Oxygen barrier and light interference packaging properties for controlling light-induced oxidation in milk

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OXYGEN BARRIER AND LIGHT INTERFERENCE PACKAGING PROPERTIES FOR CONTROLLING LIGHT-INDUCED OXIDATION IN MILK

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SCIENTIFIC ABSTRACT

Fluorescent light exposure has well documented negative effects on fluid milk through oxidation reactions. A shift to light-emitting diode (LED) lights in retail dairy cases has occurred due to increased energy efficiency, but the effects of LED light on fluid milk are not known. The objective was to study the interaction of light protective additives (LPA) with a high oxygen barrier package under fluorescent and LED lighting conditions simulating a retail refrigerated dairy case. The extent of oxidation in 2% milk packaged in polyethylene terephthalate (PET) packages with different light interference properties (UV barrier, 2.1% titanium dioxide (TiO_2) LPA, 4.0% TiO_2 LPA, 6.6% TiO_2 LPA) under light exposure up to 72h was compared to control packages (light-exposed, light-protected). Chemical measures of oxidation included dissolved oxygen content, formation of secondary lipid oxidation products, riboflavin degradation, and volatile analysis by electronic nose. Changes in dissolved oxygen content were associated closely with oxidation changes in milk over 72h. PET with 6.6% TiO_2 was the most successful package, based on triangle test methodology, protecting milk sensory quality similar to light-protected milk through 8h LED light exposure. Based on a 9-point hedonic scale, (1=dislike extremely, 9=like extremely), consumers liked milk stored under LED light more (α =0.05; 6.59 ± 1.60) than milk stored under fluorescent light (5.87 ± 1.93). LED light is less detrimental to milk quality than fluorescent light and PET with high levels of

TiO_2 can protect milk quality for short periods of time under typical retail storage conditions.

OXYGEN BARRIER AND LIGHT INTERFERENCE PACKAGING PROPERTIES FOR CONTROLLING LIGHT-INDUCED OXIDATION IN MILK

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PUBLIC ABSTRACT

Fluorescent light exposure has well documented negative effects on fluid milk by causing oxidation reactions that change milk components, create off-flavors, and degrade vitamins. Increased use of light-emitting diode (LED) lights in retail dairy cases is occurring to save energy, but the effects of LED light on fluid milk are not known. While the most commonly used milk package, made of high density polyethylene material, does not control oxygen from reaching the product, the package material used for most soda packages, polyethylene terephthalate (PET), does limit oxygen transfer in and out. This project studied if packaging that interfered with LED and fluorescent light passing through the package, while controlling the amount of oxygen, could protect milk flavor and vitamins. Chemical measures of oxidation, such as dissolved oxygen, were taken to compare to sensory testing. Sensory testing of milk found that PET with higher light interfering additives was most successful and produced milk similar to light-protected milk through 8h LED light exposure. Changes in dissolved oxygen content were associated closely with oxidation changes in milk. Consumer testing of the most successful packages found that consumers liked milk stored under LED light more than milk stored under fluorescent light. LED light is less detrimental to milk quality than fluorescent light and PET with high levels of light interfering additives can protect milk quality through typical retail storage conditions.

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CHAPTER I

INTRODUCTION

Milk consumption in the United States has been declining steadily for the past several decades. Overall, fluid milk sales were down 5.2% in 2015 (Bauer, 2016). In 2013, per capita consumption of fluid milk fell 2.8% to only 164.6 pounds (19.22 gallons). Even though reduced-fat (2%) milk consumption grew 5.8% to 58.1 pounds per person in 2013, it had been declining for three years prior (International Dairy Foods Association, 2015). However, consumption trends for all other varieties of milk continue to decline and reach new lows. If every adult drank two 8-ounce glasses of milk daily as part of the dietary guidelines for three servings a day of low-fat or fat free dairy products, then the 2013 per capita milk consumption would have been 365 pounds (43.4 gallons; United States Census Bureau, 2015). When compared to the actual per capita consumption for 2013, this suggests that each person is drinking less than one cup of milk a day.

This decline in milk consumption is troubling for dairy producers, dairy processors, and public health officials. While the dairy industry has seen some growth in recent years through value-added dairy products such as Greek yogurt and cheese, it cannot offset the failures of the fluid milk market. Additionally, the number of licensed dairy farms in the United States has continued to decline along with fluid milk consumption. In 2014, there were 45,344 licensed dairy farms in operation with 9.3 million dairy cows that produced 206 billion pounds of milk (U.S. Dairy Industry Statistics, 2015). This marks a decline of nearly 4,000 dairy farms from 2012 to 2014. In spite of this decline in dairy farm numbers, the total U.S. milk production increased 8.8%

from 2009 to 2014 (U.S. Dairy Industry Statistics, 2015). An increase in the average herd size at most dairy farms, as well as greater efficiency results in considerably more milk being produced by each cow. This large volume of milk produced in the United States needs to be processed and marketed to consumers who are not regular milk drinkers. Fluid milk marketing must be successful to keep both dairy farmers and dairy processors in business.

Milk is an important functional food, meaning that it provides health benefits beyond basic nutrition. Milk is an excellent source of three (calcium, vitamin D, potassium) of the four nutrients that are commonly lacking in Americans' diets; milk is not a good source of iron, the fourth nutrient (Dairy Council of California, 2015). One serving of reduced-fat milk provides 29% of daily intake for calcium, 30% daily intake for vitamin D, and 10% daily intake for potassium (NDL/FNIC Food Composition Database, 2011). Consuming milk regularly is an important step to building strong bones. While other foods such as spinach are also rich in calcium, the high levels of calcium in milk are more bioavailable (30-35%) due to other the combination of nutrients in milk including vitamin D, lactose, and casein phosphopeptides (Weaver, 2001). The calcium in milk acts in synergy with other crucial nutrients, including vitamin D, protein, phosphorus, magnesium, potassium, vitamin B_{12}, and zinc, that help maintain bone health (Dairy Council of California, 2015). Low-fat and fat-free dairy products, including milk, are important components of the Dietary Approaches to Stop Hypertension (DASH) diet to help reduce the risk of serious health concerns such as heart disease and stroke (Dairy Council of California, 2015). The many nutritional benefits of milk make it an important component of a healthy diet. Consumption trends of milk need to be improved to provide these beneficial nutrients that are lacking in the diets of many consumers.

Several factors are contributing to the decline of fluid milk consumption. Growing competition from plant-based “milk” beverages, such as soymilk and almond milk, in the dairy case is leading consumers to choose other products besides milk (Food Business News, 2013). Another factor is that milk is not as readily available to consumers as other beverages. Historically, milk has a much lower availability when compared to other competitive beverages, like juice and soft drinks, in vending machines, fast food chains, restaurants, and schools (Yale Rudd Center for Food Policy & Obesity, 2013; National Dairy Council, 2015). One of the biggest factors that may be contributing to declining milk consumption is poor flavor quality of fluid milk associated with post-processing light exposure prior to purchase. Designing milk packaging to protect milk quality can control this factor.

It is well documented that light exposure has detrimental effects on fluid milk composition and current packaging options are not sufficient to protect milk quality (Cladman et al., 1998; Chapman et al., 2002; Whited et al., 2002; Mestdagh et al., 2005; Duncan and Webster, 2010; Johnson et al., 2015; Walsh et al., 2015). Exposure to light including sunlight, fluorescent lights, and light-emitting diode (LED) lights leads to an oxidized off-flavor in fluid milk. Historically, fluorescent lights have been widely used in retail case lighting. However, a recent shift to using more LED lights has occurred to address both marketing and U.S. Department of Energy (DoE)-imposed mandates on energy regulation. LED lights can transmit specific ranges of light that highlight product characteristics across different products in a retail case such as meat, dairy, fruits, vegetables, and frozen foods, thus enhancing visual impressions to the consumer (White, 2012). LED lighting also provides many energy saving features compared to fluorescent lighting systems, providing retailers with avenues for addressing DoE energy regulation requirements.

Sensory testing of milk exposed to light has identified negative descriptors such as astringent, burnt protein, butterscotch, cardboard, medicinal, metallic, or plastic-like (Alvarez, 2009; Brotherson et al., 2016). Consumers can detect this off-flavor after only a short period of light exposure (Heer et al., 1995; Chapman et al., 2002). Chapman et al. (2002) found that trained panelists could detect an oxidized off-flavor in 2% milk after only 15-30 minutes of fluorescent light exposure and untrained consumers could detect this off-flavor after 54-120 minutes. The rapid onset of this noticeable defect suggests that consumers are being subjected to milk with oxidized off-flavors as a result of retail light exposure before the product is even purchased. In an early study relating overall liking and off-flavors in milk, Heer et al. (1995) identified that hedonic scores for milk exposed to light (~2000 lux for > 3 hours) decreased by about 1 integer. Off-flavors in light-exposed milk have also been associated with negative emotional language. Two emotion terms, "disgusted" and "worried", were selected when consumers tasted light-exposed milk; these negative emotions may explain why consumers are choosing other beverages over this lower quality milk (Walsh et al., 2015).

Oxidized off-flavor in milk occurs through the breakdown of proteins, amino acids, and unsaturated fatty acids by the excitation of photosensitizers such as riboflavin and the consumption of oxygen either dissolved in milk or from packaging headspace. Photosensitizers absorb visible (380-700nm) or UV light (100-400nm) and become excited, initiating oxidation reactions in fat and protein components (Airado-Rodriguez et al., 2011). Different molecules cause more extensive oxidation reactions in different visible wavelength regions, following mechanisms that are not completely understood. For example, in addition to excitation at several UV wavelengths, riboflavin (Rb) is most active near its primary visible excitation peak of 446 nm while tetrapyrrole residues including photosensitizers such as chlorophylls are thought to create oxidation products in longer wavelengths above 500 nm (Airado-Rodriguez et al., 2011; Wold et al., 2015). Packaging additives such as colored pigments can block specific regions of light from reaching milk and prevent extensive oxidation reactions in milk components. Milk packaging needs to focus on blocking the wavelengths that activate photosensitizers and are most detrimental to quality and flavor.

Historically, milk was packaged in materials such as clear glass bottles and gable top paperboard. Neither packaging material is ideal for light protection. Glass and paperboard allow approximately 100% and 4% light transmission, respectively. Webster et al. (2009) identified that packaging with < 4% transmission was needed to effectively protect milk from light. Paperboard also allows oxidation reactions to occur and has been associated with off-flavors such as "stale" (Moyssiadi et al., 2004). While clear glass provides excellent oxygen barrier properties, it does not protect milk from light exposure. Over the past few decades, the dairy industry revolutionized their packaging and plastic packages are now widely used. However, if plastic packages are unpigmented they will not protect against light and are ineffective in maintaining high quality milk over retail storage.

Two main obstacles for all plastic packaging are light transmission and oxygen permeability (Moyssiadi et al., 2004). High-density polyethylene (HDPE) and polyethylene terephthalate (PET) are two types of plastic commonly used for fluid milk packaging. Clear plastics without any added light protective pigments do not protect milk against vitamin degradation and light-induced oxidation reactions that lead to off-flavors (Cladman et al., 1998; van Aardt et al., 2001; Chapman et al., 2002; Moyssiadi et al., 2004; Mestdagh et al., 2005). Approved pigments such as titanium dioxide (TiO_2) and carbon black can be added to both HDPE and PET packages to provide light protection. Other approved pigments such as green and amber have been added to PET packages, although consumers have shown a strong preference for white packages for fluid milk packaging (Cladman et al., 1998). The addition of light-blocking pigments and UV barriers to HDPE and PET packages slows both the rate of vitamin degradation (Vitamin A and B_2 [Rb]) and lipid oxidation (Cladman et al., 1998; Moyssiadi et al., 2004; Webster et al., 2009). Amber PET and UV barrier PET packages were both able to prevent oxidized off-flavors in whole (3.25% fat) milk more than clear PET or translucent HDPE (van Aardt et al., 2001). However, it was not sufficient to completely prevent these reactions and maintain acceptable milk quality over extended storage durations.

Oxygen, which can contribute to oxidation reactions, occurs in fluid milk from permeation through the packaging material, headspace oxygen, and dissolved oxygen within the product itself. Glass serves as the standard for oxygen barrier packaging. The thickness of packaging materials is an important aspect to controlling oxygen permeation. Moyssiadi et al. (2004) found that a thickness of 300-600µm in plastic packages prevented oxygen permeation from significantly affecting milk quality. Filling the package headspace with gases such as argon and nitrogen can control headspace oxygen. Doing so changed the composition of secondary oxidation products in milk such as hexanal and pentanal (Wold et al., 2015). The importance of headspace oxygen in relation to oxidation reactions in milk is not verified. It is also unknown how small amounts of dissolved oxygen within the product itself contribute to oxidation reactions occurring as a result of light exposure. Thus, even though a plastic such as PET has excellent oxygen barrier properties, unless it is pigmented it does not reduce or prevent light-induced oxidation reactions (Cladman et al., 1998; Moyssiadi et al., 2004; Zygoura et al., 2004). To prevent decreasing milk quality, combinations of pigmented plastics and oxygen barrier properties need to be more thoroughly studied to identify the optimal container for maintaining high quality fluid milk.

1.1 Objectives & Hypotheses

Objective 1: Determine the effectiveness of pigment combinations for packaging to protect and maintain acceptable fluid milk quality for up to 72 h storage at 4°C under continuous retail case lighting.

> H_o: There will be no difference in milk quality from packaging of unpigmented PET, foil wrapped PET, unpigmented PET with UV absorber, or PET loaded with three levels (low; 2.1%, medium; 4.0%, high; 6.6%) of titanium dioxide (TiO_2), as measured by sensory evaluation, Rb degradation, secondary oxidation products, electronic nose volatile analysis, and microbial standard plate count throughout 72 h storage at 4°C under continuous retail case lighting.

H_a: There will be a difference in milk quality from unpigmented PET, foil wrapped PET, unpigmented PET with UV absorber, or PET loaded with three levels (low; 2.1%, medium; 4.0%, high; 6.6%) of titanium dioxide (TiO_2), as measured by sensory evaluation, Rb degradation, secondary oxidation products, electronic nose volatile analysis, and microbial standard plate count throughout 72 h storage at 4°C under continuous retail case lighting.

Objective 2: Investigate a new quality control method for evaluating milk quality using electronic nose technology that will detect light-induced volatiles and off-flavors in fluid milk within 4 h of light exposure.

H_o: The electronic nose will not be able to detect light-induced volatile compounds in fluid milk within 4 h of light exposure.

H_a: The electronic nose will be able to detect light-induced volatile compounds in fluid milk within 4 h of light exposure by differentiating between fresh milk and milk after retail storage.

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CHAPTER II

LITERATURE REVIEW

Milk quality is influenced by light exposure, storage conditions, and packaging materials. High flavor quality milk is described as having a bland and pleasantly sweet taste (Alvarez, 2009). Milk oxidation results from oxygen permeation through packaging and light exposure from wavelengths not blocked by the packaging, leading to a rapid development of off-flavors. These off-flavors have been described as astringent, bitter, butterscotch, cardboard, chemical oily, plastic, or stale (Alvarez, 2009; Brotherson et al., 2016). There are many different mechanisms behind the development of oxidized off-flavors that occur in different components of milk, such as fat and protein. Once the mechanisms of oxidized off-flavors are better understood, packaging materials can be developed to specifically block transmission of the most reactive light regions and protect the most photosensitive components of milk. Packaging needs to be developed to maintain product integrity while meeting consumer needs for an attractive, white package.

2.1 Light Exposure

Photosensitizers in both the visible light spectrum (380-770nm) and the UV spectrum (100-400nm) become excited through light exposure and initiate oxidation reactions that negatively affect milk quality (Ryer, 1997). Light exposure and light intensity can be quantified through different measurements. The three main terms used to measure light intensity are based on lumens. Lumen is the basic unit used to measure luminous flux, which is the total output of a light (Boyce, 2003). Luminous flux measures the electromagnetic radiation emitted by a source that is multiplied by the relative spectral sensitivity of the human visual system in the visible light spectrum (Boyce, 2003). Lux is a measure of lumens per square meter, and foot-candles are a measure of lumens per square foot (Ryer, 1997).

Most oxidation reactions in milk begin when the product is exposed to natural or artificial light. Both fluorescent and light-emitting diode (LED) artificial lights in retail dairy cases emit wavelengths in the visible light spectrum that excite photosensitizers and initiate reactions that degrade milk quality. Light exposure is expressed by absorbance and transmittance. Absorbance refers to the wavelengths of light that are absorbed by the packaging material and do not reach the product inside. Transmittance measures the wavelengths that pass through the packaging material and reach the product.

Most milk oxidation literature to date has only studied milk exposed to fluorescent light under retail conditions. There is a critical need to further investigate oxidation reactions in milk stored under LED light because the switch to LED lights in retail stores is a rapidly growing trend. Many retail stores are retrofitting freezer and refrigerator cases with LED lights. When compared to traditional fluorescent tubes, LED lights can reduce energy usage by ≥60% (Peters, 2012). LED lights have a much longer lifespan (up to 5 years) than fluorescent lights, which typically last 6-12 months before replacements are needed. LED lights can be designed to provide directional illumination of products in the retail case and can make foods and packaging look more appealing to consumers (Peters, 2012). However, LED lights transmit a different light spectrum than fluorescent lights (Figure 1), so it is unknown if the same level of light-induced oxidation will occur in milk under LED retail lighting. The light spectrums in Figure 1 cannot be compared directly to each other, but provide a better picture of the relative intensity of visible light spectrum contained in each light type.

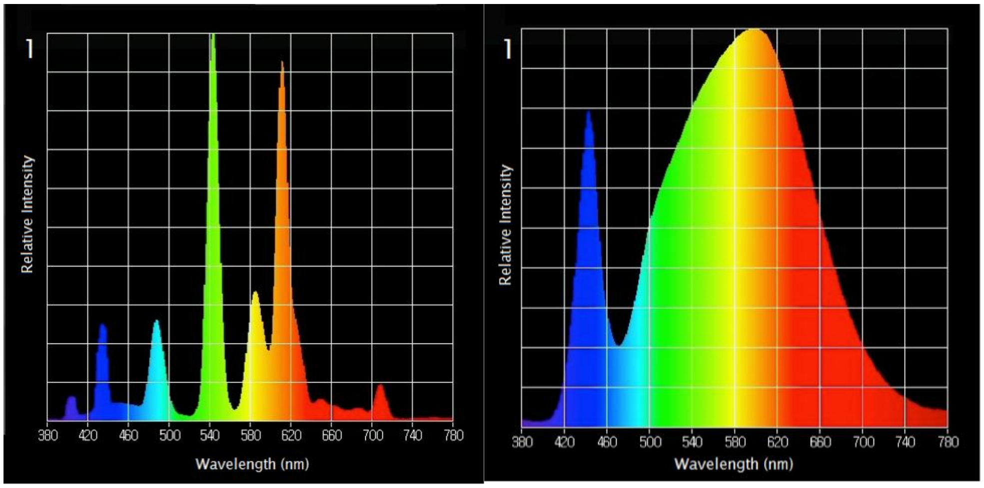

Figure 1. Relative intensity of light wavelengths in the visible light spectrum of fluorescent (left) and LED 3500K lights (right). *Image produced by Amin, K. 2016.*

Different regions of the visible light spectrum have been associated with different photosensitizers in milk. Light from the blue region (400-525 nm) contains wavelengths that are absorbed strongly by riboflavin (Rb; Vitamin B_2), which is a photosensitizer found in relatively high quantities (1.69 mg/L) in milk (Gebhardt and Thomas, 2002). These Rb peaks include 400, 446, and 570 nm (Webster et al., 2009). Both fluorescent and LED light spectrums contain transmission peaks in the blue region, which are important in studying milk's vitamin quality, especially with Rb. Light in the orange region (550-650nm) has also been shown to affect other photosensitive molecules in milk and butter such as chlorophyll, protoporphyrin, and tetrapyrroles (Wold et al., 2006; Airado-Rodriguez et al., 2011). Both light spectrums transmit relatively high amounts of orange light compared with other types of visible light. This relatively high intensity of orange light transmitted could create significant changes to photosensitizers in milk that absorb light in the orange spectrum. Even with different spectrums, any light exposure has the potential to excite photosensitizers and lead to oxidation reactions that degrade milk quality.

2.2 Oxidation Mechanisms

Oxidized off-flavors in milk occur through the breakdown of proteins, amino acids, and unsaturated fatty acids by the action of photosensitizers and oxygen. Exposure to light catalyzes the breakdown of amino acids and lipids by excited photosensitizers (Alvarez, 2009). Protein and lipid oxidation can occur simultaneously and compete for the energy of excited photosensitizers. The extent of protein oxidation under UV or fluorescent light can be detected by the formation of protein carbonyls and dityrosines as well as by measuring the molecular weight of proteins to detect polymerization and proteolysis (Scheidegger et al., 2010). Protein carbonyls have been associated with oxidized off-flavors in milk (Karagul-Yuceer et al., 2004). Photosensitizers in milk absorb light and transmit the required energy to initiate oxidation reactions in both lipid and protein components.

Photosensitizers absorb light and transmit light energy to oxygen, which becomes elevated from a stable ground state to excited sensitizers. Excited triplet sensitizers formed from stable triplet oxygen can react directly with other components in milk such as proteins or unsaturated fats and leads to formation of free radicals and volatile compounds (Wold et al., 2002; Choe and Min, 2006). These direct reactions of excited triplet sensitizers with food components are known as Type I pathways and are favored by readily oxidizable compounds, such as unsaturated fatty acids (Min and Boff, 2002). Type II pathways occur when excited triplet sensitizers transfer energy to create singlet oxygen sensitizers from stable oxygen that undergo oxidation reactions. However, water based food systems, such as milk, favor Type I pathways because of the reduced availability of oxygen, so these the Type I pathway will be the focus of our work with milk oxidation (Min and Boff, 2002).

Certain photosensitizers are able to better absorb different light wavelengths in the visible light spectrum. Specifically, light from 365-500 nm leads to the degradation of light sensitive vitamins, including riboflavin, and excites this photosensitizer to initiate reactions that create oxidized off-flavors in milk (Cladman et al., 1998; van Aardt et al., 2001; Moyssiadi et al., 2004; Webster et al., 2009). Although this light region is commonly implicated in milk oxidation, growing evidence suggests that wavelengths above 500 nm also influence milk flavor by the action of tetrapyrroles as photosensitizers (Wold et al., 2005; Airado-Rodriguez et al., 2011; Wold et al., 2015).

2.2.1 Riboflavin as a Photosensitizer

When testing absorbance, milk has a broad peak in its light absorption spectrum in the blue region from 400-525nm due to riboflavin (Rb) and beta-carotene, two prominent light absorbing milk components (Airado-Rodriguez et al., 2011; Wold et al., 2015). When Rb absorbs light, it transfers the energy from light to oxygen, converting stable triplet oxygen to excited singlet oxygen (Min and Boff, 2002). Singlet oxygen forms from the excitation of triplet oxygen by a sensitizer and light, and is highly reactive in food components (Choe and Min, 2006). The structure of Rb allows itself to react well with singlet oxygen to result in oxidation reactions, which produce compounds associated with a “sunlight-oxidized” off-flavor in milk (Min and Boff, 2002; Choe and Min, 2006). Beta-carotene is not a photosensitizer so it does not contribute to oxidation reactions causing off-flavors. In addition, beta-carotene has even been proposed to help protect against oxidation reactions since it competitively absorbs light in the same spectrum as Rb. Beta-carotene also acts as an antioxidant by scavenging free radicals and quenching singlet oxygen, reducing the amount of available reactive oxygen species (ROS) in milk (Min and Boff, 2002; Airado-Rodriguez et al., 2011).

It has long been thought that Rb is the main photosensitizer in milk (Edwards and Silva, 2001; Min and Boff, 2002; Wold et al., 2005; Lee and Min, 2009; Airado-Rodriguez et al., 2011; Wold et al., 2015). The degradation of Rb through oxidation reactions and its role as a photosensitizer are affected by light intensity, wavelengths, exposure time, and packaging materials (Choe and Min, 2006). Rb has three excitation peaks at 400nm, 446nm, and 570nm, absorbing light most strongly at 446nm (Webster et al., 2009). It is crucial that these three wavelengths are completely blocked to protect Rb and avoid excitation of this molecule. Even when transmission of these specific wavelengths is reduced to <4% light transmission, oxidized off-flavor still occurred at significant levels in milk (Webster et al., 2009). Webster et al. (2009) identified the 400nm wavelength as particularly important to block to protect Rb. Exposure to 400nm caused higher levels of oxidation than exposure to 446nm in 2% milk. There are still literature discrepancies as to which Rb excitation wavelength is more excitatory and causes higher levels of oxidation. Rb has been traditionally implicated as the main reactive molecule initiating photooxidation in milk, but recent research suggests other photo-responsive molecules may also contribute significantly to light-induced off-flavors in milk. The action of Rb mainly affects the protein component of milk due to a water-soluble nature. Other photooxidizers such as chlorophyll or other tetrapyrroles affect the fat component of milk.

2.2.2 Tetrapyrroles as Photosensitizers

Fat-soluble compounds, including residues of tetrapyrroles, have been suggested to contribute to oxidation reactions in regions of longer light wavelengths above 500nm (Airado-Rodriguez et al., 2011). This class of compounds includes chlorophyll a and b, hematoporphyrin, and protoporphyrin, which have all been found to act as sensitizers in photooxidation reactions in cheese and butter and create off-flavors (Wold et al., 2005; Wold et al., 2006). Photosensitizers including protoporphyrin, hematoporphyrin, chlorophyll a, and other tetrapyrroles have been well associated with detectable sensory differences from oxidation in dairy products (Wold et al., 2006). Chlorophyllic degradation has also been well associated with light-induced sensory off-flavors (Intawiwat et al., 2013). The tetrapyrrole chlorophyll acts as a photosensitizer, similar to Rb, and can excite ground state oxygen to form a singlet oxygen sensitizer for oxidation reactions (Choe and Min, 2006). The main difference between tetrapyrroles and Rb is the light wavelengths that they absorb, with tetrapyrroles primarily absorbing light in the orange spectrum (>500nm) and Rb primarily absorbing light in the blue spectrum (400-500 nm). However, the concentration of tetrapyrroles in dairy products varies with fat content. Tetrapyrroles are present in high fat dairy products such as cheese and butter (80% milkfat) at higher concentrations; they are found in full-fat (3.25% milkfat) milk at extremely low concentrations (0.8ppm; Wold et al., 2015). The concentration of tetrapyrroles would be so small in reduced-fat (2%) milk that it is believed these residues would not contribute significantly to oxidation. However, tetrapyrroles are still a possible source of oxidation off-flavors caused by light exposure above 500nm or between 370-

390nm when the Rb absorption spectrum (400-500nm) is being completely blocked (Webster et al., 2009).

Photooxidation reactions occur through Type I or Type II pathways by the competing action of Type I sensitizers (Rb) or Type II sensitizers (chlorophyll) (Wold et al., 2015). Type I sensitizers are mostly dominant in fluid milk because they are most successful in low levels of oxygen. The limited packaging headspace of fluid milk packages provides this low level of oxygen favored by Type I sensitizers (Wold et al., 2015). Competition from Type I sensitizers and a relative lack of longer wavelengths of light absorbed by milk lead to less excitation activity from chlorophyll and other tetrapyrroles in milk. This is the main reason that Rb has long been thought to be the main photosensitizer in milk. However, the type of pathway can change during the course of a reaction due to changing oxygen availability and the concentrations of various components, so it is possible that both pathways are occurring in milk oxidation reactions (Min and Boff, 2002).

A study by Airado-Rodriguez et al. (2011) looked at oxidation reactions in milk due to light absorbed specifically from the orange region of the visible spectrum (wavelengths > 550 nm) and found that the longer wavelengths induced more oxidized off-flavors than light from the blue region of the visible light spectrum (wavelengths < 420nm). Rb and beta-carotene cannot be excited by the orange light spectrum, except for limited excitation of Rb at its 570nm absorption peak, indicating that chlorophyll and other tetrapyrroles excited by wavelengths >550nm are mainly responsible for the development of oxidized off-flavors in milk exposed to this light region. These results suggest that chlorophyll, not Rb, is the main photosensitizer responsible for oxidation in milk when exposed to a light source, fluorescent or LED, with wavelengths longer than 550nm (Airado-Rodriguez et al., 2011). The action of different photosensitizers in milk must be distinguished and quantified to determine which parts of the light spectrum are most important to initiate oxidation in milk.

2.3 Volatile Compounds in Light-Exposed Milk

Volatile secondary oxidation products produced by photosensitizers create off-flavors in milk that are unacceptable to consumers. The formation of volatile compounds, including hexanal, pentanal, and heptanal are directly associated with fat content. Higher levels of fat produce more volatile compounds and more intense odor (Alvarez, 2009; Lee and Min, 2009). Dimethyl disulfide is another volatile compound oxidized from sulfur-containing compounds such as methionine, which come from milk proteins. Rb has been proposed to act as the photosensitizer that reacts with methionine most strongly (Min and Boff, 2002). The production of dimethyl disulfide is not significantly influenced by fat content (Jung et al., 1998; Lee and Min, 2009). Hexanal is the first volatile compound to be produced upon light exposure, with both hexanal and pentanal showing up in measurable quantities after 2 h of fluorescent light exposure. Heptanal and dimethyl disulfide are observed after 4 h and 8 h of fluorescent light exposure, respectively (Lee and Min, 2009). Concentrations of these volatile compounds in oxidized milk increased with higher levels of Rb in milk, because Rb acts as the photosensitizer to activate oxidation and begin the oxidation reactions that produce these compounds (Lee and Min, 2009). The production of hexanal is associated with light exposure in the blue region (300-580 nm), which indicates that it is the result of significant Rb sensitized oxidation reactions (Wold et al., 2015). The majority of literature indicates that light in blue region is extremely detrimental to milk quality because it activates Rb to excite stable oxygen. A complete light block package (control) has been shown to be most effective at maintaining milk quality and preventing off-flavors (Moyssiadi et al., 2004; Mestdagh et al., 2005; Webster et al., 2009). However, with current packaging technology, blocking all light wavelengths at 0% transmission is not economically feasible on a mass scale.

Most packaging does very little to protect the product against light exposure. A few packages add light protective pigments that limit light exposure between 400-500 nm but this is not completely effective in protecting milk quality. Milk packaging needs to focus on protecting the product from specific light regions during retail light exposure that excite photosensitizers, leading to the creation of secondary oxidation products and the formation of undesirable off-flavors. Since current packaging technology does not completely prevent light exposure and allows oxidation reactions in milk, it is important to measure the different volatile compounds produced and fully understand the off-flavors that are formed from milk oxidation. Analytical assays such thiobarbituric acid reactive substances (TBARS) assay measuring secondary lipid oxidation products and fluorometric assay measuring riboflavin degradation have previously been used to quantify the extent of oxidation in fluid milk and other dairy products (Mestdagh et al., 2005; Wold et al., 2005; Johnson et al., 2015; Walsh et al., 2015). These measures have sufficiently evaluated oxidation reactions in milk stored under fluorescent light, but it is unclear if they will be able to measure light-induced oxidation in milk stored under LED light.

2.3.1 Electronic Nose Technology for detecting Volatile Compounds

There is a growing need for rapid analytical methods to detect quality issues in the dairy industry. Trained sensory panels can be used to identify off-flavors and quality defects but are not always feasible because of time, resources, and budget requirements. The use of analytical methods such as gas chromatography-mass spectroscopy (GC-MS) are very effective at identifying volatile compounds related to quality issues; however, are also time-consuming and expensive to perform. New technologies, such as the electronic nose, are being applied as a rapid and economic way to detect quality issues in fluid milk through measurement of volatile compounds.

Milk contains a large number of volatiles in its headspace including acetone, hexanal, 2-butanone, toluene, limonene, heptanal, styrene, and chloroform in varying concentrations (Ampuero et al., 2003; Loutfi et al., 2015). Some of these volatiles are present in fresh milk naturally, but a significant change in the volatile profile can indicate quality defects such as production of off-flavor. Increases in the volatiles pentanal and hexanal were seen in fluid milk exposed to fluorescent retail lighting (Marsili, 1999). These two compounds are detectable by an electronic nose and could be measurable thresholds to detect oxidized off-flavors.

Electronic noses cannot be used to quantify volatile compounds, but are intended to serve as a rapid “accept/reject” or “good/bad” indicator of quality after proper training (Sensigent, 2013). Electronic noses incorporate a variety of materials and number of gas sensors with high sensitivity to read different types of volatile compounds (Ampuero et al., 2003; Ai et al., 2015; Loutfi et al., 2015). Depending on the model, an electronic nose can have from 2-32 sensors (Loutfi et al., 2015). Most electronic nose models incorporate either metal oxide semiconductor (MOS) sensors or conducting polymers. This project uses a Cyranose® 320 (Sensigent LLC, Baldwin Park, CA) that has 32 conducting polymer sensors.

Electronic nose technology has an advantage over sensory panels in that it does not require trained personnel, it takes relatively less time, and can be used on a larger number of samples because it is not fatigued as easily as the human nose (Ampuero et al., 2003). However, many factors must be controlled for electronic noses to operate correctly. Analysis must be completed immediately after sample collection and other factors such as temperature, humidity, and pH value can influence the accuracy of measurements if they are not carefully monitored and controlled (Ampuero et al., 2003). The absorption and desorption time of the electronic nose sample draw is also important to standardize to ensure that the sensors are fully saturated and able to identify changes in volatile composition. When determining a sampling plan, it is important to get at least a 6:1 ratio of data points to variables to avoid random noise and classification errors in electronic nose measurements (Goodner et al., 2001). Electronic nose data is analyzed through multivariate methods using either Euclidian distances or Mahalanobis distances (Ampuero et al., 2003; Sensigent, 2013).

