0.10	±	.01
Medium	0.62	±	.00	0.55	±	.01	0.46	±	.06	0.35	±	.09	0.25	±	.00	0.29	±	.01	0.26	±	.04
High	0.64	±	.01	0.63	±	.01	0.50	±	.01	0.99	±	.71	0.41	±	.02	0.45	±	.05	0.46	±	.00
Light-Protected Control (Foil)	0.70	±	.00	0.69	±	.01	0.65	±	.10	0.66	±	.01	0.56	±	.01	0.71	±	.01	0.71	±	.01
Omega-3 MILK (Rep. 2) Light Intensity 2385 ± 187 (Lux) Analytical Testing Only																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	0.68	±	.03	0.49	±	.02	0.35	±	.01	0.18	±	.02	0.08	±	.02	0.08	±	.04	0.06	±	.02
Low	0.49	±	.00	0.49	±	.04	0.42	±	.03	0.27	±	.03	0.21	±	.01	0.17	±	.02	0.12	±	.02
Medium	0.52	±	.00	0.57	±	.02	0.45	±	.01	0.38	±	.11	0.27	±	.01	0.21	±	.01	0.26	±	.02
High	0.53	±	.01	0.64	±	.00	0.53	±	.01	0.51	±	.00	0.37	±	.03	0.41	±	.06	0.45	±	.05
Light-Protected Control (Foil)	0.62	±	.01	0.69	±	.00	0.68	±	.06	0.60	±	.02	0.62	±	.00	0.71	±	.01	0.71	±	.01
Omega-3 Milk (Average of Rep. 1 & 2) Light Intensity 2286 ± 141 (Lux)[3]																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	X	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.
Light-Exposed Control (Clear)	0.62	±	.06	0.33	±	.23	0.25	±	.14	0.12	±	.08	0.07	±	.02	0.07	±	.02	0.06	±	0
Low	0.56	±	.10	0.43	±	.08	0.36	±	.13	0.21	±	.08	0.17	±	.06	0.13	±	.06	0.11	±	.01
Medium	0.57	±	.07	0.56	±	.01	0.46	±	.03	0.37	±	.02	0.26	±	.01	0.25	±	.07	0.26	±	0
High	0.59	±	.08	0.64	±	.01	0.52	±	.05	0.75	±	.34	0.39	±	.03	0.43	±	.03	0.46	±	.007
Light-Protected Control (Foil)	0.66	±	.06	0.69	±	0	0.67	±	.02	0.63	±	.04	0.59	±	.04	0.71	±	0	0.71	±	0

[1]Titanium Dioxide Modified High Density Polyethylene Packaging Treatment (Low, Medium, High).

[2]Mean based on 2 bottles/day/block.

[3]Overall mean based on 4 observations.

[4]Day 0 Riboflavin Concentration 0.72 µg/ml (Block A) 0.71 µg/ml (Block B)

Appendix H 1-3: Table H: Mean and standard deviation of **malondialdehyde** concentration (mg/L) of Omega-3 milk (Rep. 1), 2% milk (Rep. 2), and Average of 2% milk (Rep 1 & 2) treated over 35 days in 5 different treatment bottles; light-protected (foil wrapped), light-exposed (clear), low, medium, high TiO_2 levels.

Omega-3 MILK (Rep. 1) Light Intensity 2186 ± 869 (Lux)[2] Sensory and Analytical Testing																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	1.63	±	.04	2.12	±	.20	2.99	±	.01	4.17	±	.05	5.05	±	.04	5.25	±	.18	5.4	±	.37
Low[1]	1.47	±	.05	1.93	±	.00	2.39	±	.38	3.47	±	.04	4.08	±	.03	4.39	±	.10	4.61	±	.08
Medium	1.47	±	.11	1.84	±	.06	1.84	±	.11	2.92	±	.07	4.23	±	.09	4.48	±	.10	4.72	±	.04
High	1.45	±	.78	1.58	±	.04	1.61	±	.01	2.88	±	.11	2.39	±	.16	4.34	±	.00	3.72	±	.12
Light-Protected Control (Foil)	1.48	±	.10	1.37	±	.06	1.44	±	.06	2.06	±	.11	1.80	±	.01	2.13	±	.08	2.75	±	.02
Omega-3 MILK (Rep. 2) Light Intensity 2385 ± 187 (Lux) Analytical Testing Only																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	1.34	±	.08	3.78	±	.58	3.29	±	.12	4.43	±	.10	5.50	±	.17	5.63	±	.16	6.48	±	.11
Low	1.21	±	.06	2.32	±	.18	2.44	±	.25	3.64	±	.14	4.53	±	.13	4.55	±	.14	5.17	±	.11
Medium	.99	±	.06	2.44	±	.31	2.09	±	.03	3.04	±	.17	4.06	±	.13	4.04	±	.05	4.60	±	.02
High	.86	±	.04	1.59	±	.16	1.92	±	.02	2.64	±	.13	3.42	±	.10	3.57	±	.00	4.18	±	.09
Light-Protected Control (Foil)	.71	±	.12	1.22	±	.11	2.00	±	.10	1.92	±	.05	2.45	±	.07	2.51	±	.07	3.05	±	.09
Omega-3 Milk (Average of Rep. 1 & 2) Light Intensity 2286 ± 141 (Lux)[3]																					
PACKAGING TREATMENT	**DAY**																				
	1			**3**			**7**			**14**			**21**			**28**			**35**		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light-Exposed Control (Clear)	1.49	±	.21	2.95	±	1.17	3.14	±	.21	4.3	±	.18	5.28	±	.32	5.44	±	.27	5.94	±	.76
Low	1.34	±	.18	2.13	±	.28	2.42	±	.04	3.56	±	.12	4.31	±	.32	4.47	±	.11	4.89	±	.40
Medium	1.23	±	.34	2.14	±	.42	1.97	±	.18	2.98	±	.08	4.15	±	.12	4.26	±	.31	4.66	±	.08
High	1.16	±	.42	1.59	±	.007	1.77	±	.22	2.76	±	.17	2.91	±	.73	3.96	±	.54	3.95	±	.33
Light-Protected Control (Foil)	1.10	±	.54	1.30	±	.10	1.72	±	.40	1.99	±	.10	2.13	±	.46	2.32	±	.27	2.9	±	.21