Due to such a complex volatile profile, milk can be more challenging to analyze through an electronic nose than other foods with less complex profiles (Loutfi et al., 2015). Electronic noses have been applied to dairy products for a variety of purposes (Capone et al., 2001; Magan et al., 2001). An electronic nose was used to compare volatile compounds in raw skim milk and raw whole milk and was as effective at measuring volatiles as GC-MS (Ai et al., 2015). The shelf life of milk over 17 d was monitored by an electronic nose and compared to bacterial growth changes over the same time period. The electronic nose served as a sufficient comparison to determine the age and level of bacterial growth in milk over storage periods (Labreche et al., 2005). An electronic nose was also applied to determine rancidity and spoilage of milk over its storage period (Capone et al., 2001; Magan et al., 2001; Korel and Balaban, 2002). High quality milk was compared to milk inoculated with yeast or bacterial strains to determine the threshold of bacterial growth that the electronic nose could differentiate spoiled milk from unspoiled milk (Magan et al., 2001). All of these studies laid the groundwork for using an electronic nose as a quality check tool in the dairy industry.

Unfortunately, there are many discrepancies on electronic nose methods in literature including sample holding temperature, absorption and desorption draw times, and headspace volume for each sample. The use of different types of sensors in different electronic noses also makes direct comparison of methods and results across literature somewhat difficult. This novel technology requires further research to fully understand its capabilities and apply them most accurately to dairy products. When applied correctly, an electronic nose could be a valuable tool to rapidly identify increased levels of off-flavors in fluid milk that result from light exposure through insufficient packaging protection.

2.4 Packaging Materials

High-density polyethylene (HDPE) and polyethylene terephthalate (PET) are two types of plastics that are widely used for fluid milk packaging. PET has been gaining in popularity in recent years, as it is being more widely used for all beverage packaging over HDPE and other options such as glass and aluminum (PolyOne, 2015). HDPE and PET plastics transmit between 62-85% of light wavelengths in the visible light spectrum (Webster et al., 2009). HDPE transmits between 57-60% of light in light spectrum between 300-700 nm (van Aardt et al., 2001). Polyethylene transmits wavelengths throughout the UV and visible light spectrum, while PET absorbs UV radiation below 300 nm, preventing it from reaching the product within the package (Coltro et al., 2003). When used alone, these plastics do not provide sufficient light blocking properties to protect milk quality or flavor. When compared to pigmented packages, clear PET and HDPE packages were the least effective at preventing off-flavors and retaining vitamin content (Cladman et al., 1998; Zygoura et al., 2004; Mestdagh et al., 2005; Saffert et al., 2006).

It has been found that reducing light transmission to only 1-3% in the 400-500nm region sufficiently protects milk against developing oxidized off-flavor (Webster et al., 2009). Having a transmission of 1-3% is lower than the International Dairy Federation's light transmittance guidelines for packaging materials (Mestdagh et al., 2005). Many light protective additives (LPAs) have been tested in milk packaging to determine the effectiveness in blocking specific spectrums of light to prevent off-flavors, but most do not completely block light transmission or even reduce it to 1-3% transmission.

2.4.1 Light Protective Pigment Additives in Packaging

Titanium dioxide (TiO_2) is a white pigment that can be added to both HDPE and PET plastics to create opaque white containers with light blocking properties (Moyssiadi et al., 2004; Robertson et al., 2006; DuPont, 2007; Johnson et al., 2015). TiO_2 is a photo-responsive material and its particle size can be altered to scatter visible light and affect which light wavelengths will be transmitted through a packaging material (DuPont, 2007). TiO_2 can be formed as anatase or rutile particles. Rutile TiO_2 particles have preferable qualities to anatase particles, offering greater stability, durability, and greater ability to scatter light efficiently. The theoretical optimum size of TiO_2 particles is between 0.2-0.3 microns in diameter because smaller particles have greater light diffraction properties than larger particles (DuPont, 2007). TiO_2 has been used to create varying levels of opacity and effectively blocks light transmission between 400-500nm (Moyssiadi et al., 2004).

HDPE plastics can be formed as either monolayer or multilayer pigmented materials. Multilayer packages will typically consist of two TiO_2 layers with a layer of carbon black pigment in the middle (Moyssiadi et al., 2004; Zygoura et al., 2004). However, both types of pigmentation still result in a 28-33% loss of Rb after 7 d of storage under retail light (Moyssiadi et al., 2004). Zygoura et al. (2004) found 18.4% and 20.6% Rb loss in monolayer and multilayer TiO_2 pigmented HDPE respectively after 7 d of retail storage. This study also saw a 47.1% Rb loss in clear PET packages but only 30.9% Rb loss in PET packages pigmented with 2% TiO_2 (Zygoura et al., 2004). Saffert et al. (2006) saw a 33% decrease in Rb in whole milk packaged in clear PET packages after 10 d non-continuous fluorescent light exposure. It is evident that all TiO_2 pigmented packages provided better protection of Rb than clear packages since TiO_2, in particular, blocks wavelengths in the Rb absorption spectrum (Moyssiadi et al., 2004). When light transmission in the 400-500 nm range was reduced to only 10-15% by adding higher levels of white pigments to PET packages, a higher retention of Rb was seen in whole milk stored under fluorescent light for 10 d than in clear PET packages (Saffert et al., 2006). PET packages pigmented with TiO_2 also helped prevent lipid oxidation reactions in milk better than clear PET packages (Zygoura et al., 2004). However, TiO_2 pigmented packages do not completely prevent the development of light-induced off-flavors in milk because they cannot completely block all light wavelengths, prompting research into other pigmented plastics to best protect quality.

Other colored pigments such as green, yellow, and amber have been added to PET and HDPE packages for milk packaging. Green PET packages, which prevent most light transmission below 500nm, are more effective at preventing lipid oxidation in 2% and whole milk than clear PET packages over an 18 d storage period (Cladman et al., 1998). Yellow pigments block most light wavelengths between 400-500 nm, protecting against Rb degradation (Cladman et al., 1998; PolyOne, 2015). Amber PET packages completely block wavelengths below 450nm and partially blocks longer wavelengths between 450-700nm, but does allow some light transmission in the visible spectrum. Amber packages are more effective in protecting milk quality than clear glass, clear HDPE, or clear PET with a UV blocker (van Aardt et al., 2001). When compared to clear HDPE packages, amber PET packages also have lower increases in hexanal and dimethyl disulfide volatile compounds that contribute to oxidation off-flavor (van Aardt et al., 2001). The most ideal pigment, carbon black, blocks all light wavelengths. A triple layer combining TiO_2-carbon black-TiO_2 pigment layering in HDPE and PET packages has been tested for fluid milk packaging. It has been shown to maintain greater milk quality under continuous light exposure compared to other pigmented packages (Fonterra, 2013). The level of Rb was maintained for an extended period, with only minor decreases towards the end of a 60 d period when combinations of TiO_2 and carbon black were used in PET packaging (Mestdagh et al., 2005). However, one drawback to all colored pigment additives is that colored packages are unappealing to consumers, who prefer more attractive packaging and want white packages for milk packaging (Doyle, 2004). Pigment additions to plastic also increase the cost of packaging, by a few cents per package, which can result in increased cost to the consumer. Other barriers to pigment additions in plastic packaging include more complex recycling procedures and subsequent environmental concerns from improperly disposed plastic packages. Despite these challenges, pigments that provide light interference properties are the most effective barrier to light-induced oxidation in milk. Pigment combinations can also be supplemented with other protective features of plastic packaging, such as improved oxygen barriers to reduce the amount of atmospheric oxygen that can reach milk.

2.4.2 The Effect of Oxygen Exposure through Packaging

Apart from light exposure, the presence of oxygen is the biggest contributing factor to the formation of oxidized off-flavors in milk. PET packages are more effective at preventing oxygen-induced oxidation reactions than HDPE packages (Cladman et al., 1998; van Aardt et al., 2001). Under refrigeration conditions (4°C) PET packages have significantly lower oxygen transmission rates (OTR) than HDPE packages (van Aardt et al., 2001). The average PET OTR falls between 0.2-0.4 cm^3/(pack day atm) and HDPE OTR is 4 cm^3/(pack day atm) when measured at 23°C 50% RH (Lange and Wyser, 2003). Clear PET packages and PET packages with a UV barrier have similar oxygen transmission rates (0.85 ± 0.11 and 0.77 ± 0.10 mL/package day atm, respectively; Papachristou et al., 2006). Oxygen-binding inner layers can be added to plastic packaging materials to help remove reactive oxygen species. However, these have not been found to significantly prevent the degradation of Rb nor improve milk quality over storage under retail lights without additional LPAs (Mestdagh et al., 2005). Differences in oxygen permeability between HDPE, PET, and paperboard containers did not significantly influence the rate of lipid oxidation in whole milk over 7 d of storage (Zygoura et al., 2004). More significant differences in milk oxidation were seen from increasing the thickness of the package (from 350μm to 600μm), which helps prevent oxygen permeation through the packaging material more than the addition of an oxygen-binding inner layer (Zygoura et al., 2004). It would still be more effective to use PET packaging for fluid milk than HDPE packaging because it has lower oxygen transmission rates.

2.4.3 UV Barrier Properties for Effective Milk Packaging

PET packaging alone prevents at least 95% UV radiation, but it has been shown that without an added UV barrier, the remaining UV radiation transmitting through PET packaging can still create significant oxidation off-flavors in fluid milk (Cladman et al., 1998; Mestdagh et al., 2005). Clear PET blocks UV radiation below 300-315 nm, but allows light transmission in the UVA region (315-400nm; PolyOne, 2015). The addition of a clear UV barrier to plastic packaging blocks all wavelengths below 400nm (Coltro et al., 2003; Mestdagh et al., 2005). The addition of a UV barrier at concentrations as low as 0.080% created a 90% reduction in light transmission through PET when measured at 360nm (Coltro et al., 2003). Benzophenones and benzotriazoles are two additives commonly used as UV barriers in food packaging (Coltro et al., 2003). However, adding UV barriers to packaging by itself is insufficient in protecting optimum milk quality over periods of retail light exposure. The addition of a UV barrier to transparent PET plastic did not completely prevent the degradation of Rb in milk over continuous light storage because it did not block its absorption peaks between 430-460nm which allowed Rb to act as a photosensitizer and initiate oxidation reactions (Mestdagh et al., 2005).

UV barriers do provide additional synergistic effects to other light protecting additives that help maintain higher quality milk. Webster et al. (2009) showed that light exposure from 370-390 nm might excite photosensitizers other than Rb, which cause oxidation reactions. The action of photosensitizers in this region could be prevented by the addition of a UV barrier. This barrier could also help protect against Rb excitation at its 400 nm peak, which has been shown to be significant in causing light oxidized flavor (Webster et al., 2009). Also, when comparing plastics, milk packaged in clear PET packages with an added UV barrier had lower levels of oxidation after 7 d of light exposure than HDPE packages or clear PET packages without a UV barrier (van Aardt et al., 2001). The UV barrier provided significantly greater protection against oxidation in milk than unpigmented HDPE packages over 18 d of fluorescent light exposure (van Aardt et al., 2001). A UV barrier added to PET also helped prevent increased levels of lipid oxidation more than an oxygen-binding inner layer to PET over extended storage periods (Mestdagh et al., 2005). PET with a UV barrier protected milk quality against light exposure better than clear PET but not as effectively as pigmented PET (Cladman et al., 1998). The addition of a UV barrier is helpful, but does not control oxidation reactions in milk to sufficiently protect milk flavor.

2.4.4 Packaging Interactions with Food

Interactions occur between food and the food contact surfaces of packaging materials that can affect food quality over its shelf life. The two main types of interactions that occur between food and packaging materials are scalping (sorption) and migration. Sorption, more commonly referred to as scalping, is the transfer of components from the food product into the package. Food components that can be scalped include flavor components, fats, pigments, and organic acids (Kadam et al., 2015). When food components are scalped by packaging materials, they will no longer contribute to flavor or aroma of the food product. Migration is the transfer of components such as ingredients or additives from packaging materials to the food product (Kadam et al., 2015). Scalping and migration can lead to undesirable changes in the food, including off flavors or off odors, that are noticeable to consumers and possibly even have deleterious health effects.

The interactions of plastic packaging materials, including HDPE and PET, with food has been previously studied (Linssen et al., 1991; Caner et al., 2004; Kaplan, 2005; Khaneghah et al., 2015). These interactions can be affected by the properties of food compounds, properties of the packaging material, and storage temperature. Higher processing and storage temperatures have been shown to increase the sorption process of food compounds into packaging materials (Caner et al., 2004; Khaneghah et al., 2015). The amount of unsaturated fatty acids and degree of fatty acid unsaturation influences food and packaging interactions of edible oils stored in PET bottles. Khaneghah et al. (2015) found that oils with higher levels of polyunsaturated fatty acids (linoleic acid) underwent more extensive scalping with PET packaging than oils with lower levels of polyunsaturated fatty acids.

When dairy products (drinkable yogurts) were packaged in HDPE bottles, some scalping of flavor compounds occurred. Compounds with short carbon chains and sulfides remained in the yogurt, while compounds with longer carbon chains or branched compounds were scalped by the packaging material and did not contribute to the flavor of the food product (Linssen et al., 1991). PET can also cause scalping of certain compounds from food components, including D-limonene, into the packaging material (Caner et al., 2004). The polarity of food compounds will affect the interactions with packaging materials, with more polar aroma compounds undergoing greater scalping than less polar aroma compounds when in contact with PET packaging materials (Hernandez-Munoz et al., 2001). Despite possible interactions between plastic packaging materials and food components, plastic is still a viable option for fluid milk because of its benefits with cost, numerous packaging options, and protective influences with new light-blocking and barrier technology.

It is advantageous to combine oxygen barrier packaging characteristics with LPAs to create packaging that can sufficiently protect milk quality over typical retail storage periods. Proper packaging thickness can block oxygen permeation and carbon black pigments can prevent light permeation from detrimental wavelengths (Moyssiadi et al., 2004). The addition of a UV barrier to PET packaging blocks all wavelength transmission from 100-400nm, which can prevent excitation of photosensitizers or tetrapyrroles in that region. When UV barriers are combined with other light-blocking pigments, they can block additional regions of light that could be detrimental to milk quality (PolyOne, 2015). The more light wavelengths that are blocked, the less energy is provided to photosensitizers in milk, limiting excitation, and the subsequent molecular activation and initiation of oxidation reactions that reduce vitamin, lipid and protein quality while creating undesirable off-flavors in fluid milk. Combining a UV barrier with LPAs in a PET package could maintain more optimal milk quality over storage periods typical for consumers. TiO_2 is an optimal LPA to use, since it is able to block light exposure at higher concentrations while providing the attractive white package consumers expect for fluid milk. Unfortunately, increasing the number of additives (UV barrier, oxygen binding layers, LPAs) into milk packaging increases the cost of packaging. Even with an increased cost to the producer or processor, the added value in meeting consumer needs, which includes protective packaging to maintain freshness of product, can increase trust and desire for the product with an anticipated demand for the product (Doyle, 2004). The interaction of oxygen barrier properties in PET packaging with specific LPAs is an important consideration in preventing oxidation reactions in fluid milk that has not yet been studied.

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CHAPTER III

COMPARING EFFECTIVENESS OF HDPE PACKAGING FOR PROTECTING MILK QUALITY IN THREE STYLES OF RETAIL REFRIGERATED CASES USING LED LIGHTING

ABSTRACT

Light-emitting diode (LED) lights are a growing trend in retail refrigeration, but the effects of LED lights on light-induced oxidation in milk have not been determined. Three LED-lit commercial refrigeration cases (walk-in case, 5405 ± 836.0 lux; closed-door case, 1021 ± 17.0 lux; open-front case, 914 ± 54.4 lux) were studied to determine if light exposure (up to 8 h) caused changes in oxidation in 2% milk packaged in high-density polyethylene (HDPE) packages (light-protected, light-exposed, commercial yellow, low white (1.3%) titanium dioxide [TiO_2], high white (4.9%) TiO_2). After 8 h LED light exposure, experienced panelists described light-protected milk as overwhelmingly bland and sweet, but identified oxidized descriptors (cardboard/paper, metallic, oily) with milk packaged in HDPE packages with yellow, TiO_2, or no light protective additives (LPAs). There were higher levels of secondary oxidation products in milk stored in the walk-in retail case, which had higher light intensity than the other two retail cases, but all levels of secondary oxidation products were low compared to previous studies conducted under fluorescent light. There was a decrease in riboflavin (Rb) in most treatment packages compared to fresh milk, but all packages retained 78% or higher Rb through 8 h, indicating LED light may be beneficial for vitamin retention in milk. Retail conditions and differences in light intensity do not cause significant differences in milk quality and LED light may be less detrimental to milk oxidation than fluorescent light. More research is necessary to identify the most protective package for high quality milk.

Keywords: milk, oxidation, LED, retail refrigeration

3.1 Introduction

Fluid milk has the potential to develop off-flavors and quality defects over periods of retail storage through improper packaging protection and retail light exposure. High quality milk is described as having a bland and pleasantly sweet taste (Alvarez, 2009). When milk is exposed to retail lighting, a light-induced oxidized off-flavor develops. This off-flavor has been described as astringent, cardboard, and butterscotch in 1% milk exposed to LED lights (4,000 lux) and leads to decreased consumer acceptability of milk (Brotherson et al., 2016). Oxidation reactions occur in the protein and fat components of milk and are induced by excited photosensitizer molecules absorbing specific light wavelengths (Lee and Min, 2009; Webster et al., 2009; Wold et al., 2015). Light protective additives (LPAs) have been added to HDPE materials used for fluid milk packaging to improve light blocking characteristics and better protect milk quality (Moyssiadi et al., 2004; Zygoura et al., 2004; Johnson et al., 2015).

Historically, retail cases were lit with fluorescent lights, but due to changes in energy regulations and improved energy efficient technologies, LED lighting use in retail cases is becoming more common (White, 2012). It is unknown if there is a significant difference between oxidized off-flavor development in milk exposed to LED light or fluorescent light. It also remains to be seen if pigmented HDPE packages provide more effective protection from LED light compared to fluorescent light. There is little published on the effect of different types of retail cases and their varying refrigeration conditions (closed-door or open-front retail cases) for maintaining fluid milk quality. The three retail cases tested in this study provided a wide range of LED light intensity and different refrigeration conditions to see if either factor has a significant effect on milk quality.

The objective of this study was to evaluate the influence of LED lighting, as represented in commercial refrigeration, by three retail cases (HillPhoenix, Chesterfield, VA) on milk oxidation in 2% milk packaged in experimental HDPE packages with different light interference properties.

3.2 Materials & Methods

3.2.1 Milk Processing & Purchase

Reduced fat (2% fat) milk (high-temperature-short-time [HTST] pasteurized at 78°C for 15 sec) in half-gallon translucent HDPE packages was purchased from a local supermarket (Kroger, Blacksburg, VA). Milk was purchased directly from the dairy manager on the day of delivery soon after receipt to ensure freshness and limit light exposure in the store. Milk, covered in ice, was transported to the pilot plant (Department of Food Science & Technology, Human and Agricultural Biosciences Building 1 [HABB1], Virginia Tech, Blacksburg, VA) in coolers, providing light and temperature control.

3.2.2 Filling Milk into Treatment Packages

Milk was rapidly poured from the original packaging into each treatment package under a positive flow clean-fill laminar hood (Thermo Fisher Scientific, Waltham, MA), capped securely by hand, and stored in a dark walk-in case, until the study began, in order to prevent incidental light exposure. The treatment packages for one full replication (4 h and 8 h; n=60) were filled at the same time. This process was repeated for three replications.

HDPE resin and additives (commercial yellow pigment, titanium dioxide [TiO_2]; Ampacet, Tarrytown, NY) were made by standard extrusion blow-molding procedures into half-gallon packages (average thickness: 0.59 ± 0.06 mm) to prepare four treatment packages (Consolidated Container Co., Atlanta, GA). Treatment packages were chosen to compare to results of a previous study testing the same packages, but in front-loading, closed-door retail cases under fluorescent and LED light (Amin, 2016). The following HDPE packages were prepared for testing: translucent (no light protective additives [LPAs]), yellow (with commercial Masterbatch yellow colorant), low white (with titanium dioxide based TiPure™ Protect™ [1.3% TiO_2]), and high white (with titanium dioxide based TiPure™ Protect™ [4.9% TiO_2] [Chemours, Wilmington, DE]). The yellow package was a commercial formulation; no additional details of colorants were provided. The low white package formulation was based on commercial packages. The high white package formulation is not currently used for dairy packaging. Color values for each package (Appendix A) were measured using a CIE-L*a*b* scale on a Konica Minolta CR-300 Chroma Meter (Tokyo, Japan). Light-protected HDPE served as the control package. Light-protected HDPE packages were prepared by covering the entire translucent HDPE package with aluminum foil and a white plastic bag. Aluminum foil prevented light exposure and the white plastic bag prevented the foil from reflecting light on other packages in the retail case.

3.2.3 Retail Case Conditions and Experimental Package Treatment

The three refrigerated retail cases (HillPhoenix, Chesterfield, VA) were each equipped with 3500K LED lights. All lights in the walk-in retail case (Model 3800) and closed-door retail case (Model ONRB4) were turned on. The walk-in retail case was equipped with vertical LED lights on the left and right sides of the front of the case and horizontal LED lights on each retail shelf. The closed-door retail case was equipped with vertical LED lights on the left and right side of each retail door. After the study was completed, it was found that one of the vertical LED lights on the right hand side of the closed-door retail case was a 4000K LED light instead of 3500K LED light. This difference in type of LED light was not found to be a significant factor in the results; looking at the entirety of the retail environment, the one light difference was negligible. Only the bottom and top rows of horizontal LED lights in the front-loading, open-front retail case (Model O5DM) were turned on; the horizontal LED lights under each shelf overhang were not turned on in this case. This simulated more ideal conditions for light sensitive products in this retail case by having sufficient illumination of the products, but removing unnecessary light exposure to prevent potential quality degradation. Each package treatment (n=5) was tested in duplicate within each replication for each retail case, creating a total of ten packages in each case for each time treatment. Thirty packages were tested for each time treatment (5 treatment packages x 2 duplicates x 3 retail cases). A full replication of the two time treatments required sixty half-gallon packages of 2% milk. All milk from one complete replication was from the same processing date.

Milk was placed at eye level on the retail shelf for the walk-in case and the closed-door case. Milk was placed on the second retail shelf from the bottom of the case for the open-front case. These shelves were selected as being most common for the consumer to reach for in the store. All treatment packages were placed on the front row of the selected shelf of the retail case. Treatment packages for each retail case were placed in a randomized order (Microsoft® Excel® for Mac 2011, Version 14.5.8, Microsoft Corporation, Redmond, WA) for each time treatment. Selected treatment shelves were divided in half and randomized with one duplicate for each treatment in each half (five treatment packages on each half of the case). This ensured an equal and random distribution of the treatment packages throughout the front row of the retail case. Empty spaces in the front row, all spaces behind the front row, and all other shelves within each case were filled with water-filled HDPE half-gallon packages to simulate the refrigeration load of a full retail case. Only one time treatment (4 h or 8 h) at a time was placed in the retail cases (Figure 2).

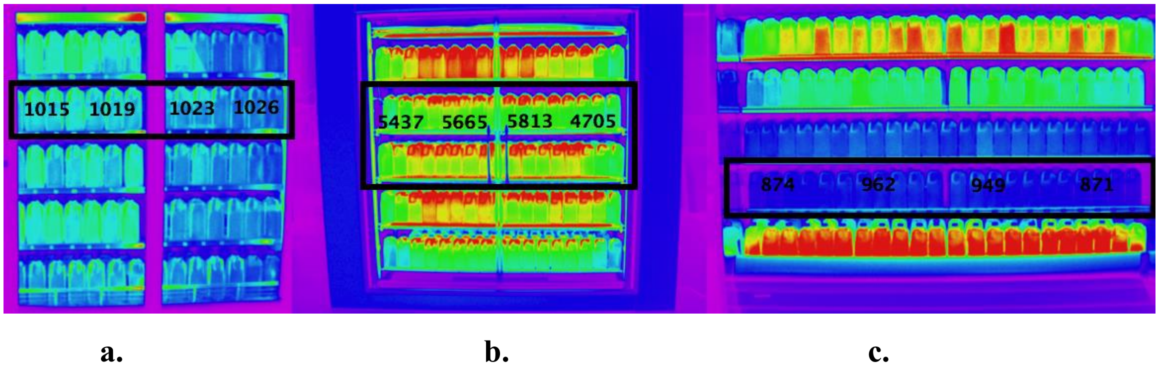


Figure 2. Layout of treatment packages in all three retail cases (a.) closed-door case, b.) walk-in case, c.) open-front case). Black squares indicate the shelves used for treatment packages. Average light readings (lux) are identified across each treatment shelf in the retail case. The colors indicate light intensity from high (red/orange) to low (blue/green). *Light intensity map photo by Jack Sjogren (HillPhoenix, Chesterfield, VA).*

3.2.4 Light Measurements and Sample Collection

Light readings were taken for each time treatment at each package position in the three retail cases using a handheld light meter (Model SN400, Extech Instruments, Nashua, NH). The light meter was placed on the overhang of each shelf in the open-front and closed-door retail cases. For the walk-in case, the light meter was placed at a 45° angle at the package neck for light intensity readings. These positions were chosen for each case to read the most intense light exposure on each treatment package, as recommended by HillPhoenix representatives. Light readings of each position in the retail case were averaged together from all replications of both time treatments (n=6) to get a light reading for each retail case position as well as a representation of how light intensity changes within the same case. Temperatures of all three retail cases were maintained at 4°C ± 1°C throughout the experiment.

Samples were collected from fresh milk (0 h light exposure) for secondary oxidation products (Thiobarbituric Acid Reactive Substances Analysis [TBARS]) and riboflavin (Rb) degradation. After each time treatment was completed, samples were collected from each package for TBARS (10 mL) and Rb degradation (10 mL), stored in polypropylene tubes (Thermo Fisher Scientific, Waltham, MA) and protected from incidental light exposure with foil wrap. Samples were frozen in a blast freezer (-20°C, Harris Environmental Systems, Andover, MA) until analyses could be completed. The treatment of milk took place over a three-week period from June-July 2015, and all analyses were completed within six weeks of sample collection.

3.2.5 Secondary Oxidation Products Measured by Thiobarbituric Acid Reactive Substances Analysis (TBARS)

The formation of secondary oxidation products including malondialdehyde (MDA) and other aldehydes were measured by TBARS analysis, as adapted from Spanier and Traylor (1991). One sample from each package was collected and analyzed for secondary oxidation products. Each packaging treatment was analyzed in duplicate within each replication for three replications (n=6). Samples were thawed in an ice water bath in the dark after being removed from the freezer. Standards were created by diluting

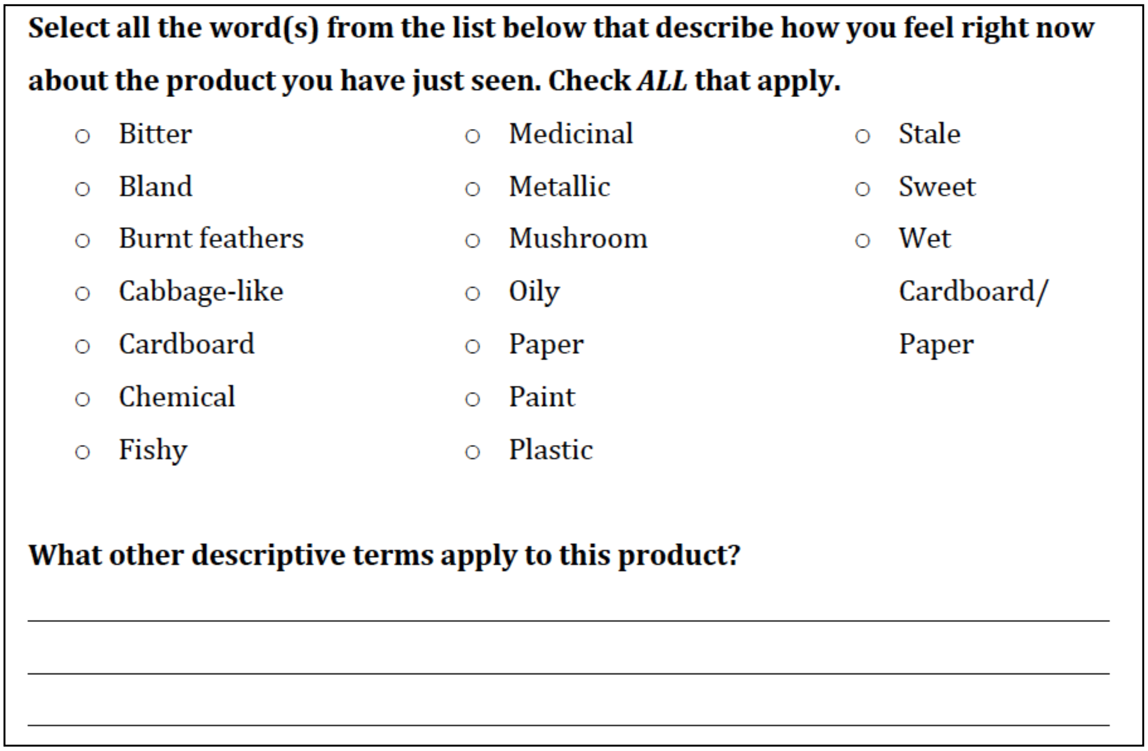
MDA stock solution to create 0, 0.5, 1.0, 2.0, and 5.0 mg MDA/mL standards for the standard curve. First, 4 mL of sodium dodecyl sulfate solution and 0.1 mL of EDTA solution were added to each 1 mL sample. Samples were mixed using a vortex mixer. All samples and standards were incubated in a 95°C water bath for 60 min and stirred every 20 min. After incubation, the tubes were cooled in an ice water bath. A prepared pyridine and butanol solution (2.5 mL) was added to each sample and standard under a fume hood. All tubes were mixed completely with a vortex and centrifuged at room temperature at 3000 rpm for 20 min. Following centrifugation, the top organic layer of solution was transferred into a cuvette and the absorbance was read with a spectrophotometer at 532 nm under the fume hood. Absorbance results were converted to mg MDA/L using the standard curve.

3.2.6 Degradation of Riboflavin (Rb) by Fluorometric Analysis

Rb concentration of milk samples was analyzed using a modified fluorometric analysis (AOAC method 970.65, 1995) and measured on Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instrument, Inc., Columbia, MD) as described by Webster et al. (2009). One Rb sample from each milk treatment package was analyzed, creating a duplicate analysis for each packaging treatment within each of three replications (n=6). Standards of 0.25, 0.50, 1.00 and 2.50 μg Rb/mL were prepared from stock Rb solution. Samples were thawed in an ice water bath in the dark after removal from the freezer. Milk samples (10 mL) were adjusted between pH 5.0-6.0 by adding approximately 0.025 mL of 10N HCl to all milk samples. Samples were autoclaved for 30 min. at 121-123°C and cooled to room temperature. The pH of samples was then adjusted between 6.0-6.5 by adding approximately 0.1 mL of 1N NaOH. Next, pH was adjusted to 4.5 to allow the proteins to precipitate out of solution by adding approximately 0.03 mL of 10N HCl. The exact volumes of all HCl and NaOH additions were recorded and used to calculate a dilution factor for each sample. All samples were centrifuged at 4000 rpm for 10 min. The supernatant was filtered from each sample using PTFE 0.45-micron filters with syringes. All collected supernatant were kept in light-protected polypropylene tubes and read in a spectrofluorophotometer (Shima Scientific Instrument, Inc., Columbia, MD). Spectrofluorophotometer readings were converted to Rb concentrations (mg Rb/L) using the standard curve and dilution factors for each sample.

3.2.7 Sensory Evaluation

An informal qualitative descriptive sensory panel was completed on the 8 h treatments for one replication. The 8 h time treatment was chosen for sensory evaluation instead of the 4 h time treatment because differences in milk flavor should be more apparent after longer light exposure. All panelists (n=10) participated extensively in sensory research on light-induced oxidation of milk for a minimum of three months prior to this study. These experienced panelists were given a ballot for each sample with a check-all-that-apply (CATA) list of oxidized milk descriptors and space to add any additional descriptive terms that applied to the product (Figure 3). Descriptive terms from the ballot were chosen from related literature on oxidized milk descriptors (Alvarez, 2009). Before beginning the sensory evaluation, a general discussion of the descriptive terms was held where terms were defined and clarified to ensure each panelist connected the same definition to each term. The panel decided to combine “cardboard” and “paper” into one term. It was also decided that “medicinal” was a more specific subcategory of "chemical." Participants were invited to add additional words, as needed, to qualitatively characterize their observations.



Select all the word(s) from the list below that describe how you feel right now about the product you have just seen. Check *ALL* that apply.

- ○ Bitter
- ○ Bland
- ○ Burnt feathers
- ○ Cabbage-like
- ○ Cardboard
- ○ Chemical
- ○ Fishy
- ○ Medicinal
- ○ Metallic
- ○ Mushroom
- ○ Oily
- ○ Paper
- ○ Paint
- ○ Plastic
- ○ Stale
- ○ Sweet
- ○ Wet Cardboard/ Paper

What other descriptive terms apply to this product?

____

____

____

Figure 3. Example of descriptive check-all-that-apply (CATA) sensory ballot developed for oxidized milk flavor evaluation.

Evaluation occurred in a group setting under white light, with panelists seated around a large table. Water and unsalted crackers were available on the table for use as palate cleansers between samples. All panelists tasted a control sample (light-protected milk) and were informed of the sample identity as the standard for high quality fluid milk. Control milk was available to panelists throughout the session as a standard for comparison. Next, panelists tasted twelve coded treatment samples and filled out a ballot for each sample. The order of the sample presentation to the panelist group was randomized, but every panelist tasted the same sample before the entire group moved on to the next sample. All samples were presented in coded, translucent HDPE half-gallon packages and each panelist poured their own sample (~2 oz.) into a clear, 8-oz. plastic sample cup. Only a randomly assigned sample code number identified samples to panelists. Panelists were instructed to expectorate samples to prevent sensory fatigue. A group discussion was held after tasting all samples to compare commonly used descriptors for oxidized off-flavor in milk. Total counts for each descriptor were tallied for each packaging treatment and means were found (Microsoft® Excel® for Mac 2011, Version 14.5.8, Microsoft Corporation, Redmond, WA). One bottle of each packaging treatment from each retail case was tested. Due to the small number of samples tasted, comparisons were only made by package and not by retail case. Word cloud figures (Wordle™, 2014) were used to generate visuals of the most frequently selected terms (chosen by ≥20% of panelists) for each package type (Figure 6). Formal statistics were not completed on the sensory evaluation due to the qualitative aspects of the descriptive sensory panel, the collection of nominal data, and the lack of statistical power from the low number of participants.

3.2.8 Statistical Methods

A 3-way analysis of variance was used to compare packages (n=5), time of light exposure (n=2), and retail case (n=3) for significant differences in oxidation measures (TBARS and Rb) using JMP (α=0.05; JMP Pro 11, SAS Institute, Cary, NC). Outliers were removed from TBARS and riboflavin assay results at 95% confidence using Dixon's Q test (Cochran and Snedecor, 1980). Tukey's honestly significant difference (HSD) test further explored significant differences within the case and package variables (JMP Pro 11, SAS Institute, Cary, NC).

3.3 Results & Discussion

Our objective was to characterize light-induced oxidation in 2% milk under LED retail light. The effects of LED lights were further explored to see if there were differences in milk quality caused by higher light intensity or different retail case conditions. The retail cases were studied to understand how light intensity varied within the same retail case as well as overall differences between the three cases tested. Sensory evaluation serves as the gold standard for detecting oxidation and its resulting off-flavors in milk. Our descriptive sensory evaluation identified differences in flavor that occurred from the different light interference characteristics of our experimental packages and different LED light intensities. Analytical measures of oxidation were performed to quantify noticeable sensory differences in different milk treatments. Chemical measures of lipid oxidation (TBARS formation and Rb degradation), which are commonly used to evaluate oxidized milk under fluorescent lights, were completed to see if they served as accurate indicators of oxidation under LED lights, or if alternative chemical analyses should be considered. This study provided a more complete picture of the effects of LED light on milk and the interactions between LED light, light exposure duration, and light interference properties from experimental HDPE packages.

3.3.1 Retail Case Light Intensity

The intensity of light over periods of retail light exposure is an important factor in determining the extent of oxidation reactions in milk components. Light intensity can vary greatly within one retail case due to the placement of light bulbs and shelves. Light intensity was determined for the retail cases tested by first finding the mean of the lux readings (n=6) at each position, then finding the mean of the average light intensity of all positions. The walk-in case had the highest average light intensity (5,405 ± 836.0 lux), nearly five times higher than the closed-door and open-front cases (1,021 ± 17.0 lux and 914 ± 54.4 lux, respectively). The average light intensity for each package position within the retail cases was calculated from individual light readings (Appendix A). The closed-door and open-front cases represented retail cases with lower LED light intensity for this study, since not all of the lights were turned on in the open-front case. The walk-in case represented a retail situation with significantly higher LED light intensity. The closed-door and open-front cases had relatively consistent light intensity (ranging from 1014-1027 ± 18.0 lux and 790-967 ± 8.3 lux, respectively), but there was more variability in the walk-in case (light intensity ranged from 3138-5995 ± 370.5 lux). The variability noted for the walk-in case may be partially attributed to measurement method. For measurements in the walk-in case, the light meter was held at the neck of the package at a 45° angle for measurements; reproducing exactly the same position for each measurement is much more difficult than placing the light meter vertically on the shelf overhang, as was completed for light measurements in the other two retail cases.

Light intensity data for each package treatment in the retail cases was also measured (Appendix A). The random placement of treatment packages throughout the retail case shelf created greater light intensity variability for the entire packaging treatment when all treatment packages are taken into account. However, this data verified that each type of treatment package received approximately the same light intensity, eliminating bias from analytical and sensory results.

3.3.2 Secondary Oxidation Product Formation measured by TBARS

The formation of secondary oxidation products can serve as an indication of oxidized off-flavor development and is important supportive information for noticeable taste defects in milk. Fresh milk in this study had an average TBARS value of 0.23 ± 0.05 mg/L, possibly measuring oxidation products that formed due to incidental light exposure in the processing plant storage cooler prior to shipping and before we received milk from the retail store. Light readings taken in the processing plant (Westover Dairy, Lynchburg, VA) storage cooler averaged 100 lux and the amount of time that milk spent in the processing cooler prior to shipment is unknown. Fresh milk was deemed acceptable for this study because it was within range of previously reported literature on milk oxidation and 0.23 mg/L is a very low concentration for oxidation products (Moyssiadi et al., 2004, Zygoura et al., 2004).

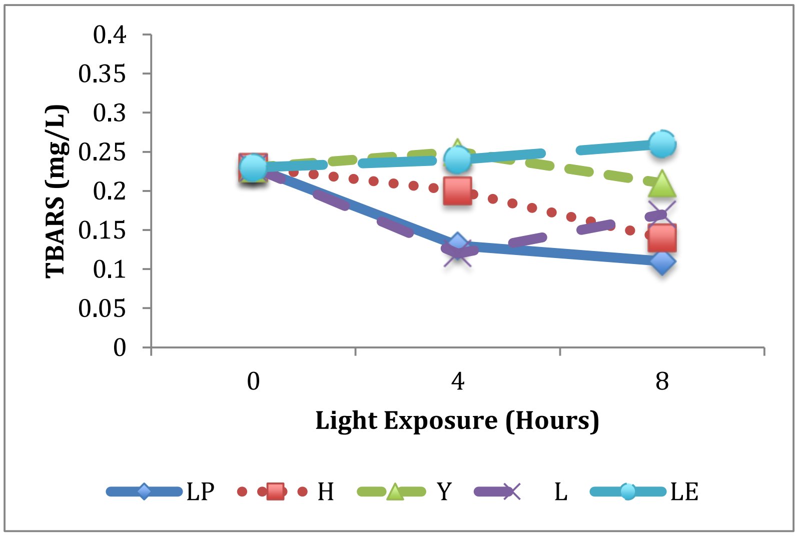
Package (pkg; $p=0.0020$) and retail case (case; $p<0.0001$) were found to have a significant effect on TBARS formation when a 3-way ANOVA compared all experimental factors ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). A 2-way pkg*case interaction ($p=0.0361$) was found in the 3-way ANOVA. There was no difference in aldehyde levels between 4 h and 8 h light exposure. This is not surprising, since previous literature has shown that the formation of oxidation end-products does not significantly increase until milk undergoes longer periods of time treatment such as 7, 10, or 18 d (Cladman et al., 1998; Walsh et al., 2015).

Due to significant pkg*case ($p=0.0361$) interaction, TBARS samples were sorted by retail case and analyzed by 2-way time*pkg ANOVA for differences ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). Package type was a significant factor for both the closed-door case ($p=0.0136$) and the walk-in case ($p=0.0281$). Tukey's HSD test for the closed-door retail case showed that the light-exposed package had significantly higher TBARS levels than the light-protected package. This followed expectations that the light-protected control milk had less light-induced oxidation as there was less light transmittance into the product. Milk in the light-protected package was not affected by differences in storage conditions between the closed-door and walk-in retail cases because it was protected from light exposure. It also followed expectations that the light-exposed package had the highest levels of secondary oxidation products because it provided the least protection from light. These results confirm that the wavelengths in LED lighting lead to oxidation reactions occurring in milk components. There were no differences in other treatment packages in the open-front or closed-door cases. In the walk-in cooler, the high white HDPE package had significantly higher levels of TBARS than the light-protected package, but none of the other packages were different. It is unclear why the high white package, with higher levels of light protective TiO_2, permitted an increase in oxidation products. Overall, all levels of oxidation products measured in this study were very low and did not indicate extensive aldehyde-producing oxidation reactions.

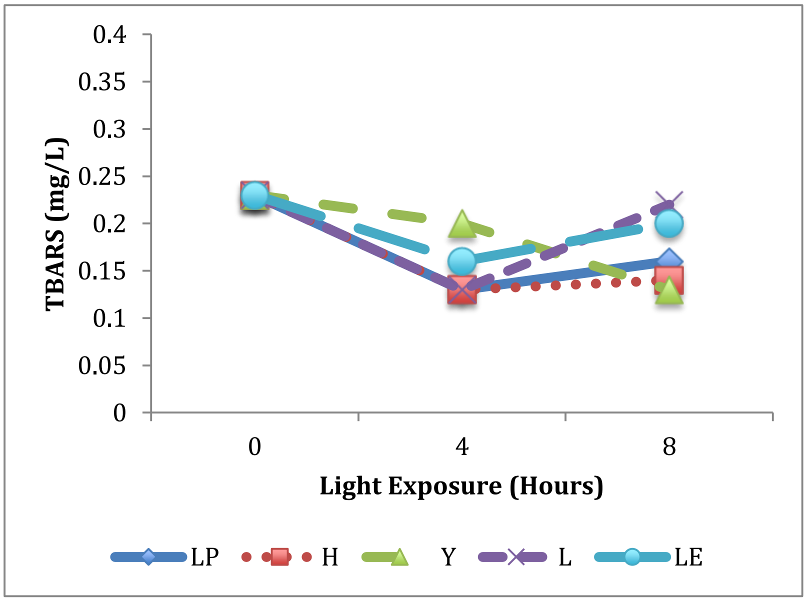
To evaluate the full effect of LED light intensity in different retail cases, the samples were sorted by package and analyzed by 2-way time*case ANOVA ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). In all packages except the light-exposed control, retail case was a significant factor in TBARS oxidation products formation. Tukey's HSD test found that the walk-in retail case caused greater TBARS formation for the light-protected control, high white package, low white package, and yellow package than either the closed-door or open-front retail cases. Higher aldehyde levels were seen in all treatments in the walk-in case, indicating a possible relationship between higher light intensity and increased oxidation reactions (Figure 4). It is unclear why some TBARS values were lower after treatment than in fresh milk, except that this methodology might not be as accurate at measuring very low levels of aldehydes and carbonyls because it is reaching the detection limit for TBARS (Mestdagh et al., 2005; Hedegaard et. al, 2006).

The concentration of secondary oxidation products after both time treatments was much lower overall than related literature on oxidized milk with similar light exposure times under fluorescent lights (Cladman et al., 1998; Zygoura et al., 2004; Johnson et al., 2015). All aldehyde levels from this experiment (Appendix A) are far below the suggested sensory quality threshold of 1.3 mg/L for UHT pasteurized 2% milk (Johnson et al., 2015), even though sensory differences were noticeable after 8 h of LED light exposure (results presented below). This suggests that TBARS, while used to measure lipid oxidation in milk stored under fluorescent lights, may not be the most appropriate measurement of lipid oxidation in milk stored under LED light. TBARS is limited because it only detects one type of oxidation end-product (aldehydes) and cannot measure oxidation through the formation of other end-products such as ketones and disulfides, which could be responsible for the detectable off-flavors in this study.

a. closed-door retail case



b. open-front retail case



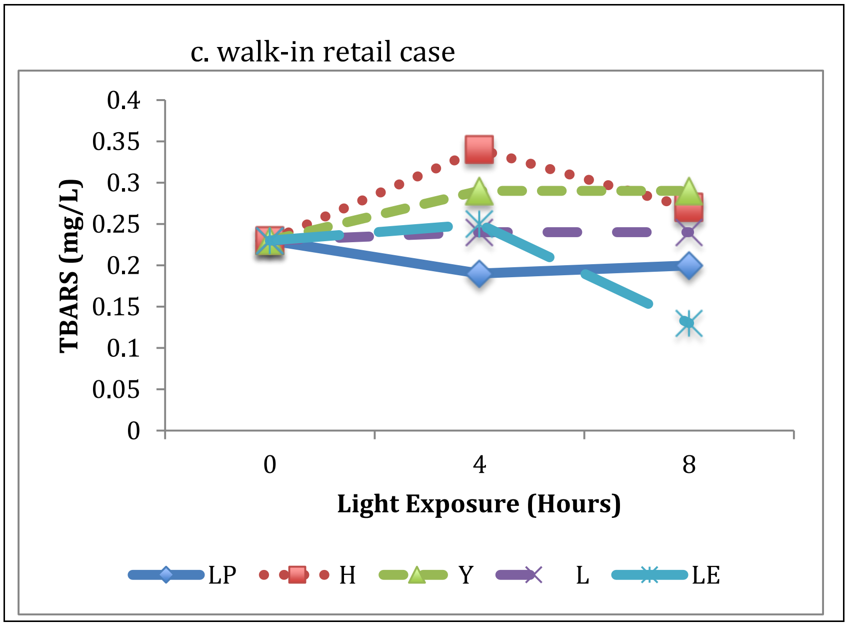
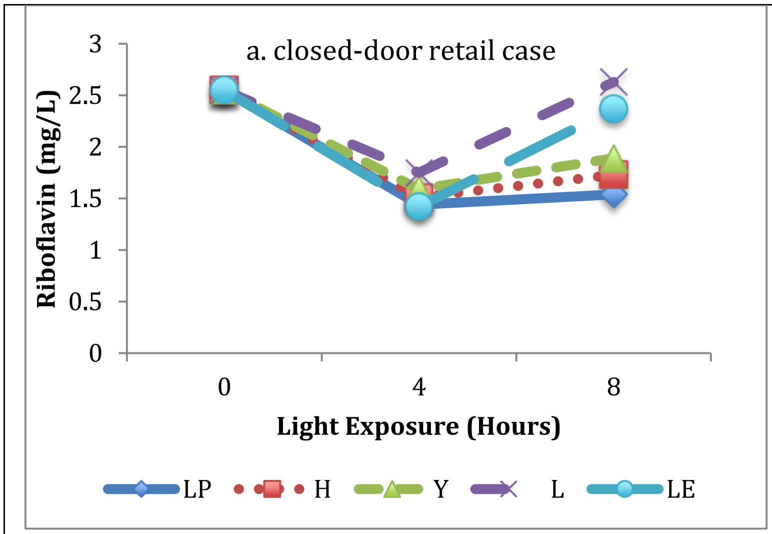


Figure 4. Average TBARS (mg/L) for treatment packages in the retail cases: a.) closed-door case, b.) open-front case c.) walk-in case. LP= light-protected HDPE with foil and plastic overlay, H= high white HDPE, L= low white HDPE, Y= yellow HDPE, LE= light-exposed [natural] HDPE).

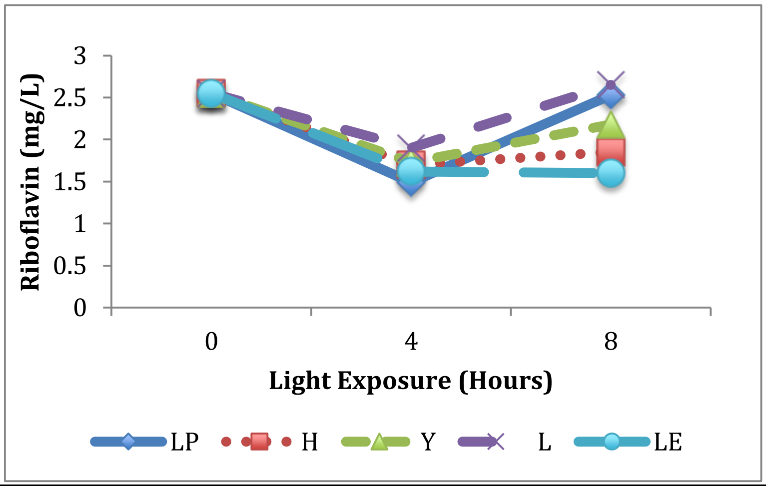
3.3.3 Rb Degradation by Fluorometric Analysis

Riboflavin (Rb) is a photosensitive vitamin that is measured as an indication of milk oxidation. As a photosensitizer, it acts to initiate type I oxidation. It is found in relatively high quantities in fluid milk. It is important to fully understand the degradation of Rb during retail storage because unfavorable storage conditions could potentially decrease the nutrient content of milk by the time consumers are drinking it. Fresh milk in this study had a riboflavin concentration of 2.55 mg/L, which is higher than previously reported values of riboflavin in milk; 1.36-1.75 mg/L (Dimick, 1982; Gebhardt and Thomas, 2002; Zygoura et al., 2004). The higher initial riboflavin concentration could have resulted from analytical variability with the fluorometric assay. This higher initial value may explain why some values found in the treatment packages of this study (Appendix A) are also higher than previously reported riboflavin values in literature.

Time (p=0.0003) and package type (p=0.0154) were identified as significant factors in riboflavin concentration by a 3-way time*case*pkg ANOVA (α=0.05; JMP Pro 11, SAS Institute, Cary, NC). Tukey's HSD test was run on the package variable and showed that low white package retained more Rb than the high white package. All other treatment packages were similar. All treatment packages had less riboflavin than fresh milk, indicating that vitamin degradation in milk occurred with increased storage under light (Figure 5). Interestingly, riboflavin concentrations were higher at 8 h light exposure than 4 h light exposure, although most were still lower than fresh milk. Higher Rb concentrations at 8 h than 4 h could be a result of the fluorometric assay detecting the formation of lumichrome and lumiflavin and measuring it as Rb (Wold et al., 2002). Previous studies have found that when Rb degrades, it creates different forms of lumichrome or lumiflavin, two compounds which can be detected by fluorescence (Wold et al., 2002). If more Rb is degrading under longer periods of light exposure (8 h) it is possible that there is increased formation of Rb degradation compounds being measured by the fluorometric assay and resulting in apparently higher levels of Rb. The relatively high riboflavin values throughout this experiment could indicate that LED lights, especially over short time durations (8 h or less), are a more optimal condition for vitamin retention in fluid milk in comparison to fluorescent lights, which have led to large decreases of riboflavin in milk (Zygoura et al., 2004; Johnson et al., 2015; Walsh et al., 2015).


a. closed-door retail case

b. open-front retail case



c. walk-in retail case

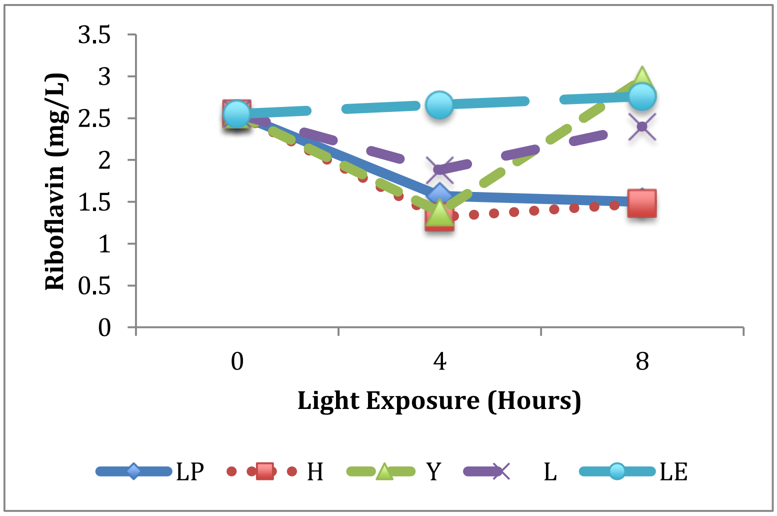


Figure 5. Average riboflavin concentration (mg/L) for treatment packages in the retail cases: a.) closed-door case, b.) open-front case c.) walk-in case. (LP= light-protected HDPE with foil and plastic overlay, H= high white HDPE, L= low white HDPE, Y= yellow HDPE, LE= light-exposed [natural] HDPE).

3.3.4 Sensory Evaluation

Light-protected milk had the fewest descriptive terms and was consistently described as bland and sweet, which is how high quality milk has been previously described (Alvarez, 2009). The most frequently selected descriptive term for all other samples after 8 h LED light exposure was sweet followed by cardboard/paper, metallic, and oily. Milk from the low white package had the most frequently selected (≥20% of panelists) oxidized descriptors of all packaging treatments. This research took place prior to publication of Brotherson et al. (2016) so the term "butterscotch" identified as an oxidized milk descriptor by that study was not included in our ballot, and was not verbalized by any panelist as a descriptor of the milk samples tested. Word clouds of frequently selected descriptors for milk from all packaging treatments can be seen in Figure 6, where sensory descriptor results were separated only by package treatment, and not by retail case. Word clouds visually demonstrate differences between packaging variables, but do not account for different light intensities and environmental conditions between the three retail cases due to the selected number of samples tasted.

When the descriptive terms were sorted by retail case, some trends were seen with the walk-in case that had higher light intensity than the other two retail cases. The higher light intensity brought out more frequent use of off-flavor descriptors including bland, painty, bitter, chemical, and wet cardboard/paper than was noticed in milk from the other two retail cases with lower light intensity. This indicates that the higher light intensity may be exciting photosensitizers to produce different compounds, such as ketones or disulfides, that could be associated with these off-flavors. Different methods of analysis should be employed to quantify these other compounds, besides aldehydes and Rb, that could be responsible for the noticeable off flavors. Possible alternative analysis methods could include measuring conjugated dienes/trienes or measuring proteolysis through changes in molecular weight from protein fragmentation and polymerization.

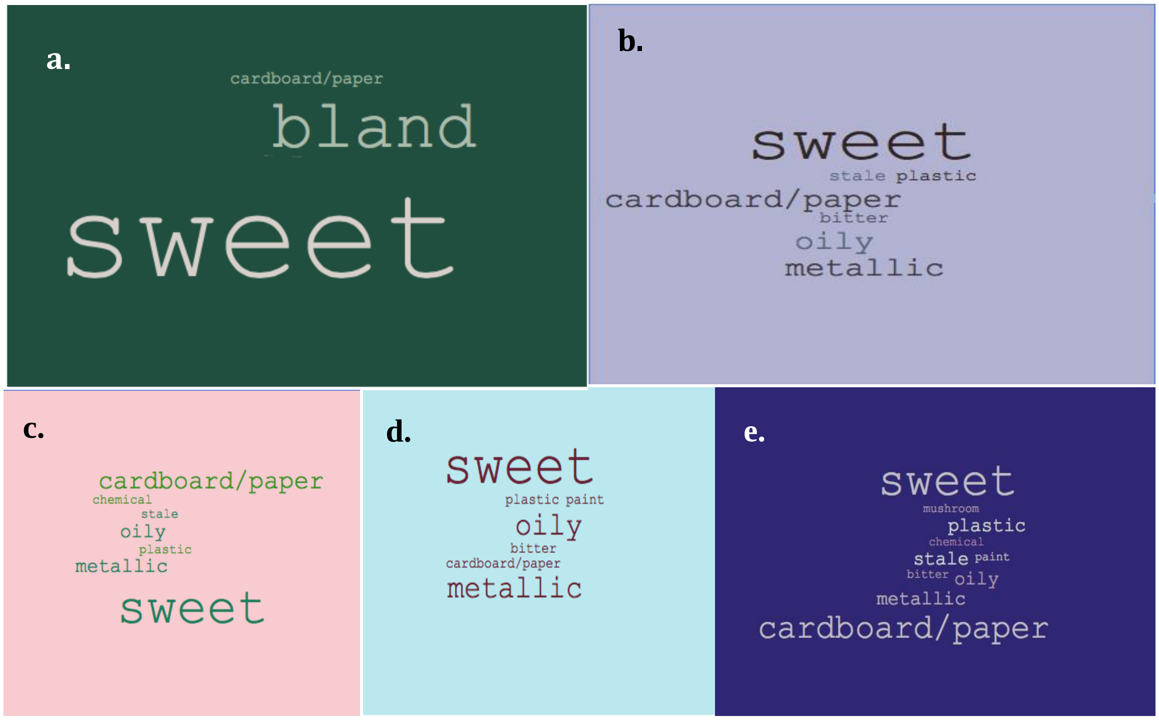


Figure 6. Word clouds comparing a.) light-protected 2% milk (control) to b.) light-exposed 2% milk in clear HDPE, c.) commercial yellow HDPE, d.) high white HDPE, and e.) low white HDPE after being exposed to 8 h of retail LED lighting. Font size of each term is relative to frequency chosen; larger font size indicated more frequent selection of a term.

The two analytical assays in our experiment did not detect the occurrence of significant oxidation reactions. The assays in this study only measured aldehyde levels and Rb content, and were unable to quantify protein oxidation or detect other lipid oxidation end-products, such as ketones, that can contribute to off-flavors. Only low levels of aldehydes were measured by TBARS, and 78% and higher concentrations of Rb were retained by all packages throughout 8 h of treatment when compared to the USDA reported Rb value in 2% milk (1.69 mg/L; Gebhardt and Thomas, 2002). Other photosensitive molecules not measured in this experiment could be responsible for initiating the oxidation reactions that created end-products in milk with noticeable off-flavors during sensory evaluation. Protoporphyrin, hematoporphyin, chlorophyll a and b, and other tetrapyrroles are all compounds in milk that are reactive to light in the orange spectrum (575-750 nm) and have been shown to play a role in oxidation (Airado-Rodriguez et al., 2011; Wold et al., 2015). Airado-Rodreguez, Intawiwat, Skaret, and Wold (2011) found that orange light caused more intense off-flavors and off-odors in cow's milk than light from the blue and violet regions (<520nm). Off-flavors could also be a result of the breakdown of proteins during proteolysis that creates disulfides. Protein carbonyl compounds form during protein oxidation and have been related to off flavors in previous work (Karagul-Yuceer et al., 2004). Changes in the composition or levels of these components could account for the cardboard/paper, metallic, or oily off-flavors that were tasted after 8 h LED light exposure, since LED light transmits relatively high amounts of light from the orange spectrum.

The sensory evaluation functioned as a 'cutting', typically used in the food industry and applied for quality control and product development methods. Panelists were familiar with oxidized milk, but were not specifically provided formal descriptive sensory training. While valuable for qualitative information, the interpretation is limited because it was not completed with a sensory panel trained specifically for dairy flavor identification and intensity. The small number of panelists (n=10), appropriate for an in-lab assessment, also provided limited insight into the milk consumer population. Even with these limitations, this descriptive work provided valuable preliminary knowledge of milk oxidation under LED light at a time when there was no literature published on the subject.

None of the packaging treatments were similar to the high quality light-protected control because they all had an increased selection of oxidized descriptors. However, the development of oxidized off flavors could have been influenced by the interaction between LED light transmission and light intereference properties (yellow pigment or TiO_2 loading) of the packaging. It is interesting to note that even when a wide range of off-flavor descriptors were chosen for oxidized milk samples, the samples were still described as sweet. This could be a result of protective packaging influences on milk flavor from light intereference properties of the HDPE packaging.

This study provides greater insight to the influence of LED light intensity on milk flavor. Previous work that studied milk oxidation and off-flavor development under LED light looked at 2% milk stored under LED lights (950 lux; Amin, 2016) or 1% milk stored under LED lights (4,000 lux; Brotherson et al., 2016). This study further explored oxidized off-flavor development in milk under the middle ground of light intensity (~1,000 lux) as well as extremely high light intensity (4,500-6,200 lux). These results suggest that 8 h of LED light exposure at different intensities is sufficient to create changes in milk composition that lead to an oxidized off-flavor, but does not render milk completely undrinkable.

3.4 Conclusions

Light intensity, as represented in the different retail cases, influences the extent of light-induced oxidation and compositional changes in milk. LED-lit retail cases can have vastly different spectrums of light intensity. There can also be substantial light intensity variability within a retail case, which affects the extent of light-induced oxidation for milk stored in different parts of the retail case. For this study, the walk-in retail case had large variability in light intensity, ranging from 1,500-6,000 lux on different shelves (Figure 2). This variability is an important consideration for dairy processors and retailers, since the areas of higher intensity (top shelf and far edges of shelves) are typically the locations where consumers reach for milk less frequently, and milk may remain under direct retail light for longer time periods and become highly oxidized before consumer purchase.

Packaging provides the first defense against light exposure and can slow the initiation of light-induced oxidation reactions. Packaging should be designed with high levels of light interference properties to protect milk for the worst case scenario, where it stays under high light intensity retail storage for periods of time before purchase by consumers. Packaging is an important factor in maintaining the quality and nutrients of milk from producer to consumer. More protective packaging options are needed than what is currently in the market to ensure that milk does not develop undesirable light-induced off-flavors before reaching consumers. Compositional changes in milk are occurring rapidly under retail light (4 h or less), and leading to the development of off-flavors that have been identified as metallic, oily, cardboard, and plastic. Designing lower light intensity LED retail cases and packaging options with greater light interference properties, such as multi-layered PET packages, has potential to more effectively maintain high quality fluid milk for consumers.

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CHAPTER IV

COMPARISON OF PET PACKAGING TO PROTECT MILK QUALITY UNDER RETAIL STORAGE CONDITIONS

ABSTRACT

A recent shift to more energy efficient light-emitting diode (LED) lights in retail dairy cases has occurred, but the effects of LED light on fluid milk in retail conditions are not known. Our objective was to determine the efficacy of polyethylene terephthalate (PET) packaging at preventing light-induced oxidation in 2% milk under LED and fluorescent retail light. Light interference properties were studied in combination with the improved oxygen barrier properties of PET. The extent of oxidation in 2% milk packaged in PET packages (clear with UV barrier, low (2.1%), medium (4.0%), and high (6.6%) TiO_2) under fluorescent and LED retail light up to 72 h was studied. Two control packages (clear PET = full light exposure and PET wrapped with foil and plastic= no light exposure) were used for comparison. Chemical measures of oxidation (α=0.05; ANOVA) included dissolved oxygen content, formation of secondary lipid oxidation products, riboflavin degradation, and headspace volatiles analysis by electronic nose. Sensory evaluation of milk (triangle test) compared milk from experimental packages to light-protected control milk for similarity (β=0.05) and to light-exposed control milk for differences (α=0.05). PET with high TiO_2 was an effective package for protecting milk sensory quality for up to 8 h under LED light (936 ± 136 lux). Changes in dissolved oxygen content over 72 h light exposure accurately indicated the analytical extent of oxidation reactions. LED light creates less detrimental retail conditions for fluid milk and

PET packages with high levels of TiO_2 could provide a more protective packaging option.

Keywords: milk, sensory evaluation, PET, oxygen

4.1 Introduction

Fluid milk sales have seen tremendous decline in the United States over the past few decades, dropping 5.2% in 2015 alone (Bauer, 2016). Part of this decline might be attributed to consumer experiences drinking lower quality milk that have turned consumers to other beverage options such as plant-based milk alternatives, juice, and/or soda. Because milk, which is a photosensitive product, has the potential to quickly develop oxidized off-flavors that are objectionable to consumers, this could be a factor contributing to the decline in consumption. Lowfat (1% milkfat) milk exposed to LED light has been described as having an increase in butterscotch, cardboard, and astringency flavors (Brotherson et al., 2016). We have also shown that light-oxidized milk exposed to high intensity LED light (~5,400 lux) is described as bitter, chemical, painty, and wet cardboard/paper (Potts, Ch. III, 2016). Oxidized off-flavors in milk stored under lower intensity LED and fluorescent lights elicit similar descriptors as the ones listed above. The flavor changes occurring in light-exposed milk elicits feelings such as disgust after as little as 8 h under fluorescent light exposure (Walsh et al., 2015). A cost-effective packaging material for milk has yet to be identified that protects milk flavor quality sufficiently through retail storage.

Retail lighting and packaging parameters influence milk quality in the early stages of shelf life prior to consumer purchase. In particular, these factors affect milk quality through oxidation reactions, influenced by available light and oxygen, that change milk composition, leading to undesirable off-flavor development. Milk packaging materials such as high-density polyethylene (HDPE) and polyethylene terephthalate (PET) have varying gas permeability properties and can be designed to include different levels of light protective additives (LPAs) that affect light transmission. PET is a more effective oxygen barrier with lower oxygen permeability at 25°C than HDPE (120-240 and 40,000-73,000 cm^3 $\mu m/m^2$ d atm, respectively; Selke et al., 2004). Thus, PET has potential to control oxygen influence and maintain milk quality more effectively than other packaging materials, such as HDPE. Titanium dioxide (TiO_2) is a white pigment that can be added to PET in varying levels to create a more opaque package and protect milk against retail case light exposure by blocking the transmission of specific wavelengths. Low to moderate levels of TiO_2 (0.6%, 1.3%, 4.3%) have seen limited success in HDPE packages for UHT processed milk (Johnson et al., 2015), in HTST processed milk (Amin, 2016) and in PET (2% TiO_2; Moyssiadi et al., 2004) in protecting milk flavor and nutrients, but the protective effects of higher levels (4.0% and 6.6% TiO_2) have not yet been studied in PET. Different types of light (fluorescent or LED) and different light intensities have potential to affect the extent of light-induced reactions in milk (Amin, 2016; Brotherson et al., 2016; Potts, 2016 (Ch III)), but it is unknown if these variables produce unacceptable sensory changes that influence the consumer. It is crucial to more fully characterize the oxidation reactions of milk under LED light, since this light source has shown potential as being less destructive to milk flavor and quality in 1% milk stored in translucent HDPE packages than fluorescent light (Brotherson, 2016).

The main objective was to determine effectiveness of pigment combinations in PET packaging providing an oxygen barrier to protect and maintain acceptable fluid milk quality through 72 h storage at 4°C under continuous retail case fluorescent and LED lighting. The effectiveness of combining pigments with oxygen barrier properties in a PET package was determined by measuring secondary oxidation products, riboflavin degradation, dissolved oxygen content, volatile profile changes by analytical methods; sensory evaluation was also completed to validate observable changes in sensory quality. A secondary objective of this study was to investigate a potential quality control method for evaluating milk quality using electronic nose (eNose) technology to detect light-induced volatile changes in fluid milk within 8 h light exposure.

4.2 Materials and Methods

4.2.1 Milk Processing

Vitamin D fortified 2% milk was processed by high-temperature-short-time pasteurization (HTST; 78°C for 15 seconds) at a Kroger dairy processing plant (Westover Dairy, Lynchburg, VA). Milk was packaged into translucent (no added LPA) HDPE packages at the processing plant, received within 1 h of processing from the dairy processing plant case, and transported to the pilot plant (Department of Food Science & Technology, Human and Agricultural Biosciences Building 1 [HABB1], Virginia Tech, Blacksburg, VA) in a dark refrigerated truck providing both light and temperature protection.

4.2.2 Packaging Treatments

Treatment packages were made (PTI Technologies, Holland, OH) following standard injection blow-molding processes to create 2L bottles, similar in shape to a standard 2L soda bottle, from PET resin (average thickness= 0.33 ± 0.04 mm). Six polyethylene terephthalate (PET) treatment packages were tested, including two control packages. The first control was light-protected (foil and plastic overwrap) PET packages, which served as a control against light exposure. The second control was clear PET packages, which served as the control with full light exposure. The four treatment packages that were tested against the two controls were a clear PET package with a UV absorber (UV barrier PET) and three TiO_2-loaded (three levels; low(2.1%); medium (4.0%); high (6.6%)) PET packages. Color values for each package were measured using a CIE-L*a*b* scale on a Konica Minolta CR-300 Chroma Meter (Tokyo, Japan). Transmittance values for each experimental package were calculated by cutting packaging materials into 2 x 2" squares and using a UV-visible spectrophotometer to test for transmittance (Cary 300 UV-Vis, Agilent Technologies, Santa Clara, CA).