[1]Titanium Dioxide Modified High Density Polyethylene Packaging Treatment (Low, Medium, High)

[2]Mean based on 2 bottles/day/block.

[3]Overall mean based on 4 observations.

Appendix I: 1-2

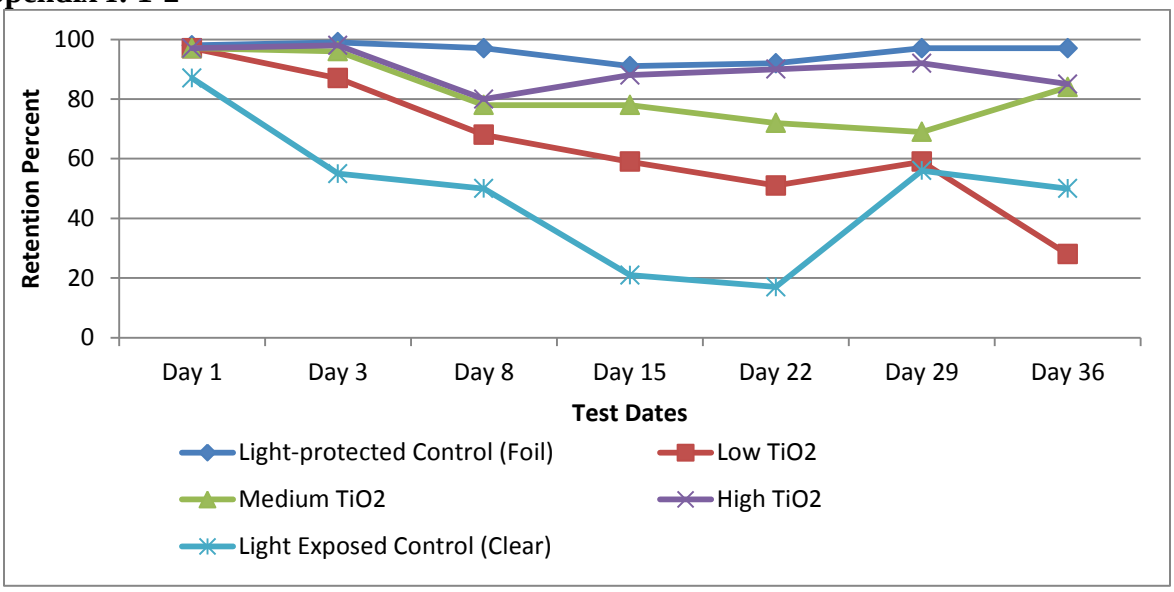

Figure I-1: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±.21 (Rep 1).

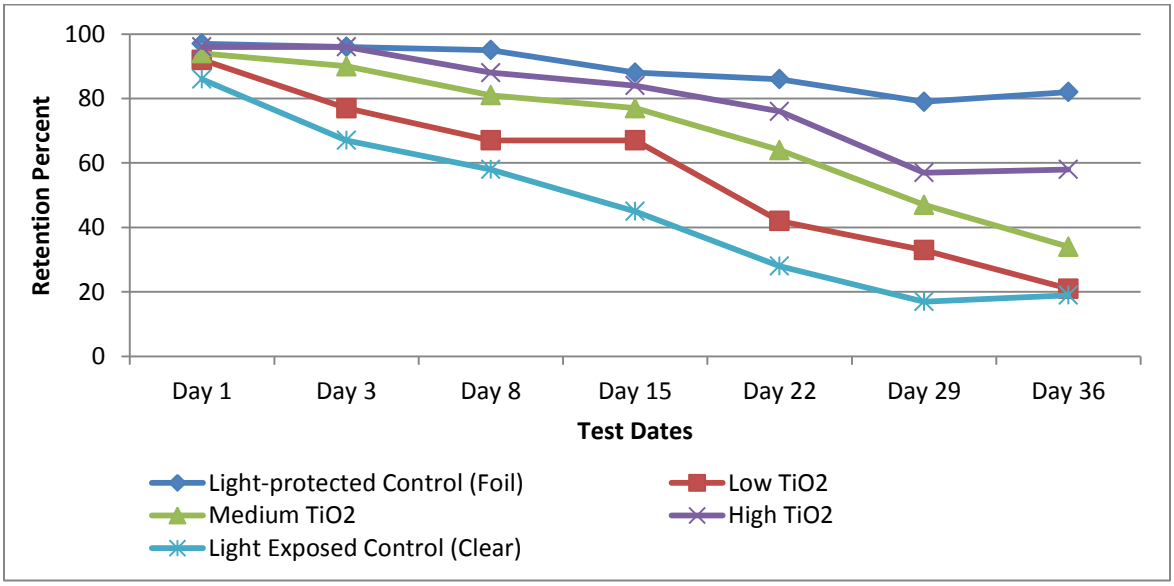

Figure I-2: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±.21 (Rep 2).

Appendix J: 1-2

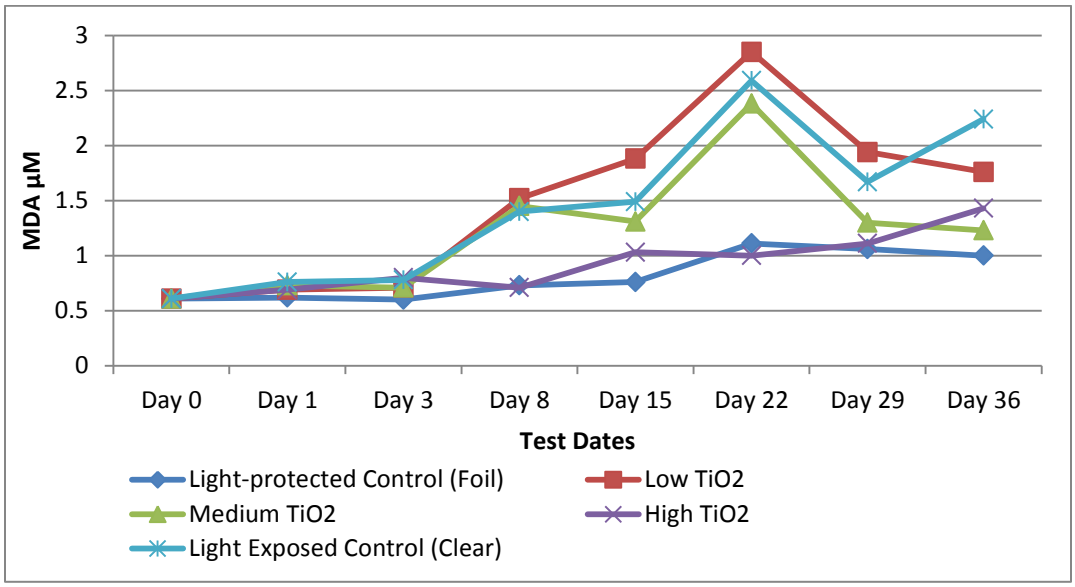

Figure J-1: Malondialdehyde concentration in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±.21 (Rep 1).

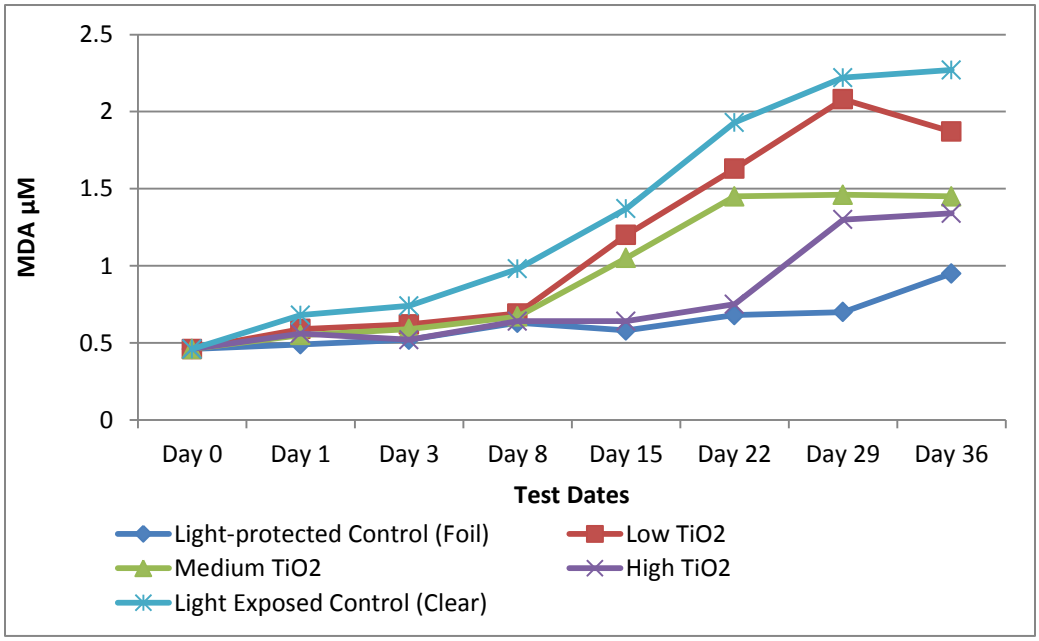

Figure J-2: Malondialdehyde concentration in in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 36 day period of 2% milk exposed to 2304±166 lux intensity at 2.85°C±.21 (Rep 2).