4.2.3 Filling Milk into Treatment Packages

Once in the pilot plant, milk was stored in a dark walk-in refrigerated case (Harris Environmental Systems, Andover, MA) and immediately filled into treatment packages. All treatment packages were filled by pressurized transfer flow from a 580L stainless steel tank. The sanitized tank was filled with commingled milk from the commercially processed and packaged HDPE packages in dark storage and flushed with pure grade nitrogen to pressurize the tank and standardize the amount of oxygen incorporated into the milk. Valve controlled plastic tubing was used to ensure each package had equivalent amounts of milk and a standardized headspace. This process also minimized air incorporation into milk, which can occur during pouring from one package to another, which was the procedure used for Potts (2016, Chapter III) and Amin (2016). Packages were filled and capped by hand under a positive flow laminar hood (Thermo Fisher Scientific, Waltham, MA) to prevent contamination during transfer to treatment packages.

This process was repeated for three replications (n= 384 packages/replication). Due to time constraints during replication 1, treatment packages for one complete replication were filled on two different days. All treatment packages were filled on one day for replications 2 and 3. All treatment packages were stored in a dark walk-in refrigerated case (Model 3800, HillPhoenix, Chesterfield, VA) until being placed in the retail case for light exposure treatment. After a prescribed light exposure time treatment was completed, sensory testing occurred within 24 hours of removal from the retail case. All treatment and sensory evaluations for one replication took place within seven days of filling the experimental packages.

4.2.4 Treatment of Experimental Packages

Packages were placed in a front loading, glass-door retail case (Model ONRB4, HillPhoenix, Chesterfield, VA) lit with either vertical fluorescent (3500K) or vertical light-emitting diode (LED) lights (3500K). It was discovered after the study was completed that one of the vertical LED lights, on the right side of the retail case, was improperly installed (4000K LED light instead of 3500K LED). This difference in type of LED light was not found to be a significant factor in the results; looking at the entirety of the retail environment, the one light difference was negligible. Length of light exposure (4, 8, 24, 72 h ± 15 min) was evaluated. The slight variability in the four time treatments is due to the amount of time required to remove an entire treatment from the retail case and place in the next treatment. The retail case was protected from incidental room light and natural light from windows by hanging dark tarps from the overhead pipes to the floor surrounding the retail case. Overhead lights within the space were turned off during this experiment.

All packages for one complete time treatment were placed in the refrigerated closed-door retail case (Model ONRB4, HillPhoenix, Richmond, VA) at the same time.

The six treatment packages were placed in a random order (JMP Pro 11, SAS Institute, Cary, NC) throughout the retail case (Appendix E). In order to study the effects of light intensity, package placement was organized to ensure that equal numbers of packages were exposed to low (<1,000 lux), medium (1,000-1,500 lux), and high (>1,500 lux) light intensity, with randomization of package treatments within each light intensity classification, throughout the fluorescent retail case. The same organization and randomization process was followed for the LED retail case but, due to lower light intensity range in this case, treatment packages were only exposed to low (<1,000 lux) and medium (1,000-1,500 lux) light intensities. Water-filled HDPE half-gallon packages were placed in one spot on each front row for each shelf in the retail case; these locations did not match the targeted light intensity criteria described above. Water-filled HDPE half-gallon packages also filled the remainder of each shelf behind the front row of treatment packages to simulate the refrigeration load of a full retail case in a grocery store. Light intensity was recorded at each package location (including water-filled HDPE half gallons) in the front row of the retail case for each time treatment using a handheld light meter (Model SN400, Extech Instruments, Nashua, NH). All light measurements in the retail case were measured in lux. Light readings were averaged together over three replications for each retail case position. At the end of the study, this created a total of 12 light readings for each position to develop an average light intensity for each treatment location.

Eight bottles of each treatment package (n=6) were completed for each time treatment in this study. Each package contained 2000 mL (65 oz.) of milk, totaling 520 oz. of milk for each treatment. This amount provided the volume necessary for sensory evaluation with enough volume left over for for analytical assessments. Three replications of each time treatment (n=4) were completed over eight weeks from September-November 2015. All milk for one complete replication was from the same processing date.

This experiment was analyzed as two separate studies (Figure 7). Study 1 looked at the effects of different treatment factors (light, time, package) on milk from individual packages, which mimics the individual packages purchased by consumers in a retail setting. Two types of light and three levels of light intensity were studied for the individual packages in Study 1 (Fluorescent light: high, medium, low; LED light: medium, low). Study 1 evaluated changes in secondary oxidation products (Thiobarbituric Acid Reactive Substances Analysis; TBARS), riboflavin degradation, changes in volatile composition (measured by electronic nose), changes in dissolved oxygen levels in milk, and changes in microbiological quality of milk from 0-18 d post processing.

Study 2 focused on the milk after individual packages from each treatment were commingled for sensory evaluation to better evaluate the effect of packaging, time, and light treatments as a whole, without the additional factors such as light intensity variability between package positions within the retail case. Study 2 measured the following: sensory evaluations of all treatments, changes in secondary oxidation products (TBARS), riboflavin degradation, and changes in volatile composition (measured by electronic nose).

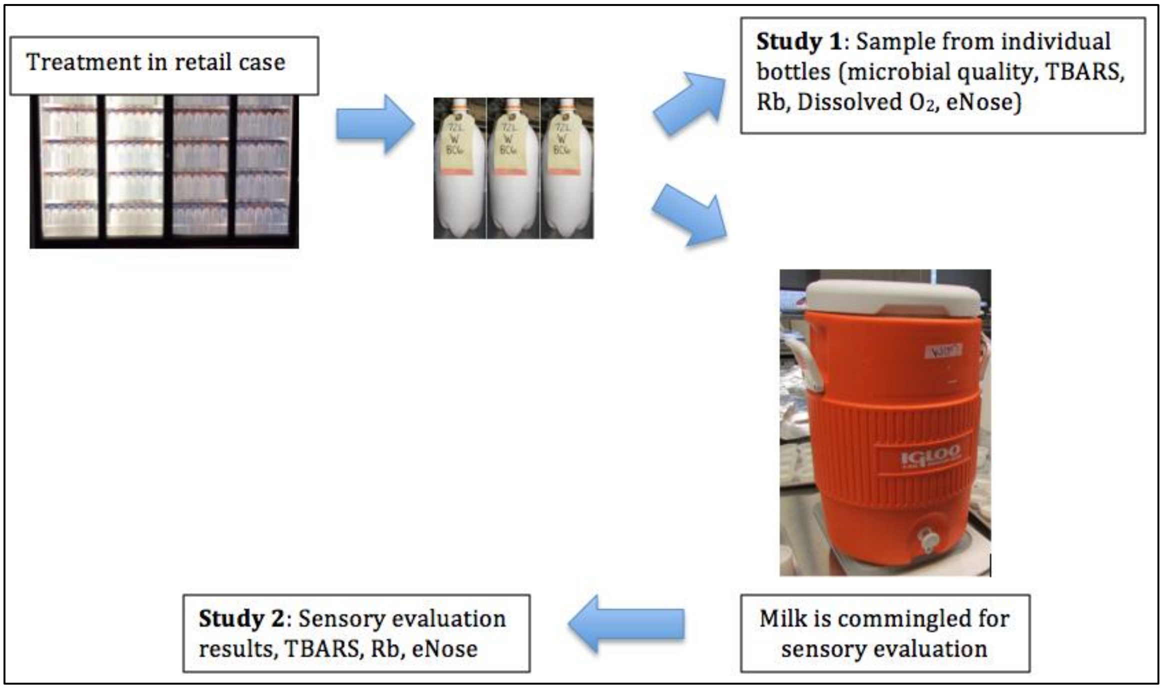


Figure 7. Flowchart of sampling plan for Study 1 and Study 2.

4.2.5 Sampling Plan

Fresh milk (0 h of light exposure) had triplicate samples collected for microbiological quality, TBARS, and Rb degradation. Ten samples were also collected for electronic nose (eNose; Sensigent LLC, Baldwin Park, CA) volatile analysis. Fresh milk underwent microbiological quality analysis to ensure that any off-flavor and quality deterioration was a result of treatment conditions and not due to low quality milk before the experiment began. TBARS, Rb degradation, and eNose samples were collected and analyzed to provide a baseline for analytical testing after experimental treatments.

United Dairy Herd Improvement Association (DHIA; Radford, VA) analyzed milk composition in duplicate for all three replications (n=6). Standard composition of 2% milk was verified by analyzing butterfat, true protein, solids-not-fat, and lactose composition by a Foss Milkoscan™ with a Fourier Transform Infrared spectrophotometer

(Eden Prairie, MN). Average milk composition was 1.96% butterfat, 3.13% true protein, 4.84% lactose, and 8.86% solids-not-fat.

4.2.5.1 Study 1 Sampling Plan

Following each time treatment (4, 8, 24, and 72 h), dissolved oxygen levels in milk were measured using a dissolved oxygen meter (LDO101, Hach, Loveland, CO). Measurements were taken immediately after each bottle for each packaging treatment was opened to avoid atmospheric oxygen levels influencing dissolved oxygen levels in milk. The meter probe was sanitized before beginning measurements and between each reading to prevent contamination. Dissolved oxygen readings were also measured prior to all other sample collection to prevent oxygen incorporation in the milk during bottle agitation that could affect dissolved oxygen levels in milk. All packages were sampled for the first two time treatments of Rep 1. However, due to time constraints, for the remainder of the experiment dissolved oxygen was measured only for four randomly selected bottles (n=8 available bottles) for each packaging treatment condition.

One sample from each bottle for each packaging treatment was collected to test for formation of secondary oxidation products by TBARS analysis (10 mL), and degradation of riboflavin (Rb) by fluorometric analysis (40 mL). Three samples from individual treatment bottles were analyzed to create a triplicate analysis for each light intensity classification (Fluorescent: high, medium, low; LED: medium, low). This created a total of nine samples from each treatment package analyzed for fluorescent light and six samples for each treatment package for LED light since the LED retail case did not have any high light intensities.

Samples (8 mL) were randomly collected from six of the eight treatment bottles for each package to complete electronic nose (Sensigent LLC, Baldwin Park, CA) volatile analysis on 8 h milk and 24 h milk. Six samples were needed to complete canonical discriminate analysis (CDA) for volatile differences. These two time treatments were chosen as the most likely to show differences in volatile profiles and as important times for consumer selection of milk. Individual packages were agitated prior to TBARS, Rb, and eNose sample collection to ensure the sample was representative of the entire package.

To test if there were significant oxidation related compositional changes in milk, two packages of milk for each packaging treatment were stored in a dark walk-in case for an 18-d shelf life study. These packages were sampled to complete microbiological analysis at 9 and 18 d to study how the microbial quality of milk changed over its shelf life. Sampling at 9 d was done to ensure that the microbiological quality of milk was acceptable for the duration of sensory testing (which was completed by 7 d post processing). Samples for TBARS analysis and riboflavin degradation were also collected at 18 d and frozen (-20°C, Harris Environmental Systems, Andover, MA) until analysis could be completed (Appendix H).

Microbiological quality samples were plated immediately following sample collection. Electronic nose samples were analyzed within six hours of collection from treatment packages. All TBARS and Rb samples were frozen (-20°C, Harris Environmental Systems, Andover, MA) until analysis was completed. All analyses were completed within eight weeks of sample collection.

<u>4.2.5.2 Study 2 Sampling Plan</u>

After samples were collected from each individual bottle, six bottles of milk from each packaging treatment were commingled into insulated beverage coolers (18.9L/ 5 gallons) for sensory evaluation. Samples for electronic nose volatile analysis (8 h and 24 h), formation of secondary oxidation products by TBARS analysis, and degradation of riboflavin (Rb) by fluorometric analysis were collected from the commingled milk for each packaging treatment as described above. Electronic nose samples (8mL) were collected in 40 mL amber glass bottles (80% headspace) and analyzed within six hours of collection from treatment packages. All TBARS and Rb samples were collected in polypropylene tubes, protected from light with a foil overwrap, and frozen in a blast freezer (-20°C, Harris Environmental Systems, Andover, MA) until analysis was completed. All analyses were completed within eight weeks of sample collection.

4.2.6 Microbiological Quality

To ensure that milk was properly pasteurized, two microbiological quality tests were performed on fresh milk (0 h light exposure) in triplicate at the beginning of each replication (n=3). Growth of aerobic organisms was measured by a standard plate count (SPC) of Petrifilm™ Aerobic Count Plates and Coliform Count Plates (3M, St. Paul, MN). These analyses were completed by standard methods (Laird et al., 2004). Aerobic Count Plates were incubated at 32° ±1°C for 48 ± 3 h and Coliform Count Plates were incubated at 37°±1°C for 24 h. Two packages from each treatment were kept in a dark walk-in cooler for 18 d following processing. One package from each treatment underwent microbiological testing as described above at 9 d post processing, and the remaining package underwent the same microbiological testing at 18 d post processing.

These tests were compared to fluid milk quality standards to ensure safety with microbial counts below 20,000 SPC/mL and below 10 CFU/mL (Jay et al., 2005).

4.2.7 Electronic Nose Volatile Analysis

Samples of 8 mL were collected for electronic nose volatile analysis in 40 mL test tubes to create 80% headspace and 20% liquid. Samples were stored in amber test tubes to protect from further light exposure and refrigerated in a dark walk-in cooler at 4°± 1°C for 30 minutes to allow sample headspace to come to equilibrium before analysis. Each sample was "sniffed" once by the preset (Table 1) Cyranose® 320 (Sensigent LLC, Baldwin Park, CA). The Cyranose® requires between 5-10 "smells" to create a cluster for each treatment. One sample was collected from six of eight bottles from each treatment to create a treatment cluster. Fresh milk electronic nose measurements created a baseline for electronic nose comparisons. All electronic nose analyses were completed within six hours of removal from the retail case. Due to time constraints and overlap with sensory evaluation, electronic nose volatile analysis was only completed for fresh milk, 8 h, and 24 h light exposure for each replication.

Table 1. Cyranose® 320 setting for the evaluation of 2% fluid milk.

Method Setting	Parameter Setting
Baseline purge	10 seconds
Sample draw	30 seconds
Air intake purge	10 seconds
Sample gas purge	60 seconds
Digital filtering	On
Substrate heater	On: 40 °C
Training repeat count	1
Identifying repeat count	1
Statistical analysis	
Algorithm	Canonical
Preprocessing	Auto-scaling
Normalization	Normal 1
Identification quantity	Medium

4.2.8 Formation of Secondary Oxidation Products Measured by Thiobarbituric Acid Reactive Substances Analysis (TBARS)

The formation of secondary oxidation products including malondialdehyde (MDA) and other aldehydes were measured by TBARS analysis as adapted from Spanier and Traylor (1991). One sample from each bottle was collected and analyzed for secondary oxidation products. Samples from each commingled milk packaging treatment were also analyzed. All treatments from individual bottles and commingled milk were analyzed in triplicate or higher. Analyses were completed as described in Chapter III.

4.2.9 Degradation of Riboflavin (Rb) by Fluorometric Analysis

Rb concentration of milk samples was analyzed using a modified fluorometric analysis (AOAC method 970.65, 1995) and measured on Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instrument, Inc., Columbia, MD; Webster et al. 2009). One sample from each bottle was collected and analyzed for Rb degradation. Samples from commingled milk packaging treatment were also analyzed. All treatments from individual bottles and commingled milk were analyzed in triplicate or higher. Analyses were completed as described in Chapter III.

4.2.10 Sensory Evaluation

Institutional Review Board approval was obtained (IRB# 11-477) from the Virginia Tech Office of Research Compliance before completing any sensory testing. Sensory testing took place in the Sensory Laboratory (Room 205; Virginia Tech Human and Agricultural Biosciences Building, 1230 Washington St SW, Blacksburg, VA). Sensory evaluation for each time treatment was completed on separate days (3 replications; n=12 d; Appendix D).

4.2.10.1 Sample Preparation and Organization

Eighteen triangle test comparisons of treatment and control milk was conducted for each light exposure time treatment (4, 8, 24, and 72 h), with nine comparisons under fluorescent light and nine comparisons under LED light. These comparisons were completed for three replications, with all replication responses combined, to achieve the targeted number of observations per comparison. Two randomized 3-digit codes were prepared for every treatment (light type*package) in each triangle test comparison and twenty randomized 3-digit codes were prepared for the control treatments to ensure a unique code was used for the control milk in each triangle test comparison (n=72 codes). A total of sixty sample cups was labeled for each treatment milk in every triangle test comparison. All coded sample cups were labeled prior to sensory evaluation.

On the day of sensory evaluation, coded sample cups were poured, capped, and stored at 3-4°C in a dark refrigerator until sensory evaluation was complete. After commingling milk from six individual bottless into an insulated 5-gallon beverage cooler, approximately 28 mL (1 oz.) was poured into individual (coded) two-ounce sample cups and capped by hand. Sufficient milk samples were prepared to complete eighteen total sensory comparisons, with each test comparing a treatment packages to a control. Similarity (n=8) and difference (n=10) comparisons were completed for each of the two lighting conditions (fluorescent and LED). During the sensory panel, samples were organized by comparison in eighteen double layered aluminum pans (9" x 13") with ice on the bottom layer and foil cover over the top layer for temperature and light control. This organization allowed for ease of panelist tray preparation since each tray contained five triangle test comparisons. Prepared trays were stored in a dark refrigerator (3-4°C) until sensory evaluation by panelist. All samples were tasted at refrigeration temperatures (4°C) to mimic typical consumer experiences drinking milk.

4.2.10.2 Panelist Timeline during Sensory Testing

Sensory evaluation took place in individual booths under white light. All responses were recorded on a touchscreen monitor. Before completing sensory evaluation, each panelist read and signed an informed consent (IRB #11-477, Appendix B). Panelists evaluated milk samples for similarities and differences by triangle test (Meilgaard et al., 2007). Each panelist completed five sets of triangle tests. Each set included three coded milk samples, representing one packaging treatment and a control, and were presented in a randomized incomplete block design (SIMS, Sensory Information Management Systems, Sensory Computer Systems, Berkeley Heights, NJ). Panelists were instructed to taste each sample from left to right, according to the sample order presented on the touchscreen monitor, and select the sample that was different by recording their decision on the monitor (see Appendix C for sample touchscreen ballot).

Unsalted crackers and water were available to panelists as a palate cleanser between samples and between triangle tests. Panelists were instructed to expectorate samples to prevent sensory fatigue.

4.2.10.3 Panelist Recruitment and Numbers

Sensory testing was completed for all three replications. Panelists were recruited from the Virginia Tech student body, faculty, staff and local community members. Panelists were recruited through the Virginia Tech Sensory Evaluation Laboratory email listserv and Facebook page. A goal of 144 panelists was targeted for each day of sensory evaluation to obtain 40 observations per comparison. With three replications of each time treatment, a total of 113-120 completions for each comparison were achieved. All data was collected and analyzed using SIMS 2000 (Sensory Information Management Systems, Sensory Computer Systems, Berkeley Heights, NJ). Proportion of discriminators for this study was estimated to be approximately 20% (Amin, 2016). A total of 120 responses created parameters of α=0.20 and β=0.05 for similarity testing and α=0.05 and β=0.20 for difference testing (Meilgaard et al., 2007). With these parameters, there needed to be 45 correct responses for the similarity comparisons and 50 correct responses for the difference comparisons (Meilgaard et al., 2007).

4.2.11 Statistical Analysis

Eighteen comparisons of PET treatment packages to light-protected and light-exposed controls were completed in three replications for each time treatment. Significant p-values ($p<0.05$) were determined from the number of correct trials for each comparison (Table 2). Differences ($p<0.05$) in the eighteen triangle tests were analyzed with the alpha and beta parameters detailed above using SIMS 2000 (Sensory Information Management

Systems, Sensory Computer Systems, Berkeley Heights, NJ). Packages were deemed successful at protecting milk quality if sensory testing results were not significantly different (similar; $p>0.05$) to light-protected milk and if they were different ($p<0.05$) from light-exposed milk under each lighting condition. Packages were considered "partially protective" if they were similar ($p>0.05$) to both light-protected and light-exposed milk, because it retained similarities to high quality milk, even if it could not be differentiated from oxidized milk. Packages were "partially ineffective" if they were different ($p<0.05$) from both light-protected and light-exposed controls because it was not similar to high quality milk but was not the same as oxidized milk. If a package was different ($p<0.05$) from light-protected milk and similar ($p>0.05$) to light-exposed milk then it is considered "completely ineffective" with detectable differences and oxidized off-flavors. Complete details of the comparisons tested, proportion of discriminators, confidence intervals, and power analysis are presented in Appendix E.

Analytical tests (TBARS, Rb, dissolved oxygen) were completed in triplicate or higher for each treatment condition. Outliers were removed from analytical test results at 95% confidence using Dixon's Q test (Cochran and Snedecor, 1980). Means and standard deviation were calculated for TBARS, Rb degradation, and dissolved oxygen (Microsoft® Excel® for Mac 2011, Version 14.5.8, Microsoft Corporation, Redmond, WA). A 3-way analysis of variance was used to measure significant changes ($p< 0.05$) in oxidation (JMP Pro 11, SAS Institute, Cary, NC). Analysis of variance was based on three replications of time of light exposure ($n=4$), type of light ($n=2$) and package type ($n=6$). Tukey's honestly significant difference (HSD) test further explored significant differences within the time and package variables (JMP Pro 11, SAS Institute, Cary, NC). Tukey's HSD test was chosen because it is more conservative and has less chance of detecting a difference when there is not (Type I error). The effect of light intensity on TBARS, Rb degradation, and dissolved oxygen was studied by analysis of covariance (JMP Pro 11, SAS Institute, Cary, NC).

Volatile analysis (electronic nose; eNose) samples were analyzed through canonical discriminate analysis (CDA; JMP Pro 11, SAS Institute, Cary, NC) and random forest model systems (R, Version 3.2.2, R Core Team, Vienna, Austria). Classification trees are used to predict membership of objects in a class based on their measurement of one or more predictor variables. A random forest is a collection of a large number of classification trees where the trees vote for the most popular class in a series (Breiman, 2001). For the eNose, the classes were comprised of the 32 volatile-detecting sensors. Trees serve as classifying systems to determine which sensors were most important at detecting volatile differences in oxidized milk. A single tree will only have slightly better accuracy in distinguishing classes than random choice, but when several trees are combined together in a random forest, there is a greatly improved accuracy in selection of the correct class (Breiman, 2001).

4.3 Results and Discussion

This study tested the efficacy of experimental PET packages in protecting milk from light-induced oxidation reactions. The effects of combining the higher oxygen barrier properties found in PET materials with the light interference properties of UV barrier and TiO_2 loading were evaluated. These results provide a more complete picture of changes to milk composition that occurred in milk stored in PET packages. There is also valuable insight of the effects of different types of retail lights with direct comparisons between milk in the same packages under both fluorescent and LED retail light. This research provides more details about possible future options for milk packaging and retail storage conditions that go beyond what is currently found in the market.

4.3.1 Study 1

4.3.1.1 Retail Case Light Intensity

Overall light intensity averaged 1447 ± 1072 lux and 936 ± 136 lux in the fluorescent and LED retail cases, respectively. The fluorescent lights were more intense, but also had more variable intensities throughout different positions within the retail case, while the LED retail case provided less intense and more consistent light exposure throughout all positions (Appendix E). Due to the randomization of treatment packages in areas of low, medium, and high light intensity, each treatment package received similar levels of light exposure and intensity throughout the experiment (Appendix E).

4.3.1.2 Microbiological Quality

Microbiological quality was measured for milk to ensure that any changes in milk quality were a result of treatment conditions and not from low quality milk. For all three replications, fresh milk had zero coliform counts and <25 cells/mL for aerobic plate counts. This is well under standard quality limits for fresh milk quality (Murphy, 2009).

The original sampling plan was to complete microbiological analysis on milk at 18 d (end of shelf life for HTST pasteurized milk) to verify microbiological quality. This sampling plan was followed for Rep 1, plating 3-fold dilutions for coliform plate counts and aerobic plate counts. There was no significant coliform growth outside of milk quality standards; however, there were significant aerobic plate counts that deemed the milk “spoiled” by 18 d with 72% of samples had >250,000 cells/mL APC by 18 d. The higher microbial loads could be due to sampling process since fresh milk was within standard quality limits.

Due to the higher than expected microbial loads, microbiological analysis was also completed at a halfway shelf life point (9 d post processing) as well at 18 d post processing for Rep 2 & Rep 3. A fourth-fold dilution was also completed for APC measurements in addition to the other three-fold dilutions to better quantify the microbial load of the milk. There was no coliform growth in any milk samples from Rep 2 or Rep 3. A small portion of samples had microbial loads greater than 20,000 cells/mL APC after 9 d (Rep 2, 10%; Rep 3, 31%). The majority of milk was deemed “spoiled” (>250,000 cells/mL APC) by 18 day microbiological testing for both Rep 2 and Rep 3.

The average APC over Rep 2 and Rep 3 was 18,000 cells/mL by 9 d post processing. Even though a small percentage of milk sampled had microbial loads higher than 20,000 cells/mL 9 d after processing, this microbial threshold is the requirement for fresh milk immediately after pasteurization (Murphy, 2009). It has been shown that flavor defects do not occur in milk until it has 10 million CFU/mL SPC (Murphy, 2009), and the milk in this study was well under those limits. These results indicate that although milk had high microbial loads at the end of its 18-day shelf life than is optimal for quality, it was still of good microbial quality through all sensory testing (completed by 7 d post processing) and microbiological quality did not influence the results of this study.

4.3.1.3 Dissolved Oxygen

Oxygen plays an important role as a reactant in light-induced oxidation reactions. Light-induced reactions in milk require a photosensitizer and oxygen (Becker et al., 2003). Once light initiates photosensitive reactions, oxygen serves to propogate them. Since oxygen is consumed as a reactant, lower levels of dissolved oxygen (DO) can indicate the occurrence of more extensive oxidation reactions. This study demonstrated several associations between DO levels in milk and the extent of light-induced oxidation reactions, making DO a valuable analytical marker of oxidation reactions in milk.

Fresh milk, in original HDPE packaging from the processing plant, had higher levels of DO than all milk following treatment (Figure 8), with an average DO concentration of 11.39 ± 0.39 ppm, which is slightly higher than values reported in previous studies (6-7.5 ppm; Rysstad et al., 1998; Moyssiadi et al., 2004; Mestdagh et al., 2005). Fresh milk and milk after 4 h light exposure in this study had comparable DO levels to fresh milk and milk stored in HDPE packaging for 4 h fluorescent and LED light exposure in a similar study (10.41-11.89 ppm; Amin, 2016).

A 3-way analysis of variance (ANOVA) found that type of light, duration of light exposure, and package were all significant ($p<0.0001$) factors in how much DO was consumed by oxidation reactions ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). However, due to the complexity of these factors, there was significant ($p=0.0122$) 3-way light*time*package (pkg) interaction. There were also significant 2-way interactions for light*time ($p<0.0001$), light*pkg ($p=0.0016$), and time*pkg ($p<0.0001$). The data was sorted by time treatment to better explore significant interactions and fully understand the effect of each factor on DO content. Analysis of covariance was performed to determine if light intensity affected dissolved oxygen levels under either type of light, and was found to be nonsignificant under both fluorescent and LED light ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC).

Packaging proved to have more significant influence than the type of light exposure on DO content after short periods of light exposure. The type of package had a significant effect on DO concentration after both 4 ($p<0.0001$) and 8 h ($p=0.0232$) of light exposure, but the type of light exposure was not significant ($p>0.05$) after these times. Tukey's honestly significant difference (HSD) test for package after 4 h of light exposure identified that light-protected PET had the highest DO levels of all treatments, and that the high white PET and medium white PET packages retained similar ($p>0.05$) amounts of DO to this light-protected control. These packages retained more ($p<0.05$) DO than the light-exposed PET and UV barrier PET. The low white PET package retained an intermediate amount of DO that was less than the light-protected PET control, not different from the other TiO_2 -loaded packages, but more than the light-exposed PET control or the UV barrier PET (Appendix E).

This observed decrease as oxygen was being consumed in oxidation reactions confirms that such reactions were occurring in the milk and becoming more extensive as a result of greater light transmittance through the packaging material. When light energy reaches milk with sufficient energy to excite photosensitizers to transmit energy and initiate oxidation reactions, the available oxygen in milk contributes to subsequent and ongoing reactions. The higher levels of TiO_2 blocked light sufficiently to limit the initiation of photosensitive molecules and the rate of oxidation in milk components. The clear PET and UV barrier PET transmitted >80% of the visible light spectrum through the packaging material, allowing light energy to excite photosensitizers and extensive oxidation to occur (Appendix E). However, all three TiO_2-loaded packages with greater light interference properties blocked the visible light spectrum, transmitting <0.05% of light through the visible spectrum (Figure 8). The transmittance data for our experimental packages suggest that light transmittance needs to be blocked to <0.01% for the visible spectrum to sufficiently prevent photosensitizer excitation and subsequent oxidation reactions. PET with 4.0% and greater TiO_2 blocks light to <0.01% transmission, and retained the most dissolved oxygen throughout light exposure, indicating less extensive oxidation. Current recommendations from the International Dairy Federation for fluid milk packaging state that light transmission should be blocked to ≤4%, but these results indicate that light transmission may need to be blocked at much lower levels to sufficiently protect milk quality (Mestdagh et al., 2005; Webster et al., 2009). This has led us to conclude that light blocking properties of the package are most critical in preventing oxidation reactions in early hours of retail storage when DO content is high and photosensitizers do not have to compete for oxygen as much. The type of light transmitted through the packaging material does not play a significant role until longer periods of retail storage have occurred.

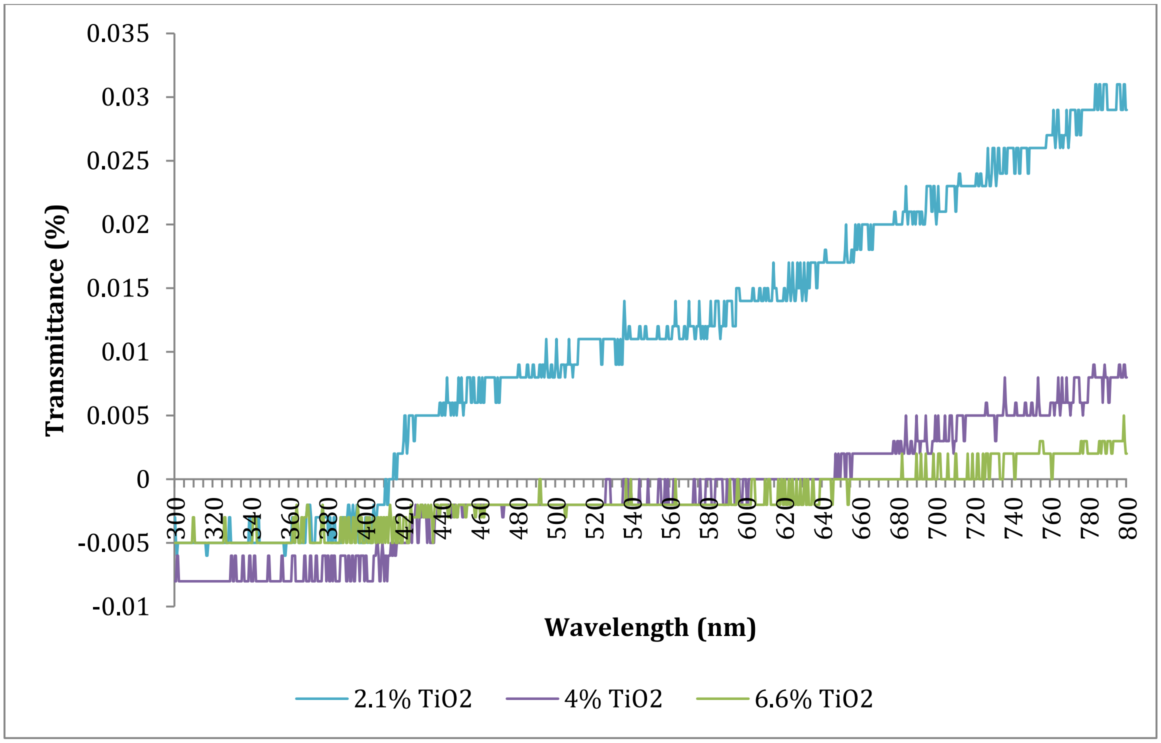


Figure 8. Light transmittance (%) of PET with low (2.1%), medium (4.0%), and high (6.6%) TiO_2 from 300-800 nm.

Both light type and package type were significant influences on DO content during longer periods of light exposure. After 24 h of light exposure, there were higher levels ($p<0.0001$) of DO under LED light than under fluorescent light, indicating that LED light initiated less extensive oxidation reactions than fluorescent light as light exposure lengthened. This trend continued as there was also significantly more ($p<0.001$) DO remaining in milk after 72 h of LED light exposure than after 72 h of fluorescent light exposure (8.34 and 7.41 ppm, respectively). This demonstrates that the type of light source is influencing the initiation and propogation of oxidation reactions by exciting photosensitizers to react with available oxygen.

Package was also a significant factor ($p<0.0001$) in DO levels after 24 and 72 h light exposure. After 24 h, the DO was significantly higher in milk packaged in the light-protected PET control and the high white PET package compared with all other packages tested (fluorescent: 9.63 and 9.49 ppm; LED: 9.71 and 9.53 ppm, respectively). The light-exposed PET control and UV barrier PET package retained the least amount of DO of all experimental packages after 24 h light exposure (under fluorescent light: 7.62 and 7.05 ppm; under LED light: 8.26 and 7.93 ppm, respectively). Tukey's HSD test for package after 72 h light exposure revealed the same trends, with the light-protected PET control and high white PET package retained significantly higher DO levels than all other treatments (fluorescent: 10.06 and 9.64 ppm; LED:10.06 and 9.65 ppm, respectively).