Appendix K: 1-2

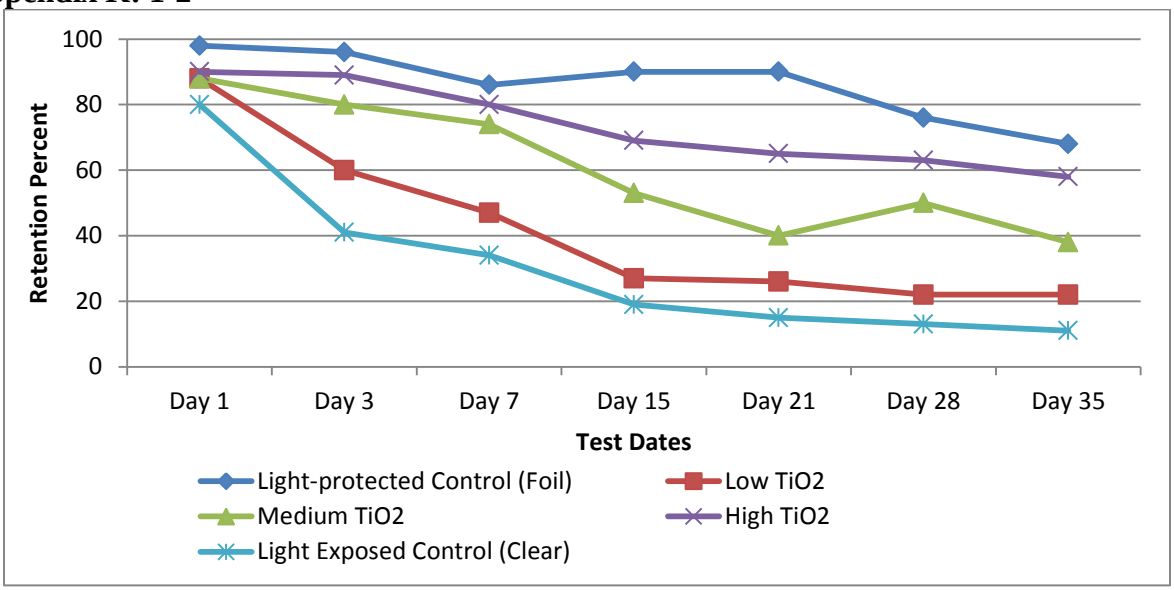

Figure K-1: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega- 3 enriched milk exposed to 2286±141 lux intensity at 2.85°C±21 (Rep 1).

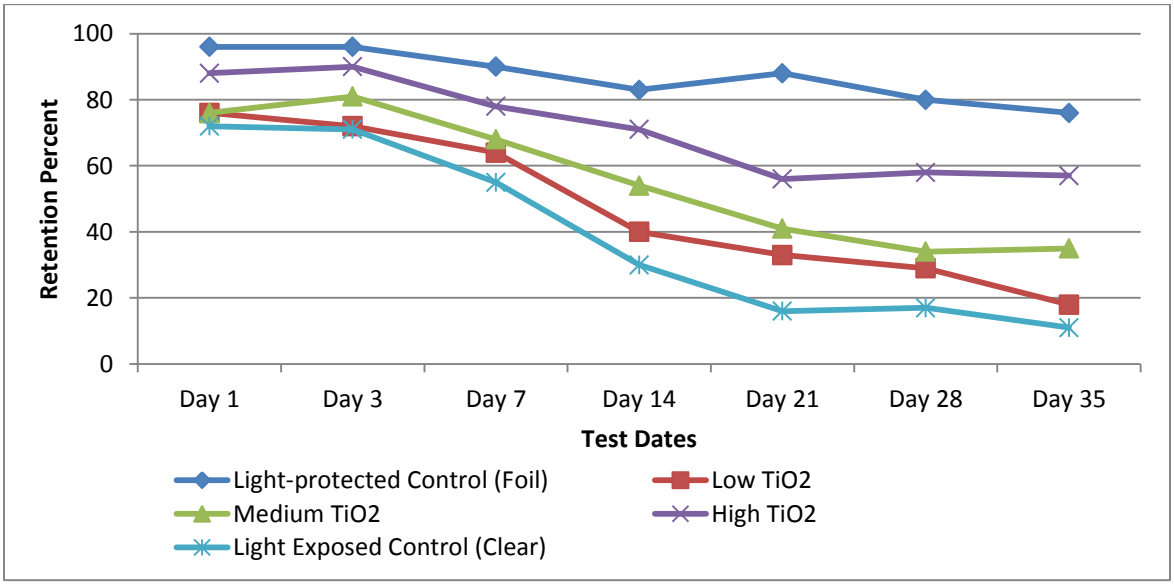

Figure K-2: Riboflavin degradation in TiO_2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of Omega-3 enriched milk exposed to 2286±141 lux intensity at 2.85°C±21 (Rep 2).

Appendix L: 1-2

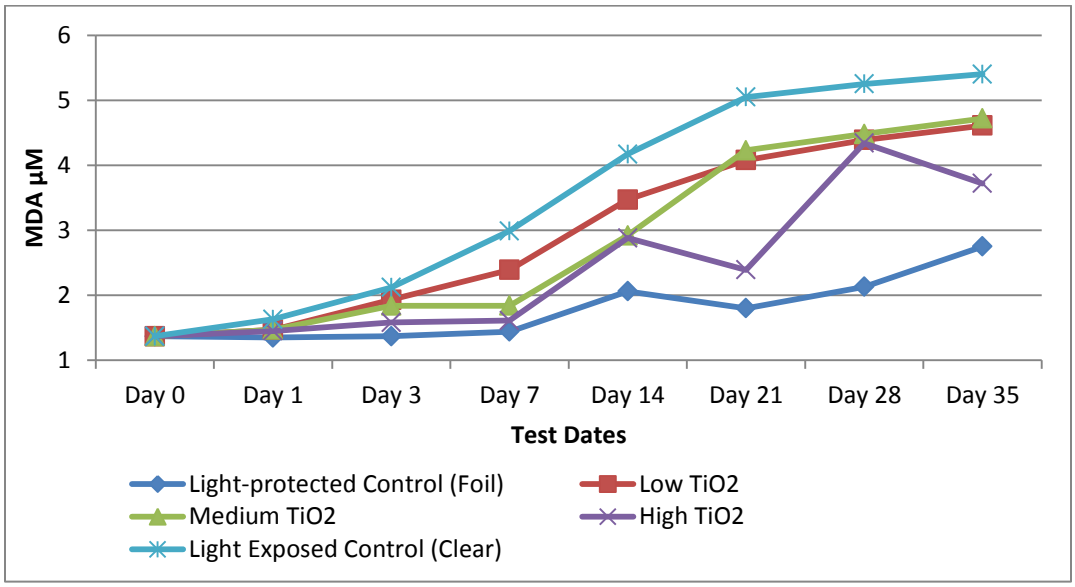

Figure L-1: Malondialdehyde concentration in TiO2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 enriched milk exposed to 2286±141 lux intensity at 2.85°C±21 (Rep 1).

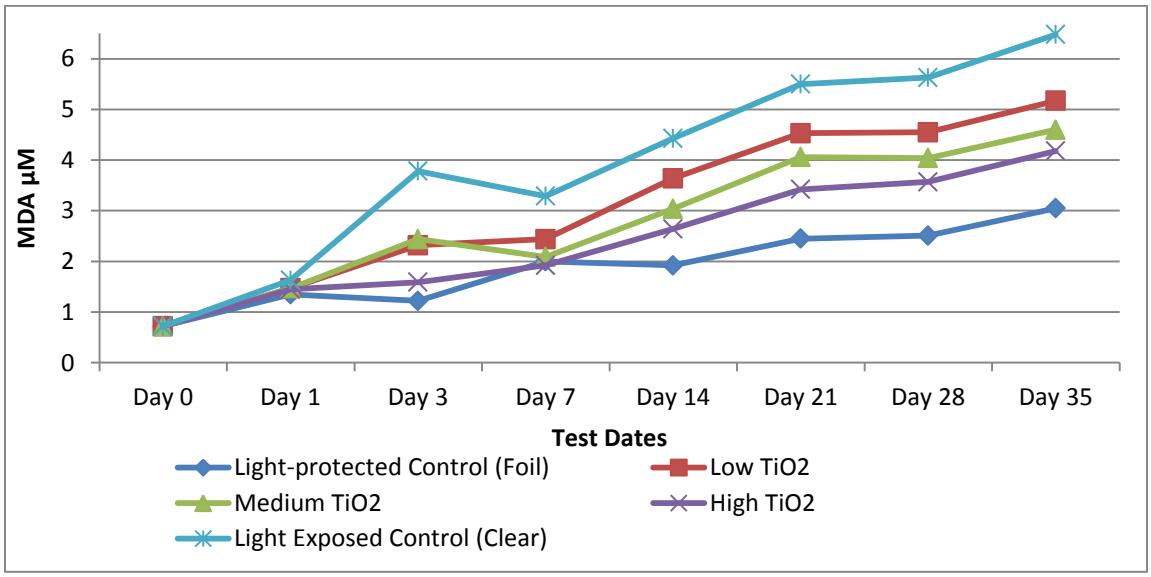

Figure L-2: Malondialdehyde concentration in TiO2-loaded packaging treatments (low, medium, high) high density polyethylene compared to light-protected (foil wrapped) and light exposed (clear) packaging over a 35 day period of omega-3 enriched milk exposed to 2286±141 lux intensity at 2.85°C±21 (Rep 2).

Appendix M: Table M: Power analysis and sensory evaluation data of 2% milk

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. Low	1	24	7	0.05	0.4	30%	0.7368	difference	0.2917	-6%	0.0928	-0.2914	0.1664
Cl vs. Med	1	26	11	0.05	0.35	30%	0.2201	difference	0.4231	13%	0.0969	-0.1045	0.3737
Cl vs. High	1	27	15	0.05	0.35	30%	0.0144*	difference	0.5556	33%	0.0956	0.0974	0.5693
F vs. Cl	1	28	11	0.05	0.35	30%	0.3141	difference	0.3929	9%	0.0923	-0.1385	0.3170
F vs. Low	1	28	13	0.35	0.05	30%	0.1039	similarity	0.4643	20%	0.0942	-0.0361	0.4290
F vs. Med	1	26	12	0.35	0.05	30%	0.1205	similarity	0.4615	19%	0.0978	-0.0489	0.4335
F vs. High	1	25	7	0.35	0.05	30%	0.7785	similarity	0.2800	-8%	0.0898	-0.3016	0.1416
Cl vs. Low	3	29	12	0.05	0.3	30%	0.2321	difference	0.4138	12%	0.0915	-0.1050	0.3464
Cl vs. Med	3	29	10	0.05	0.3	30%	0.5173	difference	0.3448	2%	0.0883	-0.2005	0.2350
Cl vs. High	3	27	18	0.05	0.35	30%	0.0004*	difference	0.6667	50%	0.0907	0.2761	0.7239
F vs. Cl	3	28	19	0.05	0.35	30%	0.0002*	difference	0.6786	52%	0.0883	0.3001	0.7356
F vs. Low	3	27	13	0.35	0.05	30%	0.079	similarity	0.4815	22%	0.0962	-0.0151	0.4595
F vs. Med	3	31	18	0.3	0.05	30%	0.004	similarity	0.5806	37%	0.0886	0.1523	0.5897
F vs. High	3	29	12	0.3	0.05	30%	0.2321	similarity	0.4138	12%	0.0915	-0.1050	0.3464
Cl vs. Low	8	36	21	0.05	0.25	30%	.0018*	difference	0.5833	38%	0.0822	0.1723	0.5777