Since the decrease in oxygen levels can be related to the extent of oxidation reactions, these results demonstrate that LPAs play an active role in preventing the initiation of oxidation reactions by blocking excitation wavelengths for critical photosensitizers. The packages that better protected against light exposure by reducing light transmission (light-protected PET control and high white PET package) had higher levels of DO because less extensive oxidation reactions occurred as a result of less light reaching the milk and beginning the chain of photosensitive reactions. When less light transmitted through the package, photosensitizers were not able to receive sufficient energy for electrons to jump to the next orbital, then transfer that energy to susceptible molecules. The packages without LPAs that allowed greater light transmission to milk (light-exposed PET control, UV barrier PET) had lower levels of oxygen because greater light transmission allowed photosensitizers to excite oxygen to react with milk components until most of the available oxygen had been used, resulting in lower DO levels. These analytical measurements of oxidation are in agreement with the oxidation flavor identification in the sensory results presented in Study 2 below.

The interaction of time and light source was significant, suggesting that LED and fluorescent lighting influence the utilization of oxygen differently. The influence of time was explored by sorting the data by package and running 2-way time*light ANOVAs for the two control packages and high white PET package, since this treatment package showed the most protective effects against milk oxidation. Time was a significant factor in DO content for the light-protected control ($p<0.0001$) and the high white PET package ($p<0.001$). For both packages, Tukey's test revealed that these packages maintained significantly higher levels of DO through 4 h of light exposure than for all other time treatments, demonstrating the adverse effects of longer periods of light exposure on milk quality. Both time ($p<0.0001$) and light type ($p<0.0001$) were significant factors for the light-exposed control, indicating that the type of light is more critical when LPAs are not present and the entire spectrum of fluorescent or LED light reaches milk. As seen above, DO levels remained significantly higher in milk exposed to LED light than milk exposed to fluorescent light, confirming that LED light exposure caused less extensive oxidation reactions in milk than fluorescent light exposure. It is possible that the LED light spectrum is less effective at exciting photosensitizers that compete for available oxygen successfully at low levels. The leveling off of DO content in milk stored under LED light for longer time periods could indicate that LED light is less excitatory to photosensitizers in milk which would result in less detrimental changes to milk quality. This difference in oxygen consumption between fluorescent and LED light could also be the result of a shift between Type I and Type II oxidation pathways that occurs as a result of oxygen concentration and competition from milk compounds (Min and Boff, 2002).

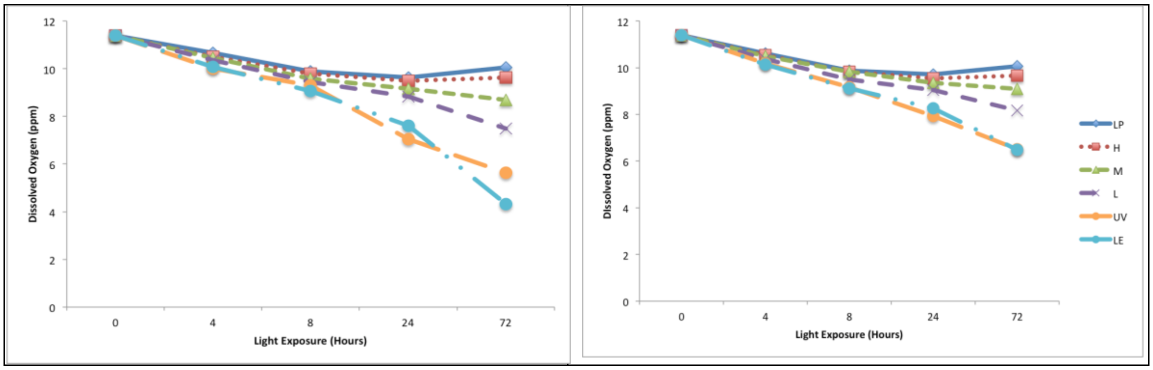


Figure 9. DO concentration (ppm) in 2% milk under retail lighting: a.) fluorescent light b.) LED light. LP= light-protected (foil and plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= clear PET package with UV barrier; LE= light-exposed PET control.

When all three factors studied are combined together, the most optimal conditions for preventing oxidation reactions in milk include storing milk in packages with high levels of LPAs under LED light for shorter periods of time (<8 h). These results demonstrate the importance of measuring DO as an indicator of the presence and availability of oxidation reactants. Previous work supports our results where UHT milk packaged in PET packages saw a decrease in DO levels as a function of storage time and light exposure (Mestdagh et al., 2005). There were slightly lower levels of DO in this study (fresh milk= 6ppm), but similar trends were seen in decreased DO levels in their PET package with a UV barrier as were seen with our UV barrier PET package (Mestdagh et al., 2005). Mestdagh et. al (2005) also found that UHT milk stored in a PET package that was not exposed to light did not see a decrease in DO over 60 d of storage, supporting our results that show that DO levels are influenced by light exposure because the oxygen is being consumed in light-induced reactions. This has led us to conclude that DO is of significant importance in milk oxidation.

4.3.1.4 Formation of Secondary Oxidation Products Measured by Thiobarbituric Acid Reactive Substances Analysis (TBARS)

Aldehyde formation from lipid oxidation products, measured by modified TBARS methods, has previously served as a benchmark for oxidized off-flavor levels in fluid milk. However, this analytical method only detected very low levels of oxidation throughout our experiment, with all aldehyde levels averaging 0.65 mg/L or less and did not compare with other oxidation measures, such as sensory evaluation (results presented below) and DO content (Appendix E). The TBARS method was originally developed to measure oxidation in meat and thus, some limitations are prevalent. Mestdagh et al. (2005) found no distinction in TBARS between milk samples that received no light exposure and milk samples that had distinct light-oxidized off-flavor after 30 h UV light exposure. This could explain why, even after 72 h of light exposure, our aldehyde levels were extremely low compared to previous results (Johnson et al., 2015; Walsh et al., 2015).

Aldehyde levels in fresh milk samples (n=9) averaged 0.17 ± 0.03 mg/L. Similar results for fresh milk have been reported in related literature, so this indicated very little oxidation prior to experimental treatment (Cladman et al., 1998; Zygoura et al., 2004). Effects of the treatment factors were compared by a 3-way light*time*pkg ANOVA for differences ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). There was no difference ($p>0.05$) in aldehyde levels between any control and experimental PET packages, but light type and time both had a significant ($p<0.0001$) effect on TBARS production. However, there was significant ($p<0.001$) 2-way time*light interaction due to the complexities of fluorescent and LED light spectrums. TBARS data was sorted by light type to better explore the effects of light on milk over different storage durations. An analysis of covariance was completed to determine if the intensity of fluorescent or LED light significantly affected aldehyde levels and found no significant difference (p>0.05) in TBARS as light intensity increased.

Even though it was not significant in the 3-way model, packaging had an effect on lipid oxidation in the 2-way pkg*time ANOVAs under fluorescent lighting (p=0.0058), but not under LED lighting (p=0.5292). Under fluorescent light, milk stored in medium white PET and UV barrier PET had significantly higher aldehyde levels than the light-exposed PET control. This did not agree with our hypothesis that the light-exposed control would have the highest TBARS levels because increased light exposure would excite more photosensitizers to initiate oxidation. It is possible that interaction occurred between milk and LPA packaging materials, such as scalping, leading to higher aldehyde levels in the white and UV fortified PET packages. Scalping can occur through migration, when substances from a package are transferred to a food product (Suloff, 2012). Acetaldehyde is a compound formed during the processing of PET packaging, and can migrate into milk through interactions between packaging materials and food, where it can contribute to off-flavors and quality degradation of the product (Suloff, 2012). It is possible that migration of acetaldehyde lead to the higher levels of aldehydes measured in the white and UV fortified PET packages.

Different trends were seen with TBARS production as the length of light exposure increased. Time did not influence TBARS production under fluorescent light (p=0.1600), but caused differences in aldehyde levels under LED light (p<0.0001). TBARS values were significantly higher after 4 h of LED light exposure than all other treatments, but by 72 h LED light exposure TBARS values were lower than all other time treatments. It is interesting to note that TBARS production was highest after only 4 h of light exposure and apparently decreased during longer light exposure. This does not correspond with the increasingly apparent sensory differences between light-protected control milk and treatment milk exposed to light, which indicated that longer periods of light exposure caused flavor differences in milk that did not occur in the light-protected milk (see sensory results). Light-induced flavor differences are typically off-flavors that result from oxidation; hence, the unexpected association we found with more obvious sensory differences and lower lipid oxidation products. However, it has been noted in previous work that TBARS results are not always well associated with sensory results (Barrefors et al., 1995), which could explain the discrepancy.

The variable composition of lipid oxidation products that changes over time could also explain why the TBARS assay detected higher concentrations at 4 h than at 8, 24, or 72 h. During lipid oxidation, peroxides are formed as primary products, but the concentration of peroxides will slope off as time increases during the lipid oxidation reactions. Secondary oxidation products, such as aldehydes, are slower to form at measurable levels during lipid oxidation reactions. The TBARS assay detects mainly aldehyde levels and is not an accurate indication of primary oxidation products or secondary products that are not aldehydes. The unexplained results from the TBARS analysis could have been measuring different types of oxidation products that resulted in higher concentrations at 4 h than at 8, 24, or 72 h.

Even with these differences, all aldehyde levels measured were very low compared to previous milk oxidation studies. Previous literature showed that TBARS results were less sensitive at low aldehyde concentrations, similar to those seen in this experiment, and are not always associated with noticeable off-flavors (Rysstad et al., 1998). TBARS values in this experiment were very low compared to TBARS levels from HDPE packages that caused noticeable differences in milk oxidation (Zygoura et al., 2004; Johnson et al., 2015; Walsh et al., 2015). All aldehyde levels measured in this experiment are well below previously suggested thresholds for taste quality by Johnson et al. (2015), who found that 1.3 mg/L was a marker of changes in UHT pasteurized milk sensory quality as a result of light exposure. This indicates that 2% milk could have a lower TBARS threshold for taste quality than UHT milk. However, due to the lack of consistent results at very low aldehyde concentrations, an alternative method of analysis might be more appropriate for future work on milk oxidation. A more complete understanding of lipid oxidation products could be gained by performing multiple methods of lipid oxidation analysis together, such as conjugated dienes/trienes to measure late forming primary oxidation products in addition to TBARS measurements for early forming secondary oxidation products. These complementary analytical assays would be time consuming and challenging at such low levels of oxidation products, but may help to better identify and distinguish volatiles that are contributing to flavor differences from milk oxidation.

4.3.1.5 Degradation of Riboflavin (Rb) by Fluorometric Analysis

One of the reasons milk is sensitive to light-induced reactions is because it contains relatively high levels of the photosensitizer, riboflavin (Rb, Vitamin B_2). Many studies have measured the degradation of Rb from light exposure as a marker of package performance in blocking light wavelengths (specifically 400, 446, and 570 nm) that excite Rb (Webster et al., 2009; Intawiwat et al., 2010; Intawiwat et al., 2013). Fresh milk in this study contained 1.24 ± 0.24 mg/L Rb. This is lower than we saw in Potts (Ch. III,

2016) and lower than some reported values that have shown 2% milk to have 1.69-1.75 mg/L Rb (Dimick, 1982; Gebhardt and Thomas, 2002; Brotherson et al., 2016), but our fresh milk Rb was within range of other studies (1.10-1.46 mg/L; Zygoura et al., 2004; Saffert et al., 2006; Amin, 2016).

The effects of light, time of light exposure, and package were studied by 3-way light*time*pkg ANOVA. The only main effect that had a significant impact ($p=0.001$) on Rb concentration was packaging type. However, there was significant ($p=0.0061$) 3-way light*time*pkg interaction ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC) when all factors were analyzed together due to the complexities of light interactions with packaging over time. The effect of light intensity was determined through an analysis of covariance for fluorescent and LED light ($\alpha=0.05$; JMP Pro 11, SAS Institute, Cary, NC). Light intensity did not significantly influence Rb degradation ($p>0.05$) for either light type. Data was sorted by light type (fluorescent or LED) because of the significant interactions and 2-way time*pkg ANOVAs were analyzed to better understand the changes of Rb under fluorescent and LED light.

Under LED light, both time ($p=0.0150$) and package ($p=0.0099$) significantly influenced Rb degradation in the 2-way time*pkg ANOVA. More Rb was retained in milk after 4 h of LED light than after 24 h of LED light exposure. This supports previous literature on Rb retention under fluorescent light, where Rb decreased as length of light exposure increased (Webster et al., 2009; Walsh et al., 2015; Brotherson et al., 2016). Under LED light, both the light-protected PET control and the high white PET package protected Rb in milk; both packages retained similarly high levels of Rb. The light-protected PET control retained higher levels of Rb than all other treatment packages and the light-exposed control. Rb content in the high white PET package was not different than the other treatment packages or light-exposed control. These results show that the higher loading of TiO_2 in the high white PET package was able to block light sufficiently to protect Rb from becoming fully excited in milk under LED lights and retain amounts of Rb that were not statistically different from the light-protected control that allowed no light to reach milk.

When analyzing the Rb samples under fluorescent light in a 2-way time*pkg ANOVA, there was significant 2-way interaction (p=0.0269). Due to the interaction, Rb samples were sorted by time to run a 2-way light*pkg ANOVA to better explore differences in treatment factors over time. Light caused significant differences in Rb levels at 4 and 24 h of light exposure. There was more Rb retained under LED light than fluorescent light after 4 h of light exposure (p=0.0023), which agrees with dissolved oxygen analytical results that indicate that LED light is less effective than fluorescent light at activating photosensitizers, such as Rb, to initiate oxidation. However, more Rb was retained under fluorescent light than LED light after 24 h (p<0.0001). This could indicate more extensive oxidation reactions from photosensitizers under LED light, but the higher levels of fluorescence products measured under fluorescent light could be a result of the fluorometric assay detecting higher levels of lumichrome and lumiflavin, which are produced as Rb degrades during oxidation reactions (Wold et al., 2002). It is possible that oxidation reactions are still occurring under fluorescent light, even though the higher levels of Rb after 24 h fluorescent light exposure indicate otherwise. After 8 h of light exposure, package type significantly effected Rb levels (p=0.0091), but type of light did not. Tukey's HSD test revealed similar trends as described above with the packages under LED light. Milk stored in the light-protected PET package retained the most Rb and the high white PET package retained a similar amount by 8 h light exposure, making high white PET the most successful packaging material tested for vitamin retention. All other packaging treatments retained significantly lower amounts of Rb than the light-protected control after 8 h light exposure.

Overall, there was a relatively high Rb retention throughout this study (Appendix E). After 72 h of light exposure, all treatment packages retained 53% or more Rb under fluorescent light and 63% or more Rb under LED light. These results are supported by Brotherson et al., (2016), who studied the effects of Rb in 1% milk under fluorescent and LED light through 24 hours and only saw a significant decrease in Rb content after 24 h fluorescent light exposure. PET with higher TiO_2-loading has protective qualities that help maintain levels of light sensitive vitamins in fluid milk by blocking light transmission to milk. It is possible that the oxidized off-flavors seen in the milk in this experiment resulted from reactions of other compounds such as chlorins and porphyrins, which have been found significant by previous work, instead of from the degradation of Rb (Wold et al., 2005; Intawiwat et al., 2013). Our experimental packages did not completely block light from the orange spectrum, since the clear PET and UV barrier PET allowed >80% transmission of this light and the TiO_2-loaded PET allowed up to 0.03% transmission from the orange light spectrum (Figure 8). Light from the orange spectrum could have excited chlorins and porphyrins to create oxidative changes in milk in our study. Fluorescent light contains more emission peak wavelengths above 500nm than LED light, which could activate chlorins and porphyrins to create more off-flavors, supporting our sensory results that showed more extensive flavor differences under fluorescent light than LED light (Intawiwat et al., 2010; Brotherson et al., 2016).

4.3.1.6 Volatile Analysis by Electronic Nose

Oxidized milk contains naturally occurring volatile compounds (Marsili, 2000; Webster et al., 2009; Brotherson et al., 2016). Changes in the volatile composition of milk can be used as an indication of off-flavor development. Milk with an oxidized off-flavor has been shown to have significantly higher concentrations of the volatiles hexanal, pentanal, octanal, and nonanal than milk with no oxidized off-flavor (Barrefors et al., 1995; Webster et al., 2009). The electronic nose (eNose) is a rapid analytical technique that can measure volatiles through a series of sensors. ENoses are not quantitative tools, but can be used as a "good/bad" or "accept/reject" decision aid (Sensigent, 2013). For the purposes of this experiment, we did not train the eNose to recognize specific volatiles; instead, we used fresh milk as a standard for non-oxidized milk quality and compared milk in treatment packages following light exposure to the fresh milk standard for differences. In this experiment, milk was analyzed for volatile composition after 8 h and 24 h of light exposure. These times were chosen as representative of the time milk remains in the retail case prior to purchase and far enough apart to have different volatile compositions.

There was data overlap from the Cyranose® because of the large number of sensors (32) contained in this eNose model. While more sensors would be appropriate for analyzing a food with very intense or complex volatile composition (i.e. wine), we found that thirty-two sensors were not necessary to analyze the relatively consistent volatile composition of pasteurized milk, and the large amount of data collected from these sensors created a lot of noise. A random forest data classification analysis (R, Version 3.2.2, R Core Team, Vienna, Austria) was completed to determine which sensors in the Cyranose® were most effective at determining differences in milk's volatile composition for each time treatment. This classification found that different sensors were more discriminatory of volatile composition in milk stored under different types of light and for different time periods. However, this classification was not able to distinguish differences between packaging types, indicating that the changes in 2% pasteurized milk volatile compositions from milk oxidation were not greatly influenced by different levels of light interference properties in PET packaging.

The length of storage under fluorescent light influenced which sensors were best at distinguishing milk volatile differences. For the milk stored under fluorescent light for 8 h, sensor 7 (S7), S9, and S11 were most discriminatory between the volatile compositions in different packages. After 24 h of fluorescent light exposure, S2, S6, S10, and S13 were best at detecting differences in volatile composition. However, for milk stored under LED light, the same three sensors (S1, S3, and S4) were crucial for determining differences between milk in experimental packages, regardless of storage duration. The importance of the sensors detailed above is supported by distinguishing sensor information for the Cyranose® 320 eNose. S5, S6, S23, and S31 can become saturated at ambient humidity for aqueous based products, resulting in lower discriminatory abilities by these sensors (Sensigent, 2013). None of these sensors were found to be significant in distinguishing fresh milk from milk after retail light exposure. If the eNose were trained with standard volatile compounds, we could better identify which volatiles, or class of volatiles, each of these discriminatory sensors detects. Future work studying volatiles in oxidized milk under similar conditions should employ the "active sensors" function of the Cyranose® 320, which allows the user to select which sensors to be active during measurement. Now that the most discriminatory sensors have been identified by random forest classification, this function could reduce the amount of noise seen in our results and allow for better differentiation in oxidation of fluid milk samples.

Changes in volatile composition were analyzed by canonical discriminate analysis (CDA; JMP Pro 11, SAS Institute, Cary, NC). The Cyranose® required a minimum of five "smell-prints" to create a cluster of Euclidean distances for each treatment's CDA. Euclidean distances measure the distance between an individual exposure and the center of all exposures in a cluster (Sensigent, 2013). The circles in the CDA plots represent 'means CL ellipses' for each treatment cluster, which is the 95% confidence region to contain the true mean of the group. CDA was completed for each treatment using six smell-prints from three replications, creating a total of 18 smell-prints for each treatment. CDA was completed using data from all 32 sensors as well as for the top 3-4 sensors identified as being the most discriminatory for each light type and time. However, even using only the top sensors, there was still substantial overlap between volatile profiles from all treatment packages, including the two controls. Due to the lack of improved discrimination using the top sensors, all CDA plots included in this work include data from all 32 sensors. Fresh milk was always significantly different from all other milk treatments (100% separation; $p<0.05$; Appendix F).

The fresh milk comparison was removed from CDA to better explore differences in volatile profiles between PET treatment packages that resulted from light exposure.

Volatile profiles were compared between treatments that underwent 8 h of fluorescent or LED light exposure (Figure 10). There was limited separation after 8 h, indicating some volatile changes from early oxidation. Under fluorescent light, the two packages that provided the least light interference properties (clear PET and UV barrier PET) were separated ($p<0.05$) from the other packages that blocked light from reaching milk. This indicated that these packages were undergoing volatile changes due to light-induced reactions that were not as extensive as the packages that prevented light from reaching milk. The fluorescent light was possibly transmitting more energy to photosensitizers in milk than the LED light, since different trends were seen in milk stored under LED light. After 8 h LED light exposure, the two controls had different ($p<0.05$) volatile profiles from all treatment packages, but not from each other. The high white PET package was also completely separated ($p<0.05$) from all other packages, indicating that it had the most different volatile profile of all milk under LED light.

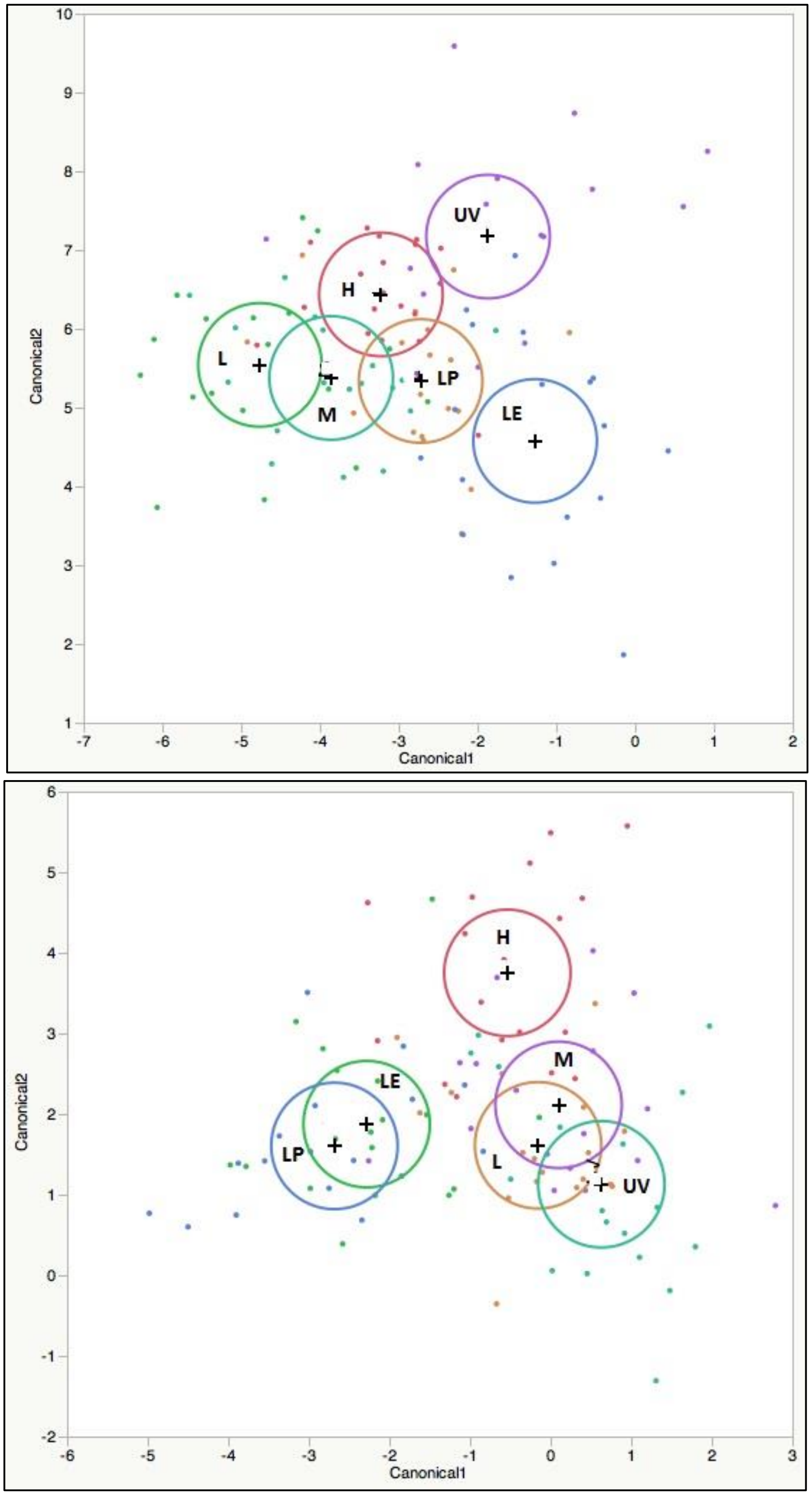


Figure 10. Canonical discrimination analysis (CDA) plots for 2% milk from PET packages after 8 h of fluorescent (top) or LED (bottom) retail light exposure using all 32 sensors. LP= light protected control, LE= light exposed control, H= high white PET, M= medium white PET, L= low white PET, UV= UV barrier PET.

There were larger separations in volatile profiles of milk after 24 h light exposure, indicating that light continued to initiate and propagate oxidation reactions that created changes in volatile compounds during longer periods of light exposure. CDA plots were compared for all PET treatment packages after 24 h of fluorescent or LED light exposure (Figure 11). Under fluorescent light, both controls (light-protected and light-exposed) had similar volatile profiles to each other ($p<0.05$), but were different from all other PET packages. The PET packages with medium and high levels of TiO_2 protected milk quality similarly, because they had similar volatile compositions after 24 h fluorescent light exposure that were different ($p<0.05$) from all other packages. Milk from the low white PET and UV barrier PET packages produced volatile compositions that were completely different ($p<0.05$) from all other PET packages. After 24 h LED light exposure, the two controls and low white PET packages were 100% separate from all other treatments. There was still substantial overlap between the high white and medium white PET packages and limited overlap with the UV barrier PET package. Both fluorescent and LED light initiate reactions in milk that change the volatile composition. These changes begin as early as 8 h of light exposure and continue through longer periods of light exposure.

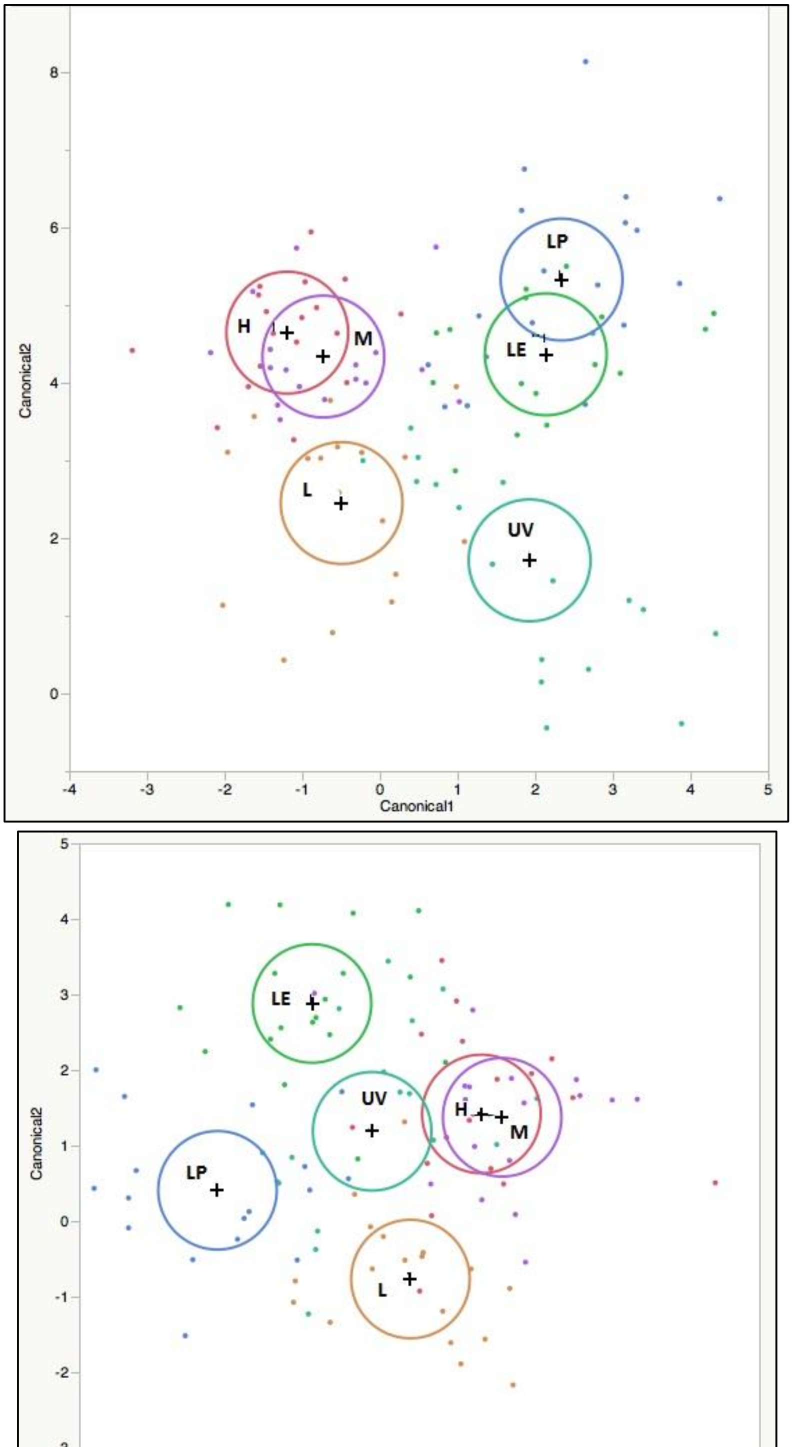


Figure 11. Canonical discrimination analysis (CDA) plots for 2% milk from PET packages after 24 h of fluorescent (top) or LED (bottom) retail light exposure using all 32 sensors. LP= light protected control, LE= light exposed control, H= high white PET, M= medium white PET, L= low white PET, UV= UV barrier PET.

The volatile profiles in this experiment could be influenced by the low temperature of the samples during measurement. Analyzing samples at lower temperatures could increase variability in the eNose functionality and results. All samples were measured at refrigeration temperature (4±1°C) to mimic the volatiles consumers smell when drinking milk, assuming the majority of milk is consumed close to refrigeration temperatures. Our eNose methodology mimicked an orthonasal approach to milk, where volatiles are detected from the environment by the external nares of the nose. Heating milk samples prior to analysis would mimic a retronasal approach, where milk is heated in the mouth and volatiles are detected through the nasopharynx to olfactory epithelium. Release of volatiles retronasally occurs when breathing during chewing (mastication) or swallowing, and is more common for food than beverages (Retronasal/Orthonasal Olfaction, 2009). This reasoning provided the justification for our orthonasal eNose methodology. However, this methodology does not follow eNose recommendations that samples be analyzed at a minimum of 22°C (Sensigent, 2013). Heating samples prior to analysis allows for more "smells" to be detected and distinguished by creating greater volatilization of compounds, resulting in increased density of molecules in headspace over the liquid, and has been employed as standard methodology in previous eNose applications to milk (Capone et al., 2001; Magan et al., 2001; Labreche et al., 2005). However, measuring volatiles at 4 ± 1°C is the most conservative situation, so any detectable differences under these conditions are indicative of differences that may be more apparent at higher temperatures.

The 100% separation seen between fresh milk and all milk following light exposure showed that milk volatile compositions changed as a result of both LED and fluorescent light exposure. There was some separation in volatile profiles when comparing only PET packages after 8 and 24 h of light exposure, but this did always not follow logical trends. More precisely identifying the exact volatiles present in milk and how they change over light exposure could better understand volatile differences from oxidation. Future studies should focus on identifying the composition of volatiles in oxidized milk and train the eNose to recognize these volatiles before testing oxidized milk samples. This study showed that the eNose has potential as a rapid quality check tool in dairy processing plants to distinguish fresh milk from “older” milk with possible quality or sensory defects. This is in agreement with research studying the differences in milk volatile composition over shelf life. Previous studies have showed that as milk ages there are corresponding increases in volatile compounds including dimethyl sulfide, ethyl acetate, 2-heptanone, and pentanal (Marsili, 2000). More detailed training of the eNose for volatile compositions in fresh milk and low quality milk, especially with the compounds listed above, is necessary before this tool can properly distinguish the protective influences of different packaging materials on milk quality.

4.3.2 Study 2

4.3.2.1 Sensory Analysis

Consumers are discriminatory of oxidized off-flavors in milk, with untrained panelists being able to detect oxidized off-flavors in as little as 54-120 minutes of light exposure (Chapman et al., 2002). This sensitivity makes sensory testing using untrained panelists one of the most important measures of oxidation in milk. Sensory testing was completed to determine if untrained panelists could detect a difference in milk flavor from experimental PET packages. This information was used to identify which package best protected milk flavor, using the light-protected control as a standard for milk flavor.

Table 2. Summary of statistical differences ($p<0.05$) for triangle test sensory comparisons.

		Time			
	Type of test[1]	**4 H**	**8 H**	**24 H**	**72 H**
Fluorescent					
LP vs. LE	Difference	0.0069*	<0.0001*	<0.0001*	<0.0001*
LP vs. UV	Similarity	0.0075*	<0.0001*	<0.0001*	<0.0001*
LP vs. L	Similarity	0.0094*	0.2119	<0.0001*	<0.0001*
LP vs. M	Similarity	0.0054*	<0.0001*	0.0154*	0.0001*
LP vs. H	Similarity	0.4721	0.1170	0.0207*	0.0004*
LE vs. UV	Difference	0.0154*	0.1977	0.0968	0.1492
LE vs. L	Difference	0.4207	<0.0001*	<0.0001*	>0.5000
LE vs. M	Difference	0.0384*	0.1459	0.1635	>0.5000
LE vs. H	Difference	0.0735	0.2546	0.0643	0.0038*
LED					
LP vs. LE	Difference	0.1020	<0.0001*	<0.0001*	<0.0001*
LP vs. UV	Similarity	0.1056	0.0005*	<0.0001*	<0.0001*
LP vs. L	Similarity	0.0735	0.2327	<0.0001*	<0.0001*
LP vs. M	Similarity	>0.5000	0.0021*	0.0008*	<0.0001*
LP vs. H	Similarity	0.2981	>0.5000	<0.0001*	0.0001*
LE vs. UV	Difference	0.4483	>0.5000	0.0060*	>0.5000
LE vs. L	Difference	0.1977	0.1611	>0.5000	>0.5000
LE vs. M	Difference	0.3707	>0.5000	0.5000	0.4483
LE vs. H	Difference	0.0104*	0.0485*	<0.0001*	0.2148

*indicates statistical difference with statistical parameters for difference tests: $\alpha=0.05$, $\beta=0.20$; similarity tests: $\alpha=0.20$, $\beta=0.05$.