Appendix M: Table M: Power analysis and sensory evaluation data of 2% milk

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. Med	8	36	26	0.05	0.25	30%	<.0001*	difference	0.7222	58%	0.0747	0.3991	0.7675
Cl vs. High	8	36	23	0.05	0.25	30%	0.0002*	difference	0.6389	46%	0.0801	0.2608	0.6559
F vs. Cl	8	35	28	0.05	0.25	30%	<.0001	difference	0.8000	70%	0.0676	0.5332	0.8668
F vs. Low	8	34	15	0.25	0.05	30%	0.1256	similarity	0.4412	16%	0.0852	-0.0484	0.3719
F vs. Med	8	34	18	0.25	0.05	30%	0.0143*	similarity	0.5294	29%	0.0856	0.0829	0.5053
F vs. High	8	35	19	0.25	0.05	30%	0.0085*	similarity	0.5429	31%	0.0842	0.1065	0.5221
Cl vs. Low	15	38	13	0.05	0.25	30%	0.5151	difference	0.3421	1%	0.0770	-0.1767	0.2031
Cl vs. Med	15	37	21	0.05	0.25	30%	0029*	difference	0.5676	35%	0.0814	0.1504	0.5523
Cl vs. High	15	37	29	0.05	0.25	30%	0.0000*	difference	0.7838	68%	0.0677	0.5087	0.8427
F vs. Cl	15	39	30	0.05	0.2	30%	0.0000*	difference	0.7692	65%	0.0675	0.4874	0.8203
F vs. Low	15	38	31	0.25	0.05	30%	0.0000*	similarity	0.8158	72%	0.0629	0.5685	0.8789
F vs. Med	15	38	20	0.25	0.05	30%	0.0109*	similarity	0.5263	29%	0.0810	0.0896	0.4893
F vs. High	15	38	16	0.25	0.05	30%	0.1644	similarity	0.4211	13%	0.0801	-0.0661	0.3292
Cl vs. Low	22	35	19	0.05	0.25	30%	0.0085*	difference	0.5429	31%	0.0842	0.1065	0.5221
Cl vs. Med	22	35	26	0.05	0.25	30%	0.0000*	difference	0.7429	61%	0.0739	0.4320	0.7966

Appendix M: Table M: Power analysis and sensory evaluation data of 2% milk

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. High	22	35	27	0.05	0.25	30%	0.0000*	difference	0.7714	66%	0.0710	0.4820	0.8323
F vs. Cl	22	36	30	0.05	0.25	30%	0.0000*	difference	0.8333	75%	0.0621	0.5967	0.9033
F vs. Low	22	36	26	0.25	0.05	30%	0.0000*	similarity	0.7222	58%	0.0747	0.3991	0.7675
F vs. Med	22	36	22	0.25	0.05	30%	0.0006*	similarity	0.6111	42%	0.0812	0.2162	0.6172
F vs. High	22	35	14	0.25	0.05	30%	0.2521	similarity	0.4000	10%	0.0828	-0.1043	0.3043
Cl vs. Low	29	38	16	0.05	0.25	30%	0.1644	difference	0.4211	13%	0.0801	-0.0661	0.3292
Cl vs. Med	29	37	20	0.05	0.25	30%	0.0075*	difference	0.5405	31%	0.0819	0.1087	0.5130
Cl vs. High	29	37	28	0.05	0.25	30%	0.0000*	difference	0.7568	64%	0.0705	0.4611	0.8092
F vs. Cl	29	37	29	0.05	0.25	30%	0.0000*	difference	0.7838	68%	0.0677	0.5087	0.8427
F vs. Low	29	38	33	0.25	0.05	30%	0.0000*	similarity	0.8684	80%	0.0548	0.6673	0.9379
F vs. Med	29	38	30	0.25	0.05	30%	0.0000*	similarity	0.7895	68%	0.0661	0.5210	0.8474
F vs. High	29	38	17	0.25	0.05	30%	0.0954	similarity	0.4474	17%	0.0807	-0.0280	0.3701
Cl vs. Low	43	48	14	0.05	0.25	30%	0.7757	difference	0.2917	-6%	0.0656	-0.2244	0.0994
Cl vs. Med	43	48	41	0.05	0.25	30%	0.0000*	difference	0.8542	78%	0.0509	0.6555	0.9070
Cl vs. High	43	48	27	0.25	0.05	30%	0.0009*	similarity	0.5625	34%	0.0716	0.1671	0.5204

[1]Calculated by Sensory Information Management Systems software

Appendix N: Power analysis and sensory evaluation data of omega-3 enriched milk

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. Low	1	25	9	0.05	0.4	30%	0.4624	difference	0.3600	4%	0.0960	-0.1969	0.2769
Cl vs. Med	1	26	11	0.05	0.35	30%	0.178	difference	0.4400	16%	0.0993	-0.0850	0.4050
Cl vs. High	1	24	6	0.05	0.35	30%	0.8618	difference	0.2500	-13%	0.0884	-0.3431	0.0931
F vs. Cl	1	25	15	0.05	0.35	30%	0.0056*	difference	0.6000	40%	0.0980	0.1582	0.6418
F vs. Low	1	25	12	0.35	0.05	30%	0.0918	similarity	0.4800	22%	0.0999	-0.0266	0.4666
F vs. Med	1	24	13	0.35	0.05	30%	0.0284*	similarity	0.5417	31%	0.1017	0.0615	0.5635
F vs. High	1	24	17	0.35	0.05	30%	0.0002*	similarity	0.7083	56%	0.0928	0.3336	0.7914
Cl vs. Low	3	26	11	0.05	0.3	30%	0.2201	difference	0.4231	13%	0.0969	-0.1045	0.3737
Cl vs. Med	3	27	6	0.05	0.3	30%	0.9281	difference	0.2222	-17%	0.0800	-0.3641	0.0308
Cl vs. High	3	26	9	0.05	0.35	30%	0.5182	difference	0.3462	2%	0.0933	-0.2110	0.2495
F vs. Cl	3	28	18	0.05	0.35	30%	0.0008*	difference	0.6429	46%	0.0906	0.2408	0.6877
F vs. Low	3	27	10	0.35	0.05	30%	0.4108	similarity	0.3704	6%	0.0929	-0.1738	0.2849
F vs. Med	3	27	12	0.35	0.05	30%	0.1537	similarity	0.4444	17%	0.0956	-0.0693	0.4026
F vs. High	3	26	8	0.35	0.05	30%	0.6793	similarity	0.3077	-4%	0.0905	-0.2618	0.1849