[1] proportion of discriminators (pd) assumed 20%; actual pd, confidence intervals, and power analysis presented in Appendix E

LP= light-protected (foil and plastic overlay) clear PET control; LE= light-exposed PET control; UV= UV barrier PET; L= low white PET; M= medium white PET; H= high white PET.

Successful package= green font, partially successful package= purple font, partially ineffective package= blue font, completely ineffective package= red font (see statistical methods section (4.2.10) for classification explanation).

Our panelists were discriminatory of light-induced oxidized off-flavors, because there was a significant difference between the light-protected and light-exposed PET controls during all time treatments, except for 4 h of LED light exposure. It is not surprising that there was no difference between the light-protected and light-exposed controls after 4 h LED light, since LED light likely did not cause as extensive compositional changes in the light-exposed control milk after only 4 h of light exposure. LED light has been shown to cause less detrimental changes to milk quality than fluorescent light in previous studies of milk under LED light (Amin, 2016; Brotherson et al., 2016). The proportion of discriminators was also much lower for this time treatment (8%) than for the other time treatments, which could account for the lack of discrimination between the two controls.

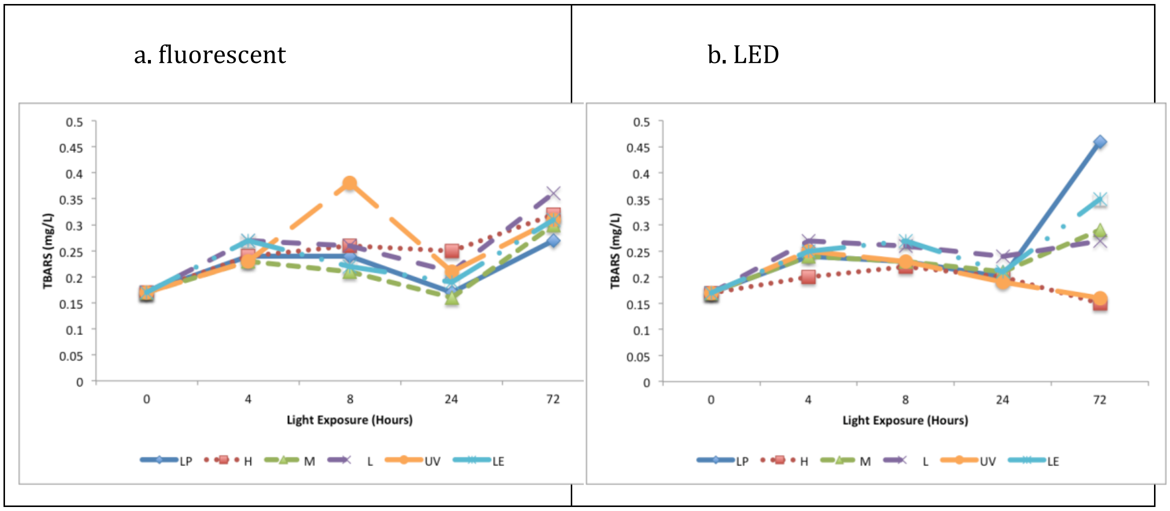
Under fluorescent retail lighting conditions, UV barrier PET performed the least successfully. After 4 h of fluorescent light exposure this package was partially ineffective and was completely ineffective under all other fluorescent time treatments. UV barrier PET had only slightly more success under LED lighting. This package was partially successful after 4 h of light exposure, but quickly failed to protect milk quality after longer lengths of retail light exposure. This supports previous work by Mestdagh et al. (2005), which showed that UV barrier PET failed to prevent differences in smell and taste of milk after 48 h of fluorescent light exposure. Our study proves that these differences are apparent long before 48 h of light exposure, becoming noticeable by as little as 8 h of fluorescent or LED light exposure. These results also continue work by van Aardt et al. (2001) who found that UV barrier PET was partially protective, creating less oxidized off-flavors in milk after 7 d of fluorescent light exposure than milk stored in HDPE packages. This study shows that although UV barrier PET may be more protective of milk flavor than HDPE packages, it is not protective enough to render milk similar to light-protected milk after 4-8 h of light exposure.

The low levels of TiO_2-loading did not offer much success in maintaining milk of similar quality to light-protected milk. The low white PET package was successful at 8 h of fluorescent light exposure, but failed to adequately protect milk quality for all other fluorescent light exposure, including 4 h. The lack of protection at 4 h would suggest that the successful protection at 8 h was not indicative of true protection of milk flavor. This could have occurred because the proportion of discriminators for this comparison was only 5%, meaning that only a small percentage of panelists were truly discriminatory of this treatment package and light-protected control milk. The low white PET package was partially successful through 8 h of LED light exposure, but became completely ineffective by 24 and 72 h. This limited sensory success is in agreement with previous research on 2% PET packages that found this level of LPAs did not adequately protect milk flavor against light-induced off-flavors (Moyssiadi et al., 2004; Zygoura et al., 2004). The medium white package was either completely ineffective or partially ineffective at all durations of fluorescent light exposure. It was partially successful at preventing light-induced oxidized off-flavors through 4 h LED light exposure, but became ineffective for all other LED time treatments. These results support previous work, which showed that TiO_2-loading of 4.3% or less in HDPE packages was not sufficient to maintain high quality UHT-processed milk during extended retail storage (Johnson et al., 2015).

The most successful package at protecting milk sensory quality and preventing oxidized off-flavors was the high white PET package. This package was partially successful through 8 h fluorescent light exposure, and completely successful at protecting milk flavor through 8 h LED light exposure. It also retained limited success through the remaining lengths of retail light exposure for both fluorescent and LED, only becoming completely ineffective at protecting milk flavor at 72 h LED light exposure. These are very promising results since 50% of milk is only under direct retail lighting for 8 h or less (Chapman et al., 2002) and more dairy retail cases are including LED lights instead of fluorescent.

4.3.2.2 Formation of Secondary Oxidation Products Measured by Thiobarbituric Acid Reactive Substances Analysis (TBARS)

Samples were taken from each commingled milk treatment from sensory evaluation and tested to determine the amount of secondary oxidation products in the milk that panelists tasted. These analyses were completed to verify that the milk panelists were drinking exhibited similar levels of oxidation after being commingled as the milk from individual packages in the retail case. Based on ANOVA evaluation of the effects of light, time, package, and interactions, the only factor that significantly influenced aldehyde levels was time (p=0.004). There were significantly higher aldehyde levels at 72 h than at 24 h. The other two time treatments were not different. This is in agreement with previous literature, which has shown increased TBARS levels after longer periods of light exposure (Walsh et al., 2015), although it is unclear why these levels were lower at 24 h than at 4 or 8 h of light exposure. As was seen with the measurements of secondary oxidation products in individual packages, TBARS were very low throughout this experiment (all averages were below 0.50 mg/L), indicating that PET packages were effective at protecting against high levels of lipid oxidation (Figure 12). It is unclear why there is such a wide spread of aldehyde concentrations after 72 h of LED light exposure, or why light-protected milk had the highest level of secondary oxidation products at this time. However, these TBARS levels are within range of a similar study of HDPE packages under fluorescent and LED light (Amin, 2016). One explanation for the unexpected TBARS values is that the TBARS assay measured more than just aldehyde concentrations. Martinez-Monteadgudo et al. (2015) found that TBARS assay also measures degradation products from Maillard reactions, such as furfural and hydroxymethylfurfural, which could react with thiobarbituric acid (TBA) and account for the higher values seen for some treatments. This assay may not be the most accurate indicator of oxidized off-flavors, because the sensory evaluation showed clear differences by 4 h and 8 h light exposure between oxidized milk and light-protected control milk that were not associated with the very low concentrations of secondary oxidation products measured in this experiment. The lack of significant oxidation products under fluorescent light does not mean that oxidation off-flavors were not present, because previous literature notes that sensory differences have been detected when there is not a corresponding increase in lipid oxidation products measured by TBARS analysis (Rysstad et al., 1998).


a. fluorescent

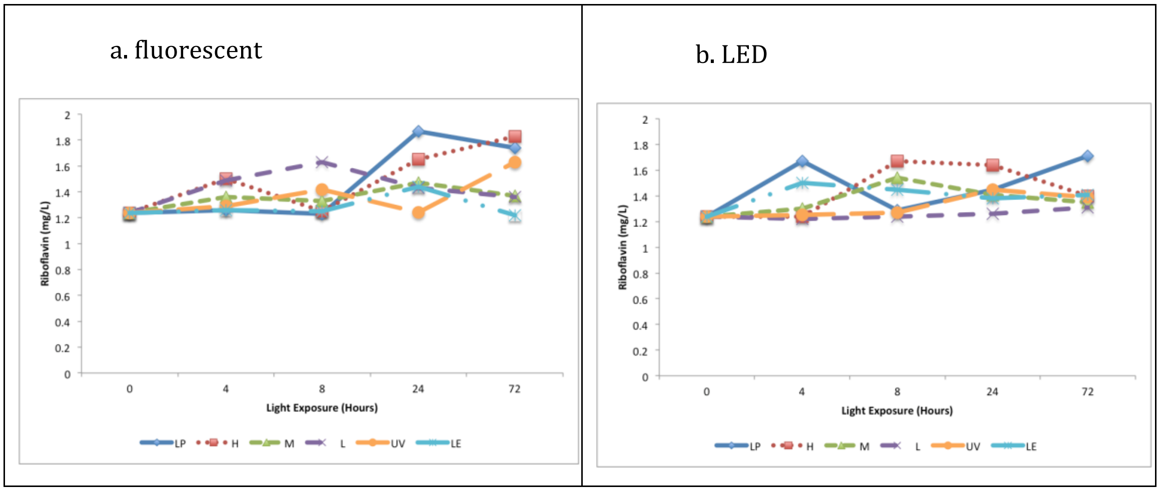
b. LED

Figure 12. TBARS formation (mg/L) in commingled milk samples from sensory evaluation comparing fresh milk to milk packaged in PET packages from all time treatments under: a.) Fluorescent light and b.) LED light. LP= light-protected (foil and

plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= UV barrier PET; LE= light-exposed PET control.

4.3.2.3 Degradation of Riboflavin (Rb) by Fluorometric Analysis

No effect was observed on Rb concentration for light, package, time or the interactions. It is unusual to not see a decreasing trend in Rb content as length of light exposure increased, but Rb remained consistent through 72 h fluorescent and LED light exposure (Figure 13). These results are in agreement with Brotherson et al. (2016) who found no significant decrease in Rb in 1% milk after 24 h of fluorescent light exposure, and only small decrease in Rb after 24 h LED light exposure (>90% retention). These results also support Saffert et al. (2006) who found that differences in Rb degradation in whole milk under fluorescent light could not be distinguished between pigmented PET packages until 7 d (168 h) of light exposure. These results are different than observed in a similar study with HDPE packages by Amin (2016). The difference in results could result from a protective interaction of improved PET oxygen barrier properties with TiO_2 light interference properties since PET has a much higher oxygen barrier than HDPE. The high levels of Rb retained in all experimental packages throughout this experiment conclude that PET is protective of vitamin content in milk with all treatments retaining over 1.00 mg/L (just under 60% of USDA reported riboflavin values for 2% milk).


a. fluorescent

b. LED

Figure 13. Riboflavin concentration (mg/L) of commingled milk samples from sensory evaluation under fluorescent (a) and LED (b) light. LP= light-protected (foil and plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= UV barrier PET; LE= light-exposed PET control.

The lack of significant results from riboflavin suggest that other photosensitizers may be more involved in oxidation reactions that are leading to the off-flavors seen in milk under fluorescent and LED light under the conditions of this study. LED light contains a broad spectrum of light wavelengths, especially from 580-620 nm (Figure 14a). This peak overlaps with the orange light region (575-750 nm), where light is absorbed by tetrapyrroles that occur naturally in cow’s milk (Airado-Rodriguez et al., 2011). Fluorescent lights contain more intense emission peaks of wavelengths over 500 nm, which could also activate other photosensitizers much more than Rb, which absorbs light most strongly at 400 and 446 nm (Figure 14b). Tetrapyrroles, particularly chloric compounds, have been found to cause more oxidized off-flavors than photosensitizers such as Rb (Airado-Rodriguez et al., 2011; Intawiwat et al., 2013). This supports our results that saw a high retention of Rb even as oxidized milk flavors became more noticeable. The effects of other photosensitizers in the orange region of visible light, such as chlorophyll should be studied further to confirm their role in oxidation and off flavor development.

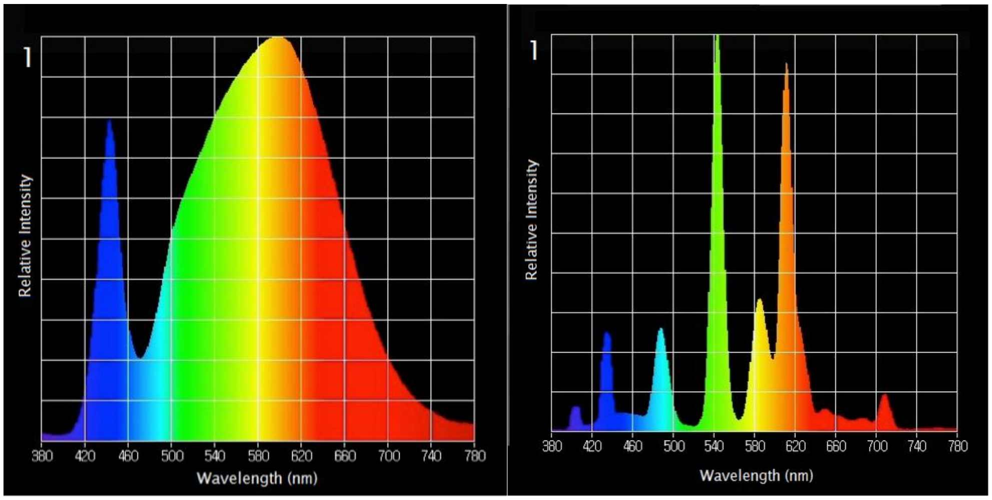


Figure 14. Relative intensity of light wavelengths in visible light spectrum of LED 3500K retail lights (14a) and fluorescent 3500K retail lights (14b). *Image produced by Amin, K. 2016.*

4.3.2.4 Volatile Analysis by Electronic Nose

CDA was completed as described in Study 1 above. Five smell-prints from three replications of commingled milk were combined together to create a cluster of fifteen smell-prints for each commingled treatment in the CDA plot. There was a greater spread and overlap of the clusters from each treatment because of the higher number of data points included, but these plots represent the milk from all three replications of sensory testing. The fresh milk had 100% separation from all treatment milk under both fluorescent and LED light after 8 h of light exposure (Appendix G). Fresh milk was removed from all CDA plots to better distinguish differences between PET packages (Figure 15). Differences in volatile profiles were becoming apparent after 8 h of light exposure. After 8 h fluorescent light exposure, the high white PET and UV barrier PET packages had completely separate ($p<0.05$) volatile profiles from all other treatments.

There was overlap between the low and medium white PET packages, but they had distinct smell-prints from all other packages. The two control packages also had overlap in their volatile profiles, but were different from all other PET packages. There were less differences in volatile profiles for the milk stored in PET packages under LED light. The only packages that were completely different from all other packages were the light-protected and light-exposed controls. The UV barrier PET had a different volatile profile than the light-protected control, light-exposed control, medium white, or high white PET packages under LED light.

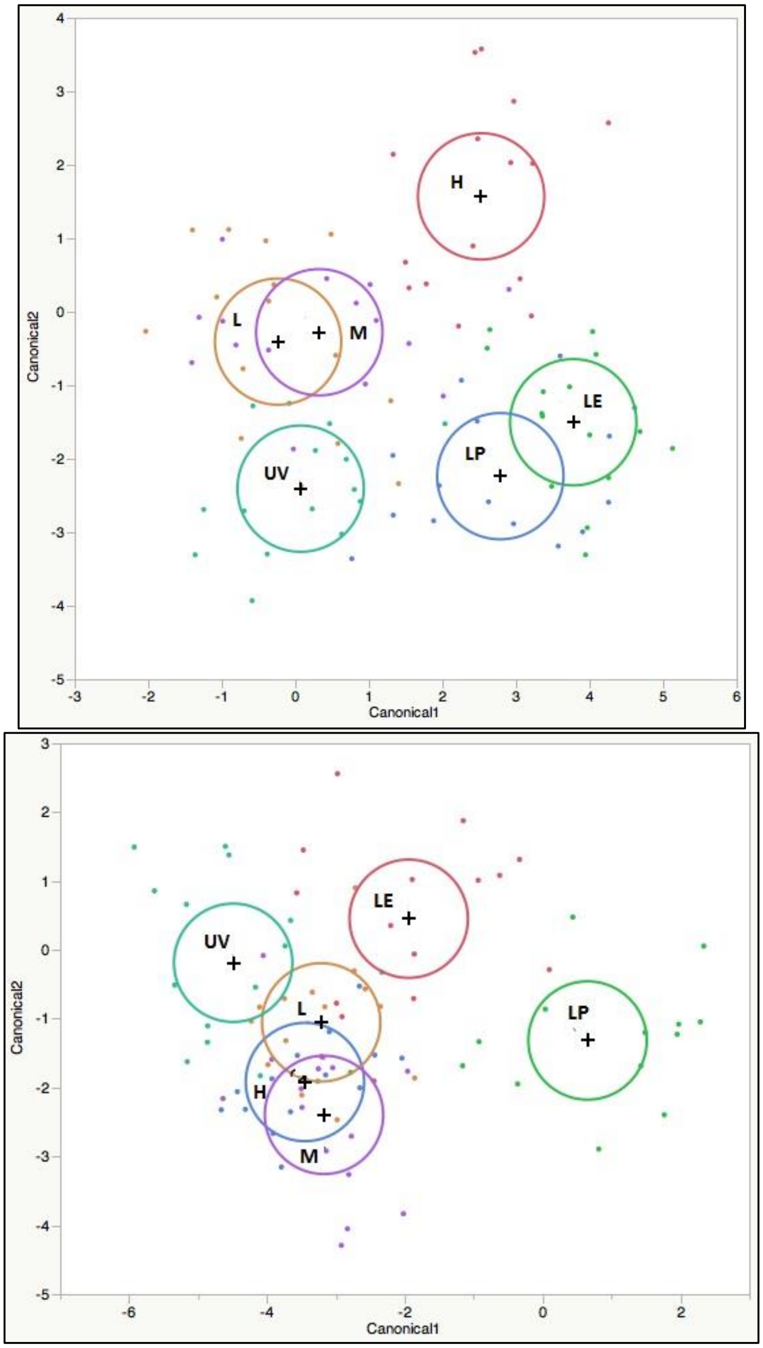
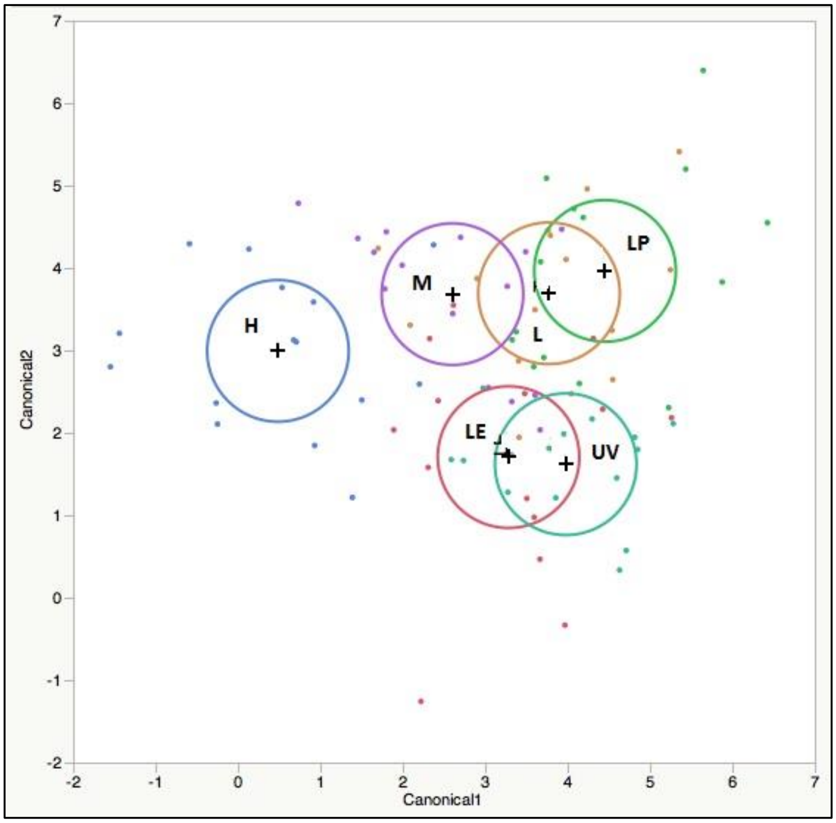


Figure 15. Canonical plots for commingled milk from PET packages after 8 h light exposure under fluorescent (top) and LED (bottom) light. LP= light-protected (foil and plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= UV barrier PET; LE= light-exposed PET control.

There was some separation seen between PET treatments after 24 h fluorescent and LED light (Figure 16). The light-protected and light-exposed controls were separate (p<0.05) from all other treatments but were overlapped with each other after 24 h fluorescent light exposure. The volatile profile from milk in the low white PET also had 100% separation from all other treatments after 24 h fluorescent light exposure. After 24 h LED light exposure the only package that was 100% separate from all other treatments was the high white PET package. The light-exposed control and UV barrier PET packages had similar volatile profiles but were different from all other treatments. This could be due to similar oxidation from the two packages that allowed the most extensive light exposure to reach milk. The light-protected control, low white PET and medium white PET had similar volatile profiles and were different from all other PET packages.



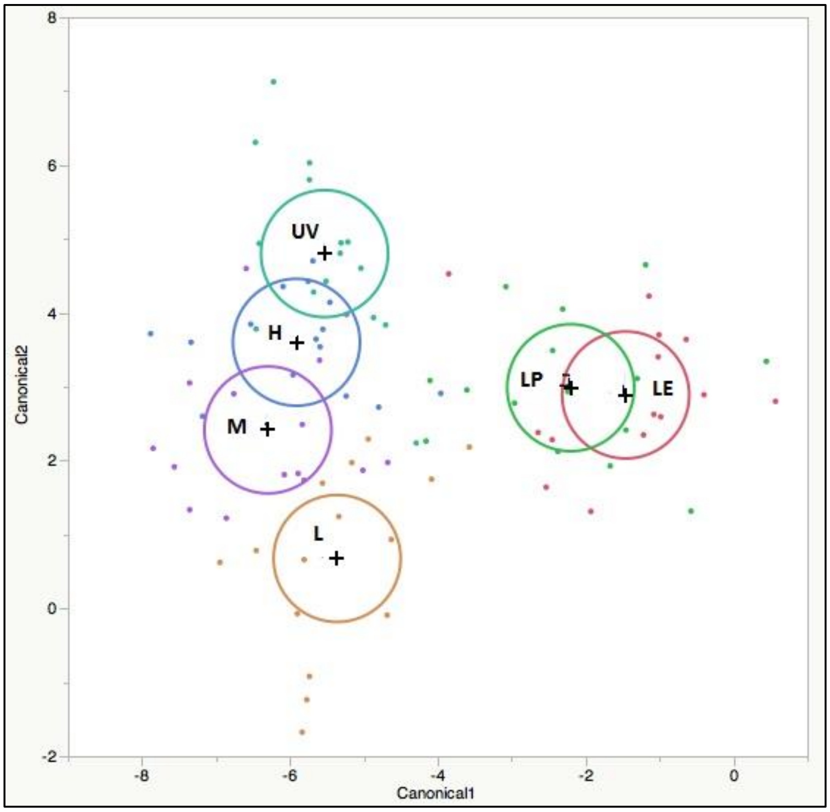


Figure 16. Canonical plots for PET packages after 24 h light exposure under fluorescent (top) and LED (bottom) light. LP= light-protected (foil and plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= UV barrier PET; LE= light-exposed PET control.

Similar trends were seen in the CDA plots between commingled milk from PET packages as in Study 1 CDA plots of milk from individual PET packages. This confirms that the commingled milk panelists were drinking is representative of milk as it left the retail case in individual packages. When fresh milk was removed from CDA, some differences were noticed in volatile profiles between milk stored in different PET packages. However, there was still overlap between the two control packages and certain treatment packages that were shown to be clearly different through sensory evaluation. Sensory panels should still be used as the most effective way to test fluid milk for undesirable off-flavors such as light-induced oxidation.

4.4 Conclusions

PET is a viable packaging option for maintaining high quality milk throughout retail storage under lights; however, the oxygen barrier alone is not sufficient to protect milk. Higher levels of titanium dioxide (6.6%) LPA block more of the light spectrum under both fluorescent and LED light and keep light-induced oxidation reactions in milk from becoming as extensive as in clear PET packages. Differences in light intensity (500-4,800 lux) did not have a significant influence on the extent of oxidation reactions in 2% milk packaged in PET packages. Even small amounts of dissolved oxygen in milk (≤12ppm) are important reactants in oxidation reactions, and measuring dissolved oxygen levels is a valid indication of the extent of light-induced oxidation and package performance at preventing these oxidation from occurring. LED light at <1,500 lux has the potential to be less destructive to milk quality, creating fewer oxidation products and retaining more vitamins over typical retail storage periods. Sensory evaluation remains the most effective way to distinguish off-flavors in milk. After as little as 4 h of direct light exposure, off-flavors become noticeable to untrained consumers, and can influence their milk drinking experiences and purchasing decisions. This reiterates the urgency of this issue and the need to revolutionize fluid milk packaging to better protect milk quality and flavor.

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CHAPTER V

CONSUMER ACCEPTABILITY OF MILK PACKAGED IN PET PACKAGES

Abstract

Oxidation reactions in milk produce off-flavors including cardboard, stale, and astringent that may influence consumer acceptability. Polyethylene terephthalate (PET) packages with high levels of titanium dioxide (TiO_2) reduce the occurrence of light-induced oxidation in milk. The type of light exposure (fluorescent or light-emitting diode [LED]) affects the type and extent of light-induced reactions in milk differently. Consumer acceptability of 2% milk packaged in PET packages under retail storage conditions (4 h light exposure, 1,500 lux) was determined for milk under fluorescent and LED light. PET treatments under LED light included light-protected control, medium white PET (4.0% TiO_2), high white PET (6.6% TiO_2), and light-exposed control. Treatments under fluorescent light were clear PET and clear HDPE to provide comparisons for different packaging materials and light types. Sensory evaluation by an untrained consumer panel (n=157) was supported by analytical measures of oxidation including thiobarbituric acid reactive substances (TBARS) assay, riboflavin degradation, electronic nose volatile analysis, and dissolved oxygen content. All milk stored under LED light had higher ratings for overall acceptability, flavor, and aftertaste than milk stored under fluorescent light. Milk stored under fluorescent light was also deemed to have too much flavor, which led to decreased acceptability. Dissolved oxygen measured differences in oxidation between LED and fluorescent light more accurately than other analytical measurements. Type of light exposure has a more significant effect on

oxidation reactions in milk than packaging TiO_2 levels. LED light is less detrimental to milk quality and flavor than fluorescent light.

Keywords: milk, oxidation, dissolved oxygen, sensory, PET

5.1 Introduction

Fluid milk sales and consumption are sectors of the dairy industry that have been declining for the past several decades. Part of the reason for this decline can be attributed to the development of light-induced oxidized off-flavors under retail lighting conditions that are unacceptable to consumers (Walsh et al., 2015; Brotherson et al., 2016). After 1% milk was exposed to LED light (4,000 lux) it led to a decrease in sweet, milkfat, and cooked/sweet flavors and an increase in cardboard, astringency, and butterscotch flavors (Brotherson et al., 2016). An effective and economical packaging option needs to be identified to protect milk from light-induced off-flavor development under retail storage conditions to deliver high quality milk to consumers and increase positive experiences with bovine milk.

Experimental milk packaging has been tested extensively to protect milk against the development of these off-flavors. Milk packages made from polyethylene terephthalate (PET) have been fortified with white titanium dioxide (TiO_2) pigments to block light wavelengths that initiate oxidation reactions in milk components. The varying effectiveness of these experimental packages has been explored under both fluorescent and LED lighting, and has found that PET packages provide effective protection under LED retail lighting conditions through at least 8 h light exposure (Chapter IV). However, there is a lack of complete understanding of consumer acceptability of milk that was stored in these experimental packages.

The objective of this study was to more fully characterize consumer acceptability of fluid milk under innovative storage conditions, including LED light and PET packages. Sensory testing was completed to explore differences in consumer acceptability of milk flavor and characteristics stored in PET (n=5) and HDPE (n=1) packages under fluorescent and LED retail lights (1,500 ± 100 lux) for 4 h. The 4 h time frame was selected because it is most characteristic of how long the majority of milk will remain under direct lighting in the retail refrigeration case before purchase. Changes in dissolved oxygen content, formation of secondary oxidation products, degradation of riboflavin, and changes in volatile composition as measured by electronic nose were completed to support sensory evaluation results.

5.2 Materials & Methods

5.2.1 Milk Processing & Filling into Treatment Packages

Vitamin D fortified 2% milk was processed (HTST pasteurization; 78°C for 15 seconds) at a local dairy processing plant (Westover Dairy, Lynchburg, VA). Milk was packaged into translucent HDPE half gallons at the processing plant and transported to Virginia Tech Human and Agricultural Biosciences Building 1 (HABB1; Blacksburg, VA) pilot plant in dark coolers with ice for light and temperature control. Milk was commingled into a 5-gallon insulated beverage cooler and gravity filled with a plastic tubing system under a positive flow clean-fill laminar hood (Thermo Fisher Scientific, Waltham, MA) into treatment packages and capped securely by hand. All treatment packages were stored in a dark walk-in refrigerated retail case (Model 3800, HillPhoenix, Chesterfield, VA) until treatment. All treatment packages (n=80) were filled at one time.

Six treatment packages were prepared for this experiment. PET packages (n=5) and HDPE packages (n=1) were both blow molded from resin (PET= standard injection blow-molding procedures; PTI Technologies, Holland, OH; HDPE= standard extrusion blow-molding procedures; Ampacet, Tarrytown, NY). Two treatment packages, clear

HDPE and clear PET, were placed under fluorescent light. Four treatment packages were placed under LED light: light-protected PET, light-exposed PET, medium white (4.0% TiO_2) PET, and high white (6.6% TiO_2) PET. Light-protected PET had foil wrap for complete light protection and a white plastic overlay to prevent light reflection onto other packages in the retail case. The medium white PET and high white PET package were selected from the results of Chapter IV as the most successful options at protecting milk against light-induced oxidation. They were compared to light-protected and light-exposed controls under LED light as standards for high and low taste quality milk. The light-exposed PET and light-exposed HDPE controls under fluorescent light were selected to explore differences between oxidized milk under fluorescent and LED lights that could result from different packaging materials. The light-exposed HDPE control also provided some comparisons to a similar study by Amin (2016).

5.2.2 Treatment of Experimental Packages

All treatment milk was placed in retail cases under targeted light intensities of 1,500 ± 100 lux from 3500K LED lights for 4 h. Light intensity was measured in lux at each position using a handheld light meter (Model SN400, Extech Instruments, Nashua, NH). Six positions in the fluorescent closed-door retail case (Model ONRB4, HillPhoenix, Chesterfield, VA) matched the light intensity criteria. Sixteen positions in the LED open-front retail case (Model O5DM, HillPhoenix, Chesterfield, VA) matched the light intensity criteria for this study. Thirteen bottles of each PET treatment and fifteen bottles of the HDPE treatment were needed to meet the necessary volume (800 oz.) for sensory evaluation. The same number of each treatment package was placed in the retail cases at each time in a randomized order (Microsoft® Excel® for Mac 2011, Version 14.5.8, Microsoft Corporation, Redmond, WA). Five replications of each 4 h time treatment were completed in the fluorescent retail case to treat all fluorescent packages. Four replications of each 4 h time treatment were completed in the LED retail case to treat all LED packages. All replications were completed over three days.

5.2.3 Sampling Plan

Fresh milk (0 h light exposure) had samples collected in triplicate for microbiological analyses, TBARS (10 mL), and Rb degradation (30 mL). Dissolved oxygen in fresh milk was measured in triplicate. Microbiological analyses (standard plate count of Petrifilm™ Aerobic Count Plates and Coliform Count Plates [3M, St. Paul, MN]) were completed for fresh milk as described in Chapter IV to ensure that any changes in milk were a result of treatment conditions and not from starting with low quality milk. Samples (n=10; 8 mL) were also collected from fresh milk for electronic nose (eNose) volatile analysis to provide a baseline for volatile changes.

Following treatment, all packages were removed from the retail cases and placed in a dark walk-in refrigerated retail case (Model 3800, HillPhoenix, Chesterfield, VA) until transport for sensory evaluation. Chemical oxidation measures were taken including dissolved oxygen content, electronic nose (eNose) volatile analysis, formation of secondary oxidation products (TBARS), and riboflavin (Rb) degradation. The dissolved oxygen content of each package was measured using a dissolved oxygen meter (LDO101, Hach, Loveland, CO) as described in Chapter IV. Volatile analysis by an eNose was completed as described in Chapter IV. TBARS (10 mL) and Rb degradation (30 mL) samples were collected and frozen as described in Chapter IV. All analyses were completed within three weeks of sample collection.

5.2.4 Sensory Evaluation

Milk was transported in experimental packages to the NC State University Sensory Service Center (Raleigh, NC) for sensory testing within one day of all treatments being completed. Milk was transported in coolers packed with ice for light and temperature control. Once milk was received at NC State, it was stored in a dark, walk-in refrigerated retail case (UDS-4/Brown, Salisbury, NC) until consumer sensory panels were completed, within 48 h of transport.