Appendix N: Con't

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. Low	7	35	16	0.05	0.25	30%	0.0867	difference	0.4571	19%	0.0842	-0.0221	0.3935
Cl vs. Med	7	36	25	0.05	0.25	30%	0*	difference	0.6944	54%	0.0768	0.3522	0.7311
Cl vs. High	7	35	22	0.05	0.25	30%	0.0003*	difference	0.6286	44%	0.0817	0.2413	0.6444
F vs. Cl	7	34	24	0.05	0.25	30%	0*	difference	0.7059	56%	0.0781	0.3660	0.7516
F vs. Low	7	35	21	0.25	0.05	30%	0.0011*	similarity	0.6000	40%	0.0828	0.1957	0.6043
F vs. Med	7	36	19	0.25	0.05	30%	0.0125*	similarity	0.5278	29%	0.0832	0.0864	0.4970
F vs. High	7	36	11	0.25	0.05	30%	0.6967	similarity	0.3056	-4%	0.0768	-0.2311	0.1478
Cl vs. Low	14	38	19	0.05	0.25	30%	0.0246*	difference	0.5000	25%	0.0811	0.0499	0.4501
Cl vs. Med	14	38	22	0.05	0.25	30%	0.0016*	difference	0.5789	37%	0.0801	0.1708	0.5661
Cl vs. High	14	37	30	0.05	0.25	30%	<.0001*	difference	0.8108	72%	0.0644	0.5573	0.8751
F vs. Cl	14	39	34	0.05	0.25	30%	<.0001*	difference	0.8718	81%	0.0535	0.6756	0.9398
F vs. Low	14	38	21	0.25	0.05	30%	0.0044*	similarity	0.5526	33%	0.0807	0.1299	0.5280
F vs. Med	14	36	22	0.25	0.05	30%	0.0006*	similarity	0.6111	42%	0.0812	0.2162	0.6172
F vs. High	14	38	21	0.25	0.05	30%	0.0044*	similarity	0.5526	33%	0.0807	0.1299	0.5280
Cl vs. Low	21	34	17	0.05	0.25	30%	0.0327*	difference	0.5000	25%	0.0857	0.0384	0.4616

Appendix N: Con't

Paired Comparison	Light Exposure at time testing (days)	Total number of appraisals (number)	Total number of correct appraisals (number)	α	β	Proportion of discrim-inators - Preset	p-value[1]	Testing	Proportion correct	Proportion of discrim-inators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Cl vs. Med	21	34	11	0.05	0.25	30%	0.6117	difference	0.3235	-1%	0.0802	-0.2127	0.1833
Cl vs. High	21	34	24	0.05	0.25	30%	<0.0001*	difference	0.7059	56%	0.0781	0.3660	0.7516
F vs. Cl	21	35	29	0.05	0.25	30%	<0.0001*	difference	0.8286	74%	0.0637	0.5857	0.9000
F vs. Low	21	35	30	0.25	0.05	30%	<0.0001*	similarity	0.8571	79%	0.0591	0.6398	0.9317
F vs. Med	21	34	30	0.25	0.05	30%	<0.0001*	similarity	0.8824	82%	0.0553	0.6872	0.9599
F vs. High	21	34	15	0.25	0.05	30%	0.1256	similarity	0.4412	16%	0.0852	-0.0484	0.3719
Cl vs. Low	28	34	11	0.05	0.25	30%	0.6117	difference	0.3235	-1%	0.0802	-0.2127	0.1833
Cl vs. Med	28	33	13	0.05	0.25	30%	0.2852	difference	0.3939	9%	0.0851	-0.1190	0.3008
Cl vs. High	28	33	24	0.05	0.25	30%	<0.0001	difference	0.7273	59%	0.0775	0.3996	0.7822
F vs. Cl	28	35	28	0.05	0.25	30%	<0.0001	difference	0.8000	70%	0.0676	0.5332	0.8668
F vs. Low	28	35	30	0.25	0.05	30%	<0.0001	similarity	0.8571	79%	0.0591	0.6398	0.9317
F vs. Med	28	35	30	0.25	0.05	30%	<0.0001	similarity	0.8571	79%	0.0591	0.6398	0.9317
F vs High	28	34	20	0.25	0.05	30%	0.002	similarity	0.5882	38%	0.0844	0.1741	0.5906
Cl vs. Low	36	48	20	0.05	0.25	30%	0.1423	difference	0.4167	13%	0.0712	-0.0506	0.3006
Cl vs. Med	36	48	42	0.05	0.25	30%	<0.0001	difference	0.8750	81%	0.0477	0.6947	0.9303

[1]Calculated by Sensory Information Management Systems software.

Appendix O: Statistical Contrast for 2% Milk

F statistic and p values for specific contrasts with riboflavin concentration as response. $\alpha = 0.05$ in 2% milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-36)	0.3454	0.5586
Light-protected (Day 1) vs Light-exposed (Day 1-36)	326.1	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-36)	164.8	<0.0001*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-36)	42.04	<0.0001*
Light-protected (Day 1) vs High TiO2-loaded (Day 1-36)	7.88	0.0064*
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	2.79	0.0990
Light-exposed (Day 1-22) vs All TiO2-loaded packaging (Day1-22)	303.88	<0.0001*
Light-protected (Day 15) vs High TiO2-loaded (Day 15)	1.43	0.2354

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with malondialdehyde concentration as response. $\alpha = 0.05$ in 2% milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-36)	0.31	0.8598
Light-protected (Day 1) vs Light-exposed (Day 1-36)	65.80	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-36)	50.06	<0.0001*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-36)	21.33	<0.0001*
Light-protected (Day 1) vs High TiO2-loaded (Day 1-36)	2.69	0.1056
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	1.06	0.3076
Light-protected (Day 36) vs High TiO2-loaded (Day 36)	77.01	<0.0001*
Light-exposed (Day 1) vs High TiO2-loaded (Day 36)	4.99	0.0286*

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with hexanal concentration as response. $\alpha = 0.05$ in 2% milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-36)	0.13	0.7257
Light-protected (Day 1) vs Light-exposed (Day 1-36)	20.62	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-36)	18.9	<0.0001*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-36)	1.18	0.2856
Light-protected (Day 1) vs High TiO2-loaded (Day 1-36)	.7073	0.4061
Light-exposed (Day 15) vs Light-protected and All TiO2-loaded packaging (Day 15)	53.72	<0.0001*
Light-protected (Day 1) vs High TiO2-loaded (Day 29)	.89	0.3531
High TiO2-loaded (Day 1) vs High TiO2-loaded (Day 36)	.89	0.3531

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with pentanal concentration as response. $\alpha = 0.05$ in 2% milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-36)	0.04	0.8457
Light-protected (Day 1) vs Light-exposed (Day 1-36)	1.65	0.2082
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-36)	2.46	0.1259
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-36)	0.79	0.3817
Light-protected (Day 1) vs High TiO2-loaded (Day 1-36)	0.17	0.6808
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	0.02	0.8783
Light-protected (Day 1) vs High TiO2-loaded (Day 36)	0.51	0.4812
High TiO2-loaded (Day 1) vs High TiO2-loaded (Day 36)	0.04	0.4812

*pvalue <0.05 indicates statistical significant difference.

Appendix P: Statistical Contrast for Omega-3 Milk

F statistic and p values for specific contrasts with riboflavin concentration as response. $\alpha = 0.05$ in omega-3 milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-35)	0.21	0.6506
Light-protected (Day 1) vs Light-exposed (Day 1-35)	59.40	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-35)	40.39	<0.0001*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-35)	17.33	<0.0001*
Light-protected (Day 1) vs High TiO2-loaded (Day 1-35)	2.52	0.1172
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	1.51	0.2231
Light-protected (Day 21) vs High TiO2-loaded (Day 21)	3.67	0.0595
Light-exposed (Day 28) vs All TiO2-loaded packaging (Day 28)	80.93	<0.0001*

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with malondialdehyde concentration as response. $\alpha = 0.05$ in omega-3.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-35)	39.16	<0.0001*
Light-protected (Day 1) vs Light-exposed (Day 1-35)	1098.32	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-35)	597.05	<0.0001*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-35)	597.05	<0.0001*
Light-protected (Day 1) vs High TiO2-loaded (Day 1-35)	240.05	<0.0001*
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	0.0843	0.7724
Light-protected (Day 7) vs High TiO2-loaded (Day 7)	3.57	0.0629
High TiO2-loaded (Day 1) vs High TiO2-loaded (Day 35)	601.22	<0.0001*

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with hexanal concentration as response. $\alpha = 0.05$ in omega-3 milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-35)	0.04	0.8382
Light-protected (Day 1) vs Light-exposed (Day 1-35)	22.87	<0.0001*
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-35)	15.69	0.0004*
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-35)	1.46	0.2356
Light-protected (Day 1) vs High TiO2-loaded (Day 1-35)	0.6307	0.4325
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	0.00	0.9983
Light-protected (Day 1) vs High TiO2-loaded (Day 28)	0.2963	0.5896
High TiO2-loaded (Day 1) vs High TiO2-loaded (Day 35)	6.19	0.0178*

*pvalue <0.05 indicates statistical significant difference.

F statistic and p values for specific contrasts with pentanal concentration as response. $\alpha = 0.05$ in omega-3 milk.

Contrast	F statistic	p value
Light-protected (Day 1) vs Light-protected (Day 3-35)	0.16	0.6962
Light-protected (Day 1) vs Light-exposed (Day 1-35)	3.61	0.0656
Light-protected (Day 1) vs Low TiO2-loaded (Day 1-35)	2.83	0.1012
Light-protected (Day 1) vs Medium TiO2-loaded (Day 1-35)	1.09	0.3027
Light-protected (Day 1) vs High TiO2-loaded (Day 1-35)	0.72	0.4023
Light-protected (Day 1) vs Light-exposed and All TiO2-loaded packaging (Day1)	0.00	1.000
Light-protected (Day 1) vs High TiO2-loaded (Day 28)	2.92	0.0962
High TiO2-loaded (Day 1) vs High TiO2-loaded (Day 35)	2.92	0.0962

*pvalue <0.05 indicates statistical significant difference.