On the day of sensory testing, 4 gallons of milk from individual bottles for each treatment were commingled into 5-gallon insulated beverage coolers (Igloo Products Corp, Katy, TX) and commingled milk (89 mL) was poured into three-digit coded 6 oz. Styrofoam sample cups. Samples were maintained at <10°C and protected from light during preparation and serving. Milk was served to the panelists at 8-12°C (just above refrigeration temperatures) as standard protocol for the NC State Sensory Service Center to allow for the most optimal flavor profiling. This temperature is slightly higher than used in our previous sensory work (approximately 4°C; Chapter IV).

All panelists completed a digital consent form before participating in the sensory evaluation (Appendix J). Panelists (n=157) tasted all six samples of milk in a randomized complete block design and evaluated each sample for liking (appearance, aroma, flavor, mouthfeel/thickness, aftertaste), aftertaste intensity, and freshness perception on a 9-point hedonic scale (1=dislike extremely; 9=like extremely; Appendix J). Two just-about-right (JAR) questions were also asked to see how panelists rated their perception of flavor and mouthfeel/thickness/viscosity on a JAR scale (3=just right; Appendix J).

5.2.5 Electronic Nose Volatile Analysis

A minimum of 5 "smells" was needed to create an accurate cluster for each treatment in each replication; therefore, samples (8mL) were only collected in 40 mL amber bottles from the first three full replications (n=12 packages of each treatment) to measure changes in volatile composition. Two samples were collected from each package to achieve the necessary threshold, since there were four packages of each treatment in the LED case and three packages of each treatment in the fluorescent case during each full replication. ENose volatile analysis was completed by the Cyranose® 320 (Sensigent LLC, Baldwin Park, CA) as described in Potts (Ch. IV, 2016).

5.2.6 Formation of Secondary Oxidation Products Measured by Thiobarbituric Acid Reactive Substances Analysis (TBARS)

The formation of aldehydes and other secondary oxidation products from lipid oxidation reactions were measured by TBARS analysis as adopted from Spanier and Traylor (1991) and described in Potts (Ch. III, 2016). One sample from each PET (n=13) and HDPE (n=15) package was analyzed for secondary oxidation products.

5.2.7 Degradation of Riboflavin (Rb) by Fluorometric Analysis

The degradation of Rb in milk was measured through a modified fluorometric analysis (AOAC method 970.65, 1995; modified method described in Potts, Ch. III, 2016) and measured on Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instrument, Inc., Columbia, MD). One sample was analyzed from each PET (n=13) and HDPE (n=15) treatment package.

5.2.8 Statistical Analysis

The analysis of sensory data included both liking attributes and just-about-right attributes. Liking attributes were measured on a nine-point hedonic scale (1=dislike extremely, 9=like extremely). Liking attributes evaluated for differences ($p<0.05$) using analysis of variance (ANOVA) and Fisher's LSD in XLSTAT (Addinsoft, New York, New York). Milk attributes were scored on just-about-right (JAR) scales (1 or 2=too little, 3=just right, 4 or 5=too much). JAR attributes were analyzed for differences ($p<0.05$) using the k proportion test in XLSTAT (Addinsoft, New York, New York).

Analytical tests (TBARS, Rb, dissolved oxygen) were completed in triplicate or higher for all treatments. Outliers were removed from the data set at 95% confidence using Dixon's Q test (Cochran and Snedecor, 1980). One-way ANOVA measured significant ($p<0.05$) changes in oxidation (JMP Pro 11, SAS Institute, Cary, NC). Analysis of variance was based on the different combinations (n=6) of package (pkg) and light type since there were not adequate data points to complete a two-way ANOVA comparing light*pkg as separate factors. Means and standard deviation were calculated for TBARS, Rb degradation, and dissolved oxygen (Microsoft$^{®}$ Excel$^{®}$ for Mac 2011, Version 14.5.8, Microsoft Corporation, Redmond, WA). Electronic nose samples were analyzed through canonical discrimination analysis using means CL ellipses (CDA; JMP Pro 11, SAS Institute, Cary, NC).

5.3 Results and Discussion

This study provided more detailed information consumer acceptability of milk from the most protective PET packaging options for fluid milk. The results of Potts (Ch. IV, 2016) demonstrated that higher levels of TiO_2-loading in PET blocked more light and produced milk that tasted similar to light-protected milk, which is why the medium white PET and high white PET were selected as the experimental PET packages tested in this study. This study focused on consumer acceptability of milk, and determined if consumers found milk packaged in PET acceptable. This study provided realistic comparisons by choosing parameters (4 h light exposure, 1,500 lux) that produced milk similar to what consumers typically experience in retail conditions. It also provided valuable insight with direct comparison of PET to HDPE, the most common milk packaging material, under fluorescent light, which has been the traditional lighting used in retail dairy cases.

Milk was deemed to be of high microbiological quality for this study. When measured in triplicate, there was zero growth on Petrifilm™ Coliform Count Plates and <25 cells/mL growths on Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN). This verified that any changes in milk flavor or quality were a result of treatment conditions. Light intensity throughout this study matched the targeted criteria, with fluorescent light averaging 1,482 ± 50 lux and LED light averaging 1,458 ± 44 lux. The random placement of treatment packages in each retail case ensured that each treatment received similar light intensity (averaging 1,451-1,495 lux), and any differences in milk flavor or quality resulted from the type of light or package tested (Appendix I).

5.3.1 Sensory Evaluation

The consumer acceptability panel provided insight to how the average fluid milk consumer responded to milk that has undergone typical retail lighting conditions, and if experimental PET packages improved acceptability by protecting against light-induced oxidized off-flavors. Consumers were screened for regular milk consumption habits before participating in sensory evaluation. Of the 157 participants, ranging from 18-65 years old, 61.1% were female. Participants responded as drinking milk at least 2-3 times per month (5.1%) or two or more times per week (74.5%). These participants were also frequent purchasers of milk, with 67.5% of panelists saying that they purchase milk one or more times per week. They were representative of the general population, since 47.8% of panelists said that they consume reduced fat (2%) milk more frequently than any other fat content milk and 2% milk is the most widely consumed fluid milk in the United States (International Dairy Foods Association, 2015).

Milk stored in all of the PET packages under LED light were liked similarly well ($p>0.05$), with mean scores in the 'liked slightly' to 'like moderately' range (hedonic score= 6.4-6.7) for overall liking, indicating that the presence or absence of LPAs in PET did not have a significant impact on consumer acceptability after only 4 h of light exposure. Acceptability of milk from the clear PET and HDPE packages stored under fluorescent light scored significantly ($p<0.05$) lower than all packages under LED light (5.7 and 6.1 hedonic mean scores, respectively; 'like slightly'). The same trends were seen for flavor and aftertaste acceptability, with all milk stored under LED light scoring in the same acceptability range ($p>0.05$). Consumers also scored milk flavor and aftertaste in the clear HDPE and PET packages stored under fluorescent light significantly ($p<0.05$) lower. This study found higher acceptability scores than were reported by Brotherson et al. (2016) for 1% milk stored under higher intensity LED light (4,000 lux) for 12 or 24 h (hedonic score= 5.9 and 5.4, respectively). These results indicate that higher light intensity and longer durations of light exposure have the potential to decrease consumer acceptability of milk.

Fluorescent light had a significant effect on acceptability. Overall acceptability of milk packaged in the clear PET package (no LPAs) dropped nearly a full integer (from

6.6 to 5.7) from being stored under fluorescent light instead of LED light. Acceptability of milk flavor and aftertaste from milk packaged in the clear PET packages dropped more than a full integer when the milk was stored under fluorescent light instead of LED light. The significantly lower acceptability of milk, particularly its flavor and aftertaste, under fluorescent light clearly demonstrates that different types of light have different effects on light-induced oxidation reactions in milk. Fluorescent light initiates reactions that cause more noticeable and less acceptable changes in milk to consumers.

Both of the treatments under fluorescent light (clear PET and HDPE) had lower aroma acceptability than milk stored in the clear PET package under LED light. Milk from clear PET stored under fluorescent light showed the only difference ($p<0.05$) in aftertaste intensity. This treatment had less acceptable aftertaste intensity, following this milk's trend of lower acceptability in many attributes. There was no detectable difference ($p>0.05$) in the appearance of samples for any of the treatments.

Participants also rated the perception of freshness for each sample. All of the milk in PET packages stored under LED light, including the clear PET package, scored higher ($p<0.05$) for freshness perception than milk from the clear PET package stored under fluorescent light. The perception of freshness corresponded well with acceptability scores for other milk attributes and is an important consideration for consumers. The type of light milk is stored under had a significant impact on the freshness perception of milk after only 4 h of light exposure.

Participants rated milk flavor and mouthfeel for acceptability on a just-about-right scale (JAR; 1 or 2=too little, 3= just right, 4 or 5= too much). The JAR score for milk flavor and mouthfeel was compared to the participant's overall acceptability score for the same sample after sensory evaluation to see how individual attributes influenced overall acceptability of each treatment. Participants perceived milk stored under fluorescent light as having ‘too much’ flavor compared to milk stored under LED light, which affected their acceptability of milk. When panelists scored milk as having too much flavor on the JAR scale, it caused significant penalty in their overall liking score of the same milk sample. Panelists who thought milk stored in the clear PET under fluorescent light had ‘too much’ flavor rated the overall acceptability of this milk 2.5 integers lower than panelists who thought this milk sample’s flavor was ‘just right’. Likewise, panelists who thought milk stored in clear HDPE packages under fluorescent light had ‘too much’ flavor rated their overall acceptability of milk stored in clear HDPE packages under fluorescent light 2.3 integers lower than panelists who though this milk sample had a ‘just right’ flavor. When rating the mouthfeel of milk, 15.9% of panelists thought that milk stored in HDPE under fluorescent light had ‘too much’ mouthfeel, and this led to a 0.2 integer decrease in their overall acceptability of this milk treatment.

Changes occurred in milk that affected flavor and quality after as little as 4 h of light exposure, which were noticeable to untrained consumer panelists and had direct influence on overall acceptability of the milk stored in these conditions. These results demonstrate that milk stored under LED light has clear advantages for maintaining consumer acceptability over milk stored under fluorescent light. The light blocking additives included in PET did not have a significant impact on maintaining more acceptable milk through 4 h of light exposure, but the type of light milk is exposed to impacted many aspects of milk acceptability. Light interference properties in packaging may provide more significant protection to maintain consumer acceptability if milk was stored under higher intensity lights for longer durations of time.

5.3.2 Dissolved Oxygen

Dissolved oxygen in milk serves as a reactant to propagate light-induced oxidation reactions, and has been previously related to differences in oxidation by different types of light and different levels of packaging protection against light (Potts, Ch. IV, 2016). The measurement of dissolved oxygen content in milk provided supportive analytical measurements of oxidation that were associated with sensory results. Fresh milk had higher levels of dissolved oxygen then all milk after 4 h of light exposure, with 11.27 ± 0.06 ppm, indicating light-induced reactions were occurring and consuming oxygen as a reactant. After treatment, there were significant differences in dissolved oxygen levels (Figure 17) that are associated with the changes in consumer acceptability of milk, proving the value of this analytical measure as an indication of the severity of oxidation reactions in milk.

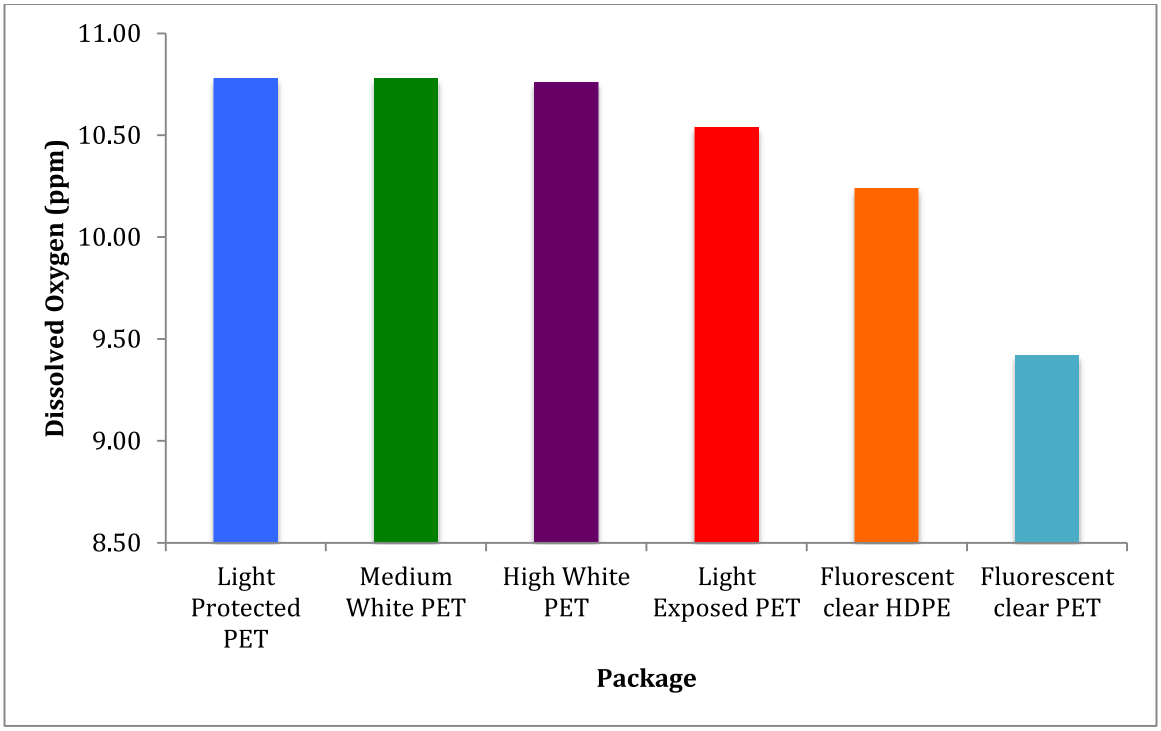

Figure 17. Dissolved oxygen content (ppm) in 2% milk after 4 h light exposure. HDPE and PET treatments were stored under fluorescent light and light protected PET, medium white PET, high white PET, and light exposed PET were all stored under LED light.

All of the milk stored under LED lights had similarly high levels of dissolved oxygen after 4 h of light exposure, indicating that less extensive oxidation reactions occurred in this milk than in milk stored under fluorescent lights. This was confirmed by the higher acceptability scores from sensory testing for milk stored under LED light. Following the same trends as in the sensory acceptability testing, milk from HDPE packages had higher levels of dissolved oxygen than milk from PET packages when both were stored under fluorescent light (Appendix I). These results demonstrate that the type of packaging material has a significant effect on the extent of oxidation reactions when milk is stored under fluorescent light. It is possible that some of the natural opacity from translucent unpigmented HDPE packages helped block light wavelengths better than the clear unpigmented PET packages. The type of light is also very important, since milk stored in clear PET packages under LED light underwent significantly less oxidation reactions than milk stored in the same package under fluorescent light.

These analytical results confirmed the differences in milk that were detected by the consumer panel, and demonstrated the importance of light type by showing the significant changes that occurred after as little as 4 h light exposure. These results are in agreement with previous work (Brotherson et al., 2016, Potts, Ch. IV, 2016) which suggests that LED lights are less detrimental to milk, and create less extensive off-flavors from oxidation reactions that are unacceptable to consumers.

5.3.3 Formation of Secondary Oxidation Products measured by Thiobarbituric Acid Reactive Substances (TBARS) assay

The formation of secondary oxidation products has previously served as an indication of the extent of oxidation reactions in fluid milk (Mestdagh et al., 2005; Johnson et al., 2015; Walsh et al., 2015). However, in all of these studies milk was stored under fluorescent light and for >4 h. Potts (Ch. III and IV, 2016) and Amin (2016) found that the TBARS assay was only able to detect very low levels of secondary oxidation products in 2% milk stored under fluorescent and LED light at different light intensities that did not correlate well with apparent sensory results. This study detected similarly low TBARS values. Fresh milk had 0.07 ± 0.00 mg/L TBARS and TBARS values ranged from 0.14-0.22 mg/L after 4 h light exposure. There was no difference ($p>0.05$) in aldehyde levels between any packages (Appendix I). These results confirm the need to find a more reliable analytical indicator of oxidation reactions that occur in milk stored under LED lights, such as changes in dissolved oxygen content.

5.3.4 Degradation of Riboflavin (Rb) by fluorometric analysis

In this study, we found that riboflavin did not undergo significant changes after short periods (4 h) of light exposure. Fresh milk contained 1.44 ± 0.01 mg/L riboflavin, which is within normal ranges of previously reported riboflavin values in milk (1.36-1.75 mg/L; Dimick, 1982; Gebhardt and Thomas, 2002; Zygoura et al., 2004; Amin, 2016; Brotherson et al., 2016). After 4 h light exposure, there was no degradation ($p>0.05$) in riboflavin content in milk in any of the packages or under either type of light exposure (all treatments 1.44-1.46 mg/L; Appendix I).

5.3.5 Volatile Analysis by Electronic Nose

Canonical discrimination analysis (CDA) was used to create a cluster of "smells" for each treatment to compare to fresh milk for differences in volatile profiles as described in Potts (Ch. IV, 2016). As seen with our previous study (Potts, Ch. IV, 2016), fresh milk always showed 100% separation ($p<0.05$) from all milk after light exposure. When all six treatments were compared together, there was a large amount of overlap because of random noise from measuring all 32 sensors for each "smell" (Appendix I). Treatments were separated by light type to better compare differences in volatile profiles under fluorescent and LED light, as completed in Chapter III (Potts, 2016).

There was 100% separation between the two treatments under fluorescent light from each other and from fresh milk ($p<0.05$; Figure 18). There was a large difference in volatile profile between the two treatments and fresh milk, which is in agreement with sensory results, showing that the two treatments under fluorescent milk underwent the most oxidation. The eNose was discriminatory enough to clearly detect differences in volatile profile after only 4 h of fluorescent light exposure. It was also able to distinguish differences in volatile profile between the two packaging materials, making it as sensitive as the consumer sensory panel, which found differences ($p<0.05$) in aroma between unpigmented HDPE and PET under fluorescent light and clear PET under LED light. The differences in aroma compounds between milk stored in the two packaging materials could be a result of different scalping interactions between the packaging and milk.

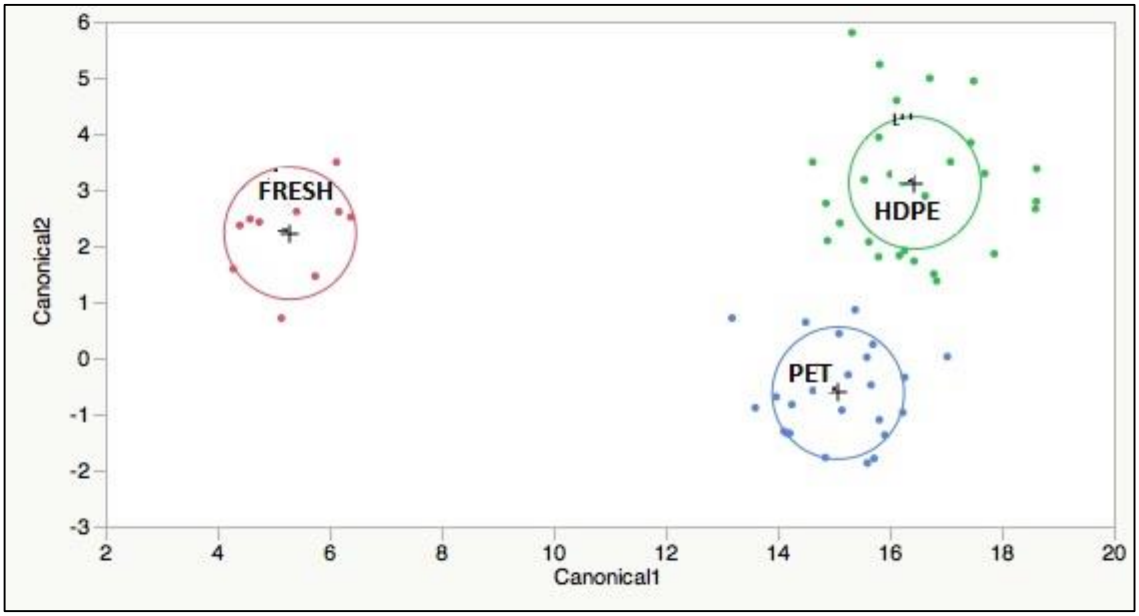

Figure 18. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of the two treatments (clear PET and clear HDPE) after 4 h fluorescent light exposure for differences.

The four PET treatments under LED light were also compared to fresh milk for volatile differences and found 100% separation between fresh milk and all milk after 4 h LED light exposure (Appendix I). When fresh milk was removed from the CDA plot, 100% separation was seen between the four PET treatments under LED light (Figure 19). This indicates that the eNose may be more discriminatory than sensory panels, finding significant differences ($p<0.05$) between PET packages under LED light after just 4 h, when the consumer sensory panel was not able to detect differences between milk under LED light.

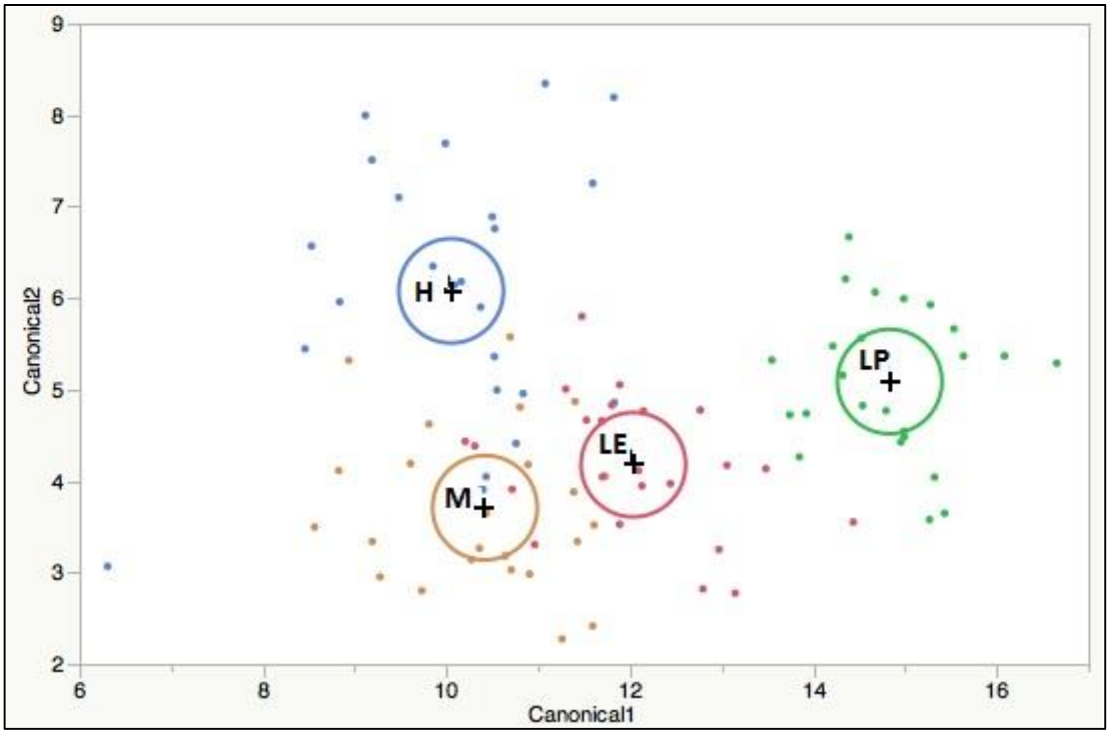

Figure 19. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of the four treatments (LP=light-protected PET, LE= light-exposed PET, M= medium white PET and H= high white PET) after 4 h LED light exposure for differences.

The eNose was sensitive enough to detect differences in volatile profile after just 4 h of light exposure. It was able to distinguish ($p<0.05$) volatile differences between different types of packaging materials (PET and HDPE) and from the same packaging material (PET) with different LPAs under LED light. The eNose is discriminatory enough to be used as a quality check tool to differentiate fresh milk from older milk that has undergone light exposure after as little as 4 h of light exposure, and has potential to identify milk stored in different types of packaging materials. Future work should focus on training the eNose to recognize specific volatile compounds to identify which volatiles are changing under fluorescent and LED light exposure.

5.4 Conclusions

The type of light that milk is stored under has a significant effect on milk quality oxidation. Fluorescent light causes significant decreases in milk acceptability, flavor, and aftertaste after as little as 4 h when packaged in unpigmented packages with no light interference properties. This decreased acceptability can be related to decreasing milk trends over the past several decades, where fluorescent retail lights and translucent HDPE packages for milk were the norm. LED light is much less detrimental to milk quality and flavor than fluorescent light, as shown by higher consumer acceptability of milk stored under LED light. Regardless of the presence of absence of LPAs in the PET packages, milk stored under LED lights maintained higher acceptability and liking than milk stored under fluorescent light.

Milk can be packaged in unpigmented PET packages and stored under LED lights (<1,500 lux) and still be acceptable to consumers. However, many retail cases have higher light intensity, and not all retail stores have switched to LED lights. Packaging is the first line of defense for maintaining milk quality, and packages should contain high levels of light interference properties to protect milk from worst-case storage conditions, such as long periods under fluorescent light. Processors and retailers can play their part in maintaining high quality milk from cow to consumer by packaging milk in protective packages and designing low intensity LED retail cases. The most optimal storage conditions for maintaining high quality milk are packaging milk in PET packages under LED light for short periods of time. If milk flavor can be improved through packaging and retail case improvements, consumer acceptability and milk sales can be increased.

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APPENDICES

APPENDIX A

Complete Results for Chapter III

Table 3. Average ± SD light intensity (lux) for each position within each retail case (closed-door case= 14 test locations, open-front case= 22 test locations, walk-in case= 16 test locations).

	Closed-Door Case	Open-Front Case	Walk-In Case
1	1014 ± 18.0	790 ± 7.8	4828 ± 274.3
2	1015 ± 18.0	825 ± 7.8	5455 ± 380.2
3	1016 ± 18.0	865 ± 6.6	5575 ± 235.1
4	1017 ± 18.0	898 ± 6.8	5897 ± 490.8
5	1018 ± 18.0	923 ± 8.0	5933 ± 213.9
6	1019 ± 18.0	942 ± 11.1	5337 ± 388.2
7	1020 ± 18.0	953 ± 11.6	5568 ± 511.4
8	1021 ± 18.0	963 ± 13.3	5820 ± 237.3
9	1022 ± 18.0	967 ± 11.8	5940 ± 222.3
10	1023 ± 18.0	965 ± 9.8	5763 ± 374.3
11	1024 ± 18.0	962 ± 9.2	5733 ± 431.1
12	1025 ± 18.0	952 ± 8.1	5815 ± 236.5
13	1026 ± 18.0	951 ± 7.6	5995 ± 227.7
14	1027 ± 18.0	952 ± 6.5	5475 ± 193.4
15		951 ± 6.1	4210 ± 474.4
16		946 ± 7.4	3138 ± 1037.4
17		940 ± 6.0	
18		925 ± 7.3	
19		903 ± 6.9	
20		873 ± 6.5	
21		841 ± 6.5	
22		813 ± 9.2	

Table 4. Average ± SD light intensity and median light intensity (lux) for each package treatment in each retail case. LP=light-protected, H= high white, W= low white, Y= commercial yellow, LE= light-exposed

			LP	**H**	**W**	**Y**	**LE**
Closed-door Case	**4 H**	Mean ± SD	1019 ± 17.5	1019 ± 17.3	1019 ± 18.2	1018 ± 17.4	1020 ± 19.1
		Median	1014	1012	1011	1011	1011
	8 H	Mean ± SD	1023 ± 17.7	1021 ± 19.1	1022 ± 18.7	1023 ± 17.9	1021 ± 18.6
		Median	1026	1025	1023	1027	1026
Open-front Case	**4 H**	Mean ± SD	909 ± 58.9	935 ± 40.3	957 ± 15.5	903 ± 61.2	914 ± 40.0
		Median	930	938	956	917	924
	8 H	Mean ± SD	893 ± 45.8	928 ± 58.3	894 ± 75.6	880 ± 75.6	914 ± 56.7
		Median	888	953	919	888	927
Walk-in Case	**4 H**	Mean ± SD	5285 ± 468	5680 ± 359	5483 ± 117	5083 ± 776	5362 ± 544
		Median	5405	5745	5450	5235	5490
	8 H	Mean ± SD	5303 ± 1297	5333 ± 628	5550 ± 597	5795 ± 545	5745 ± 970
		Median	5750	5625	5575	5810	6155

Table 5. Average TBARS ± SD (mg/L) for each packaging treatment in each time period and each case (Chapter III).

	Light-protected	High white	Low white	Yellow	Light-exposed
Closed-door Case					
4	0.13 ± 0.05	0.20 ± 0.04	0.12 ± 0.05	0.25 ± 0.09	0.24 ± 0.16
8	0.11 ± 0.05	0.14 ± 0.07	0.17 ± 0.08	0.21 ± 0.17	0.26 ± 0.15
Open-front Case					
4	0.13 ± 0.05	0.13 ± 0.04	0.13 ± 0.06	0.20 ± 0.08	0.16 ± 0.03
8	0.16 ± 0.06	0.14 ± 0.06	0.22 ± 0.10	0.13 ± 0.05	0.20 ± 0.09
Walk-in Case					
4	0.19 ± 0.04	0.34 ± 0.18	0.24 ± 0.03	0.29 ± 0.11	0.25 ± 0.03
8	0.20 ± 0.03	0.27 ± 0.09	0.24 ± 0.10	0.29 ± 0.05	0.13 ± 0.05

Table 6. Average riboflavin concentration ± SD (mg/L) for each packaging treatment in each time period and each case (Chapter III).

	Light-protected	High white	Low white	Yellow	Light-exposed
Closed-door Case					
4	1.44 ± 0.37	1.51 ± 0.20	1.75 ± 0.42	1.59 ± 0.35	1.42 ± 0.28
8	1.54 ± 0.45	1.73 ± 0.34	2.63 ± 157	1.89 ± 0.56	2.37 ± 1.51
Open-front Case					
4	1.49 ± 0.34	1.70 ± 0.62	1.90 ± 0.54	1.73 ± 1.09	1.62 ± 0.27
8	2.53 ± 1.23	1.85 ± 0.25	2.65 ± 1.22	2.18 ± 0.96	1.60 ± 0.41
Walk-in Case					
4	1.57 ± 0.61	1.32 ± 0.27	1.88 ± 0.46	1.38 ± 0.48	2.66 ± 1.34
8	1.50 ± 0.30	1.48 ± 0.29	2.40 ± 1.06	2.96 ± 1.61	2.76 ± 1.30

Table 7. L*a*b* color values for experimental HDPE packages. L*= black to white; a*= green to red; b*= blue to yellow.

	L	**a**	**b**
Clear	89.03	0.78	-2.65
Low white	96.46	0.15	-0.74
High white	97.48	-0.05	-0.52
Yellow	87.95	-12.31	69.76

APPENDIX B

Virginia Polytechnic Institute and State University

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

Title Project: Packaging Protection of Flavor Quality in Milk
Investigators: Kemia Amin, Hayley Potts, and Susan E. Duncan, PhD, RD

I. Purpose of this Research/Project

You are invited to participate in a study about the protection relationship of flavor and nutrient quality in oxidative flavor milk, as influenced by packaging. Packaging design is an important parameter in protecting milk quality. This study will help identify key variables in protecting nutritional value and flavor quality of these products.

II. Procedures

After you provide consent by signing this form, return it through the hatch in front of you. If you choose to not provide consent, you may leave the sensory laboratory after you return the unsigned form through the hatch. If you consent to participate, you will register on the touchscreen monitor and complete the demographic and general questionnaire. After the questionnaire, there will be a series of five sensory triangle tests, which will take approximately 20 minutes total time to complete. You will be presented with five separate sets of three milk samples each. For each set, you need to taste samples from left to right, and will be asked to identify the different sample among the three milk samples.

Please follow these steps when you taste the milk:

1) Smell the sample and take a generous sip, roll the milk around in the mouth, and then expectorate.
2) After tasting, draw a breath of air slowly through the mouth and then exhale slowly though the nose.
3) Record your response on the touchscreen monitor for the sample that is different.

Between each set, you need to rinse the mouth, eat the crackers to clean the palate, and wait one minute before evaluating the next set of samples.

III. Risks

There are no more than minimal risks for participating in this study. Participants with unknown allergies to dairy products may be at risk. If you have known allergies to any of these products, please withdraw from the study at this time. If you have gluten sensitivity, you may choose to decline participation or to choose not to eat the crackers provided between each sample set. Your participation in this study will provide valuable information about flavor quality of milk products, which will be useful to the food/dairy and packaging industries. If you would like a summary of the research results, please contact the researcher at a later time.

V. Extent of Anonymity and Confidentiality

The results of your performance as a panelist will be kept strictly confidential except to the investigators. 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 snack for participating in this study. In addition, you can have a participation punch card marked. Participation in this panel gives you 2 punches on the card. Getting 10 punches/marks and turning in your punch card, with your name and contact information, rewards you with a $10 gift card from selected retail stores. Your completed card is also your entry into a semester long drawing for a $100 gift card.

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. There may be reasons under which the investigator may determine you should not participate in this study. If you have allergies to dairy products, or are under the age of 18, you are asked to refrain from participating.

VII. Subject's Responsibilities

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

> Smell and taste the milk products and identify the odd sample based on aroma and taste.

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

-----------For human subject to keep------------

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject. I may contact:

Kemia Amin, Investigator	kamin2@vt.edu
Hayley Potts, Investigator	hpotts45@vt.edu
Susan Duncan, Faculty/ Investigator	(540) 231-8675; duncans@vt.edu

David Moore
Chair, Virginia Tech Institutional Review (540) 231-4991;
moored@vt.edu
Board for the Protection of Human Subjects
Office of Research Compliance
1880 Pratt Drive, Suite 2006 (0497)
Blacksburg, VA 24061

APPENDIX C

Scorecard placed on SIMS touchscreen monitor

There are three samples in each of the 5 triangles for you to evaluate. Please make sure that the sample code matches the sample code on the cup.

Two of these samples are alike (same). Taste the samples in the order indicated and identify the odd (different) sample. Rinse your mouth with 2 oyster crackers and water between triangles.

Triangle 1

☐ Code 1 ☐ Code 2 ☐ Code 3

Triangle 2

☐ Code 1 ☐ Code 2 ☐ Code 3

Triangle 3

☐ Code 1 ☐ Code 2 ☐ Code 3

Triangle 4

☐ Code 1 ☐ Code 2 ☐ Code 3

Triangle 5

☐ Code 1 ☐ Code 2 ☐ Code 3

APPENDIX D.

Sensory panel schedule for Replication 1.

Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Milk Pickup 5PM							
8HR IN @ 10 PM	8HR OUT @ 6AM 8HR Sensory Panel 11am-5pm						
	4HR IN @ 12PM 4HR OUT @ 4PM	4HR Sensory Panel 10am-5pm					
		72HR IN @ 3PM			72HR OUT @ 3PM	72HR Sensory Panel 10am-5pm	
					24HR IN @ 6PM	24HR OUT @ 6PM	24HR Sensory Panel 10am-5pm

Sensory panel schedule for Replications 2 & 3

Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
Milk Pickup 4PM	BOTTLE ALL TRTS							
	4 HR IN @ 11:30AM 4 HR OUT @ 3:30PM	4HR Sensory Panel 10am-5pm						
		72HR IN @ 2PM			72HR OUT @ 2PM	72HR Sensory Panel 10am-5pm		
					24HR IN @ 2PM	24HR OUT @ 2PM	24HR Sensory Panel 10am-5pm	
							8HR IN @ 6AM 8 HR OUT @ 2PM	8 HR Sensory Panel 10am-5pm

APPENDIX E
Complete results from Chapter IV

Figure 20. The front loading, closed-door retail case with fluorescent (3500K, left two doors) and LED (right two doors; 3500K on left and center positions 4000K on far right position) lighting that was used for the study. Light intensity readings (lux) are represented for each position throughout the retail case. Treatment packages were not placed on the bottom row of the case due to lower light intensity and increased light intensity variability in these locations. Treatment packages were randomly placed throughout the front row of this case.

Table 8. Average light intensity (lux; mean ± SD, median) for each packaging treatment (n=24). LP= light-protected (foil and plastic overlay) PET control; H= high white PET package; M= medium white PET package; L= low white PET package; UV= UV barrier PET; LE= light-exposed PET control.

			LP	**H**	**M**	**L**	**UV**	**LE**
Fluorescent	**4 H**	Average ± SD	1659 ± 1276	1425 ± 957	1624 ± 1444	1297 ± 878	1363 ± 935	1393 ± 1058
		Median	1154.5	1274.5	851.5	1148.5	1052.0	894.0
	8 H	Average ± SD	1589 ± 1229	1223 ± 647	1466 ± 1264	1207 ± 841	1487 ± 974	1567 ± 1288
		Median	997.5	1109.5	1012.0	979.5	1350.0	1049.5
	24 H	Average ± SD	1199 ± 704	1516 ± 1045	1475 ± 1141	1064 ± 719	1614 ± 919	1525 ± 1309
		Median	1012.0	1214.5	940.5	831.0	1513.0	900.0
	72 H	Average ± SD	1531 ± 1256	1282 ± 641	1285 ± 1051	1283 ± 941	1674 ± 1446	1483 ± 1156
LED		Median	1054.5	1163.5	914.0	1035.5	1264.5	928.0
	4 H	Average ± SD	944 ± 169	892 ± 151	937 ± 124	887 ± 183	957 ± 161	924 ± 150
		Median	933.0	933.5	972.0	901.0	967.0	934.0
	8 H	Average ± SD	961 ± 142	921 ± 135	964 ± 144	927 ± 141	954 ± 129	966 ± 114
		Median	935.5	904.5	976.0	917.0	970.5	961.0
	24 H	Average ± SD	951 ± 136	936 ± 145	964 ± 122	943 ± 117	958 ± 127	956 ± 128
		Median	935.0	941.5	960.5	928.5	937.0	948.5
	72 H	Average ± SD	959 ± 107	932 ± 157	948 ± 150	943 ± 117	962 ± 142	944 ± 129
		Median	957.5	950.0	967.0	955.5	952.0	932.0

Table 9. Average TBARS results (mg/L) ± SD from the individual packages of each packaging treatment after each time treatment under fluorescent and LED light.

Fluorescent	4	8	24	72
LP	0.30 ± 0.19	0.21 ± 0.04	0.38 ± 0.31	0.32 ± 0.19
H	0.21 ± 0.06	0.23 ± 0.06	0.37 ± 0.27	0.42 ± 0.33
M	0.34 ± 0.15	0.39 ± 0.23	0.34 ± 0.23	0.45 ± 0.36
L	0.34 ± 0.21	0.28 ± 0.09	0.29 ± 0.11	0.21 ± 0.11
UV	0.31 ± 0.08	0.32 ± 0.15	0.47 ± 0.28	0.42 ± 0.17
LE	0.23 ± 0.08	0.25 ± 0.08	0.23 ± 0.07	0.20 ± 0.03
LED	4	8	24	72
LP	0.65 ± 0.26	0.38 ± 0.28	0.34 ± 0.16	0.23 ± 0.05
H	0.50 ± 0.27	0.55 ± 0.07	0.31 ± 0.11	0.26 ± 0.06
M	0.59 ± 0.18	0.28 ± 0.06	0.34 ± 0.23	0.21 ± 0.01
L	0.56 ± 0.42	0.52 ± 0.15	0.53 ± 0.32	0.23 ± 0.05
UV	0.63 ± 0.22	0.27 ± 0.13	0.55 ± 0.32	0.21 ± 0.01
LE	0.58 ± 0.24	0.26 ± 0.07	0.42 ± 0.19	0.20 ± 0.01

Table 10. Average riboflavin results (mg/L) ± SD from the individual packages of each packaging treatment after each time treatment under fluorescent and LED light.

Fluorescent	4	8	24	72
LP	1.09 ± 0.24	1.56 ± 0.48	1.53 ± 0.21	1.93 ± 1.24
H	1.01 ± 0.22	1.28 ± 0.25	1.47 ± 0.27	1.55 ± 0.98
M	1.07 ± 0.27	1.07 ± 0.27	1.49 ± 0.29	1.09 ± 0.16
L	1.39 ± 0.58	1.22 ± 0.24	1.62 ± 0.27	0.90 ± 0.11
UV	1.25 ± 0.53	1.40 ± 0.22	1.43 ± 0.18	1.50 ± 0.77
LE	1.14 ± 0.28	1.33 ± 0.38	1.38 ± 0.20	1.16 ± 0.39
LED	4	8	24	72
LP	2.05 ± 1.27	1.74 ± 0.44	1.41 ± 0.41	1.27 ± 0.22
H	1.42 ± 0.26	1.41 ± 0.27	1.16 ± 0.15	1.25 ± 0.25
M	1.38 ± 0.31	1.22 ± 0.09	1.10 ± 0.20	1.39 ± 0.29
L	1.31 ± 0.31	1.35 ± 0.23	1.06 ± 0.22	1.39 ± 0.27
UV	1.36 ± 0.22	1.28 ± 0.11	1.31 ± 0.27	1.24 ± 0.25
LE	1.37 ± 0.32	1.32 ± 0.15	1.20 ± 0.30	1.27 ± 0.37

Table 11. Average dissolved oxygen results (ppm) ± SD from the individual packages of each packaging treatment after each time treatment under fluorescent and LED light.

Fluorescent	4	8	24	72
LP	10.66 ± 0.23	9.88 ± 1.16	9.63 ± 0.39	10.06 ± 0.32
H	10.49 ± 0.22	9.79 ± 1.17	9.49 ± 0.13	9.64 ± 0.35
M	10.44 ± 0.19	9.58 ± 1.14	9.17 ± 0.40	8.69 ± 0.60
L	10.36 ± 0.23	9.43 ± 1.26	8.84 ± 0.48	7.48 ± 1.23
UV	10.00 ± 0.34	9.30 ± 0.46	7.05 ± 1.19	5.63 ± 0.50
LE	10.07 ± 0.31	9.07 ± 1.01	7.62 ± 0.46	4.33 ± 1.25
LED	4	8	24	72
LP	10.60 ± 0.25	9.87 ± 1.05	9.71 ± 0.21	10.06 ± 0.32
H	10.53 ± 0.21	9.82 ± 1.23	9.53 ± 0.18	9.65 ± 0.45
M	10.48 ± 0.25	9.82 ± 1.02	9.34 ± 0.21	9.08 ± 0.33
L	10.37 ± 0.28	9.48 ± 1.21	9.05 ± 0.25	8.16 ± 0.48
UV	10.17 ± 0.36	9.16 ± 1.21	7.93 ± 0.33	6.50 ± 0.48
LE	10.14 ± 0.27	9.11 ± 1.01	8.26 ± 0.43	6.48 ± 0.48

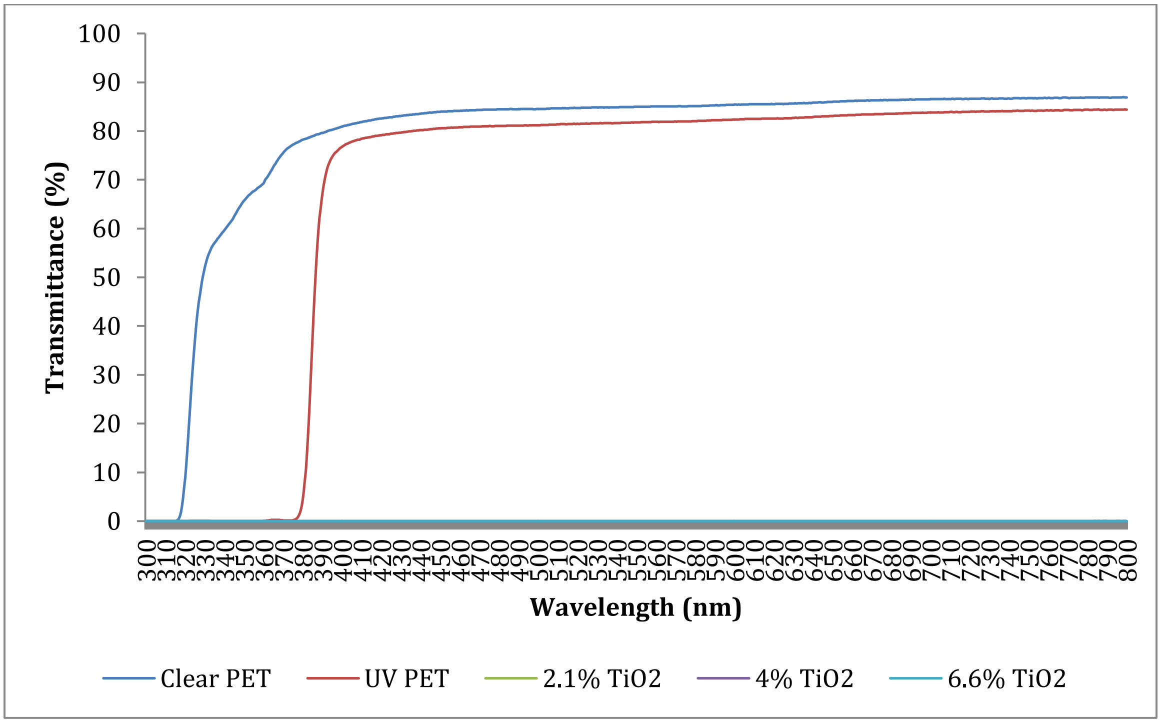

Figure 21. Light transmittance (%) of experimental PET packages in the visible light spectrum (300-800 nm).

Table 12. L*a*b* color values for experimental PET packages. L*= black to white; a*= green to red; b*= blue to yellow.

	L	**a**	**b**
Clear	91.40	1.73	-1.74
Clear with UV barrier	91.31	1.83	-1.92
Low white	94.78	0.29	-0.64
Medium white	96.31	0.17	-0.75
High white	97.20	0.01	-0.37

Table 13. Complete sensory evaluation statistics for each comparison and each time treatment tested during this experiment. Preset proportion of discriminators for each comparison is 20%. LP= light-protected (foil and plastic overlay) PET control; H= high white PET; M= medium white PET; L= low white PET; UV= UV barrier PET; LE= light-exposed PET control.

Comparison	**Testing**	**Time of Light Exposure**	**alpha**	**beta**	**Trials**	**Successes**	**p-value**	**Actual proportion of discriminators**	**Std. Deviation**	**One Sided Upper Confidence Limit**	**One Sided Lower Confidence Limit**
Fluorescent											
LP vs. LE	Difference	4	0.05	0.20	113	50	0.0069	16%	0.0133	0.1856	0.1418
LP vs. UV	Similarity	4	0.20	0.05	116	51	0.0075	16%	0.0130	0.1704	0.1485
LP vs. L	Similarity	4	0.20	0.05	117	51	0.0094	15%	0.0129	0.1647	0.1429
LP vs. M	Similarity	4	0.20	0.05	117	52	0.0054	17%	0.0128	0.1775	0.1559
LP vs. H	Similarity	4	0.20	0.05	116	39	0.4721	0%	0.0149	0.0168	-0.0082
LE vs. UV	Difference	4	0.05	0.20	117	50	0.0154	14%	0.0131	0.1625	0.1195
LE vs. L	Difference	4	0.05	0.20	117	40	0.4207	1%	0.0146	0.0369	-0.0112
LE vs. M	Difference	4	0.05	0.20	117	48	0.0384	12%	0.0133	0.1373	0.0934
LE vs. H	Difference	4	0.05	0.20	116	46	0.0735	9%	0.0137	0.1173	0.0723
LED											
LP vs. LE	Difference	4	0.05	0.20	113	44	0.102	8%	0.0142	0.1074	0.0607
LP vs. UV	Similarity	4	0.20	0.05	116	45	0.1056	8%	0.0138	0.0936	0.0702
LP vs. L	Similarity	4	0.20	0.05	116	46	0.0735	9%	0.0137	0.1064	0.0833
LP vs. M	Similarity	4	0.20	0.05	116	38	>0.5000	-1%	0.0151	0.0041	-0.0213
LP vs. H	Similarity	4	0.20	0.05	115	41	0.2981	3%	0.0146	0.0470	0.0225
LE vs. UV	Difference	4	0.05	0.20	115	39	0.4483	1%	0.0149	0.0333	-0.0159
LE vs. L	Difference	4	0.05	0.20	116	43	0.1977	6%	0.0142	0.0793	0.0327
LE vs. M	Difference	4	0.05	0.20	115	40	0.3707	2%	0.0147	0.0460	-0.0025

LE vs. H	Difference	4	0.05	0.20	115	50	0.0104	15%	0.0132	0.1739	0.1305
Fluorescent											
LP vs. LE	Difference	8	0.05	0.20	115	60	<0.0001	28%	0.0120	0.3024	0.2628
LP vs. UV	Similarity	8	0.20	0.05	114	59	<0.0001	28%	0.0122	0.2866	0.2660
LP vs. L	Similarity	8	0.20	0.05	114	42	0.2119	5%	0.0145	0.0648	0.0405
LP vs. M	Similarity	8	0.20	0.05	115	56	<0.0001	23%	0.0125	0.2409	0.2199
LP vs. H	Similarity	8	0.20	0.05	114	44	0.117	8%	0.0141	0.0908	0.0671
LE vs. UV	Difference	8	0.05	0.20	116	43	0.1977	6%	0.0142	0.0793	0.0327
LE vs. L	Difference	8	0.05	0.20	115	58	<0.0001	26%	0.0122	0.2767	0.2364
LE vs. M	Difference	8	0.05	0.20	116	44	0.1469	7%	0.0140	0.0920	0.0459
LE vs. H	Difference	8	0.05	0.20	116	42	0.2546	4%	0.0143	0.0667	0.0195
LED											
LP vs. LE	Difference	8	0.05	0.20	115	57	<0.0001	24%	0.0124	0.2638	0.2232
LP vs. UV	Similarity	8	0.20	0.05	115	55	0.0005	22%	0.0126	0.2280	0.2068
LP vs. L	Similarity	8	0.20	0.05	115	42	0.2327	5%	0.0144	0.0599	0.0357
LP vs. M	Similarity	8	0.20	0.05	113	52	0.0021	19%	0.0130	0.2012	0.1793
LP vs. H	Similarity	8	0.20	0.05	113	33	>0.5000	-6%	0.0164	-0.0482	-0.0757
LE vs. UV	Difference	8	0.05	0.20	114	29	>0.5000	-12%	0.0174	-0.0898	-0.1470
LE vs. L	Difference	8	0.05	0.20	114	43	0.1611	7%	0.0143	0.0893	0.0423
LE vs. M	Difference	8	0.05	0.20	113	32	>0.5000	-8%	0.0166	-0.0479	-0.1026
LE vs. H	Difference	8	0.05	0.20	113	46	0.0485	11%	0.0139	0.1334	0.0878
Fluorescent											
LP vs. LE	Difference	24	0.05	0.20	117	87	<0.0001	62%	0.0099	0.6317	0.5991
LP vs. UV	Similarity	24	0.20	0.05	116	57	<0.0001	24%	0.0123	0.2474	0.2267
LP vs. L	Similarity	24	0.20	0.05	116	61	<0.0001	29%	0.0119	0.2988	0.2788
LP vs. M	Similarity	24	0.20	0.05	117	50	0.0154	14%	0.0131	0.1520	0.1300

LP vs. H	Similarity	24	0.20	0.05	116	49	0.0207	13%	0.0133	0.1448	0.1225
LE vs. UV	Difference	24	0.05	0.20	118	46	0.0968	8%	0.0136	0.1071	0.0624
LE vs. L	Difference	24	0.05	0.20	116	58	<0.0001	25%	0.0122	0.2701	0.2299
LE vs. M	Difference	24	0.05	0.20	117	44	0.1635	6%	0.0139	0.0870	0.0412
LE vs. H	Difference	24	0.05	0.20	115	46	0.0643	10%	0.0137	0.1226	0.0774
LED											
LP vs. LE	Difference	24	0.05	0.20	116	61	<0.0001	29%	0.0119	0.3083	0.2692
LP vs. UV	Similarity	24	0.20	0.05	117	63	<0.0001	31%	0.0116	0.3175	0.2979
LP vs. L	Similarity	24	0.20	0.05	116	66	<0.0001	35%	0.0114	0.3631	0.3438
LP vs. M	Similarity	24	0.20	0.05	117	55	0.0008	21%	0.0125	0.2156	0.1946
LP vs. H	Similarity	24	0.20	0.05	114	61	<0.0001	30%	0.0120	0.3127	0.2925
LE vs. UV	Difference	24	0.05	0.20	115	51	0.006	17%	0.0131	0.1867	0.1437
LE vs. L	Difference	24	0.05	0.20	117	38	>0.5000	-1%	0.0150	0.0119	-0.0375
LE vs. M	Difference	24	0.05	0.20	114	38	0.5	0%	0.0152	0.0250	-0.0250
LE vs. H	Difference	24	0.05	0.20	117	59	<0.0001	26%	0.0120	0.2762	0.2366
Fluorescent											
LP vs. LE	Difference	72	0.05	0.20	118	66	<0.0001	34%	0.0113	0.3576	0.3203
LP vs. UV	Similarity	72	0.20	0.05	120	72	<0.0001	40%	0.0108	0.4091	0.3909
LP vs. L	Similarity	72	0.20	0.05	120	65	<0.0001	31%	0.0113	0.3220	0.3030
LP vs. M	Similarity	72	0.20	0.05	120	58	0.0001	23%	0.0120	0.2351	0.2149
LP vs. H	Similarity	72	0.20	0.05	119	57	0.0004	22%	0.0121	0.2287	0.2083
LE vs. UV	Difference	72	0.05	0.20	119	45	0.1492	7%	0.0137	0.0897	0.0447
LE vs. L	Difference	72	0.05	0.20	120	39	>0.5000	-1%	0.0146	0.0115	-0.0365
LE vs. M	Difference	72	0.05	0.20	120	39	>0.5000	-1%	0.0146	0.0115	-0.0365
LE vs. H	Difference	72	0.05	0.20	118	53	0.0038	17%	0.0126	0.1945	0.1529
LED											
LP vs. LE	Difference	72	0.05	0.20	118	58	<0.0001	24%	0.0121	0.2572	0.2174
LP vs. UV	Similarity	72	0.20	0.05	119	67	<0.0001	34%	0.0112	0.3540	0.3351
LP vs. L	Similarity	72	0.20	0.05	117	70	<0.0001	40%	0.0110	0.4067	0.3881
LP vs. M	Similarity	72	0.20	0.05	118	65	<0.0001	33%	0.0114	0.3359	0.3167

LP vs. H	Similarity	72	0.20	0.05	120	58	0.0001	23%	0.0120	0.2351	0.2149
LE vs. UV	Difference	72	0.05	0.20	117	34	>0.5000	-6%	0.0159	-0.0380	-0.0902
LE vs. L	Difference	72	0.05	0.20	118	38	>0.5000	-2%	0.0149	0.0076	-0.0415
LE vs. M	Difference	72	0.05	0.20	118	40	0.4483	1%	0.0146	0.0324	-0.0155
LE vs. H	Difference	72	0.05	0.20	117	43	0.2148	5%	0.0141	0.0745	0.0281

APPENDIX F.

CDA plots from Ch. IV, Study 1 eNose volatile analyses of individual PET packages after 8 and 24 h compared to fresh milk.

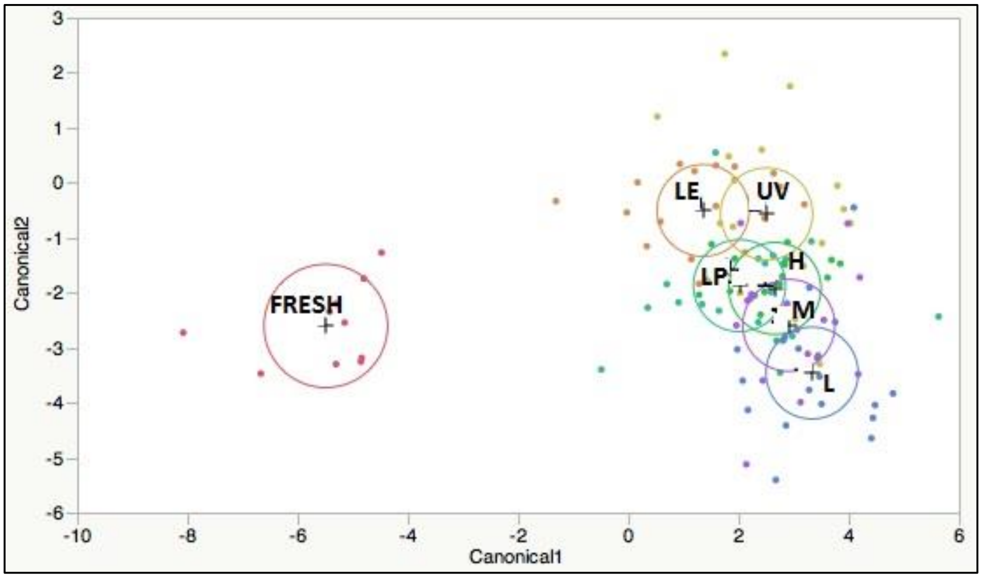

Figure 22. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles in PET packages to fresh milk after 8 hours of fluorescent light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

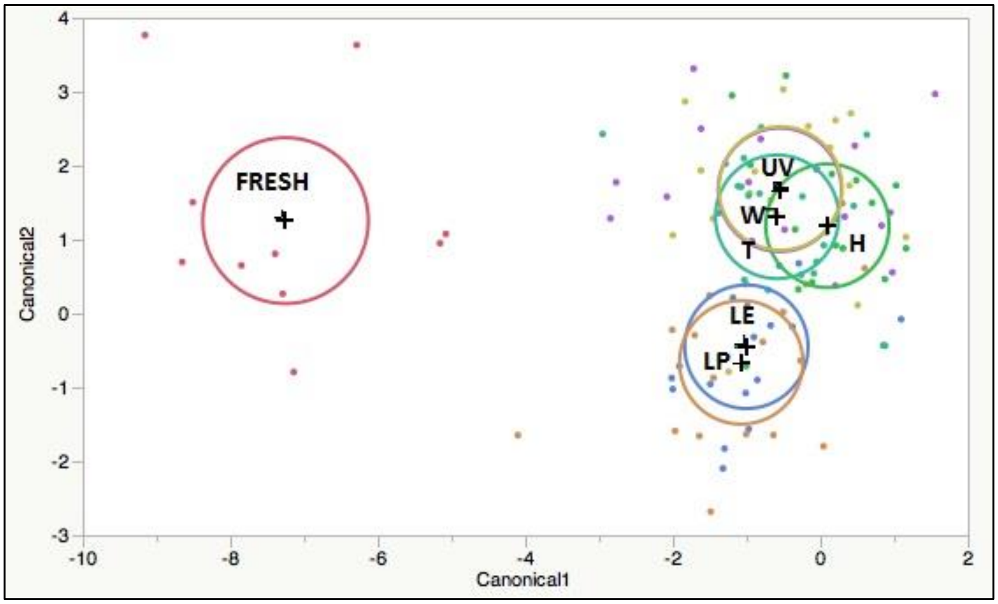

Figure 23. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles in PET packages to fresh milk after 8 hours of LED light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

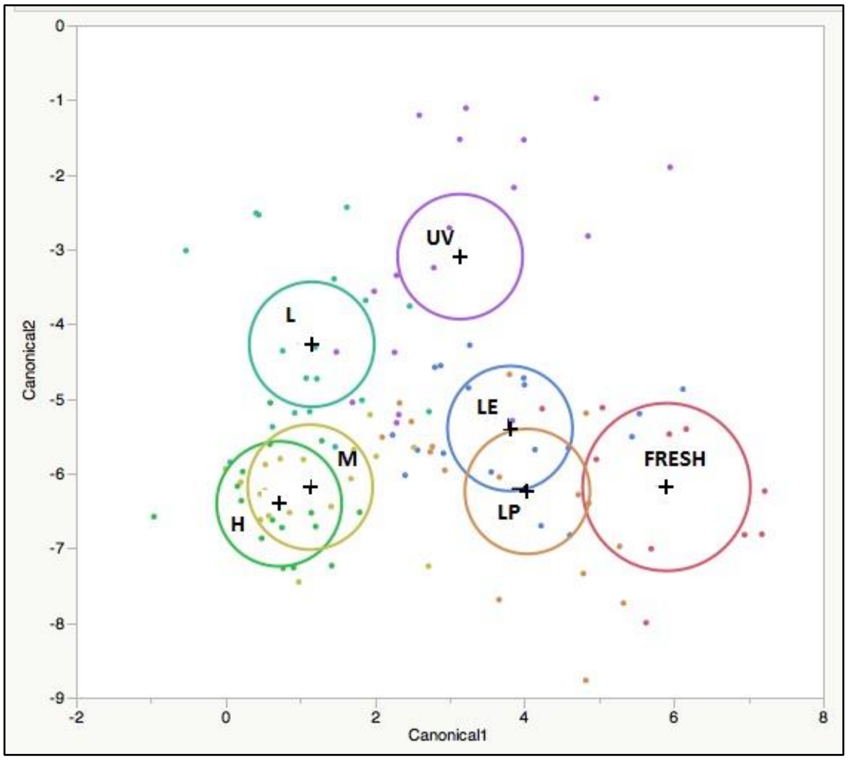

Figure 24. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles in PET packages to fresh milk after 24 hours of fluorescent light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

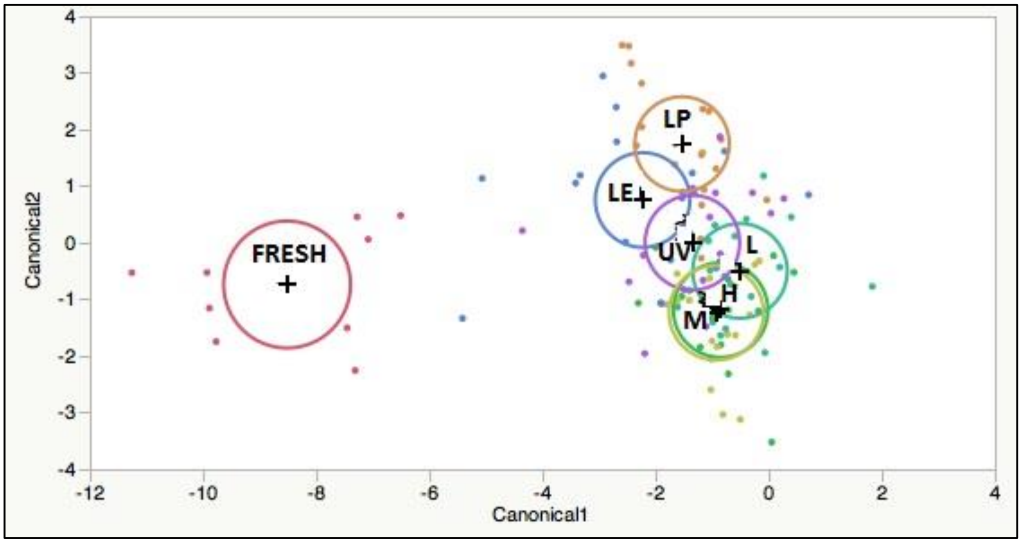

Figure 25. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles in PET packages to fresh milk after 24 hours of LED light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

APPENDIX G.

CDA plots from Ch. IV eNose volatile analyses of commingled PET packages after 8 and 24 h compared to fresh milk.

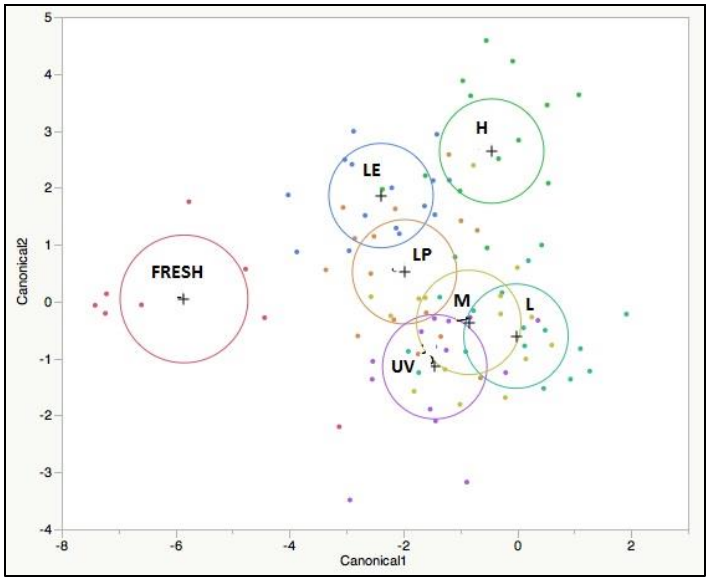

Figure 26. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of commingled PET treatments to fresh milk after 8 h of fluorescent light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

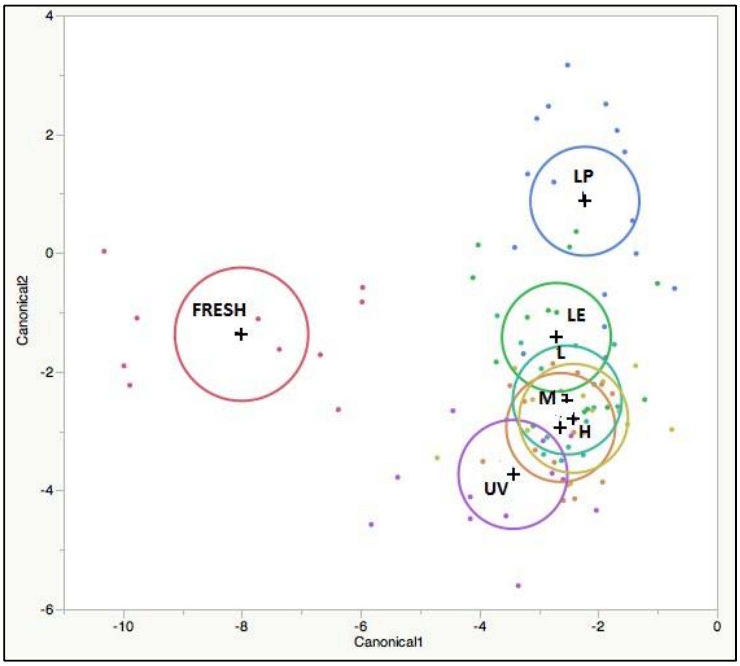

Figure 27. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of commingled PET treatments to fresh milk after 8 h of LED light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

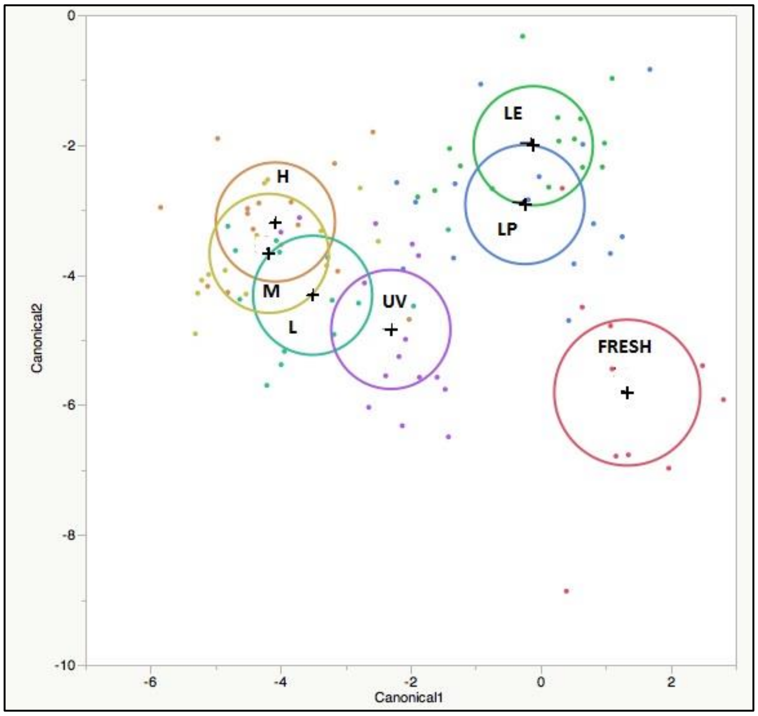

Figure 28. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of commingled PET treatments to fresh milk after 24 h of fluorescent light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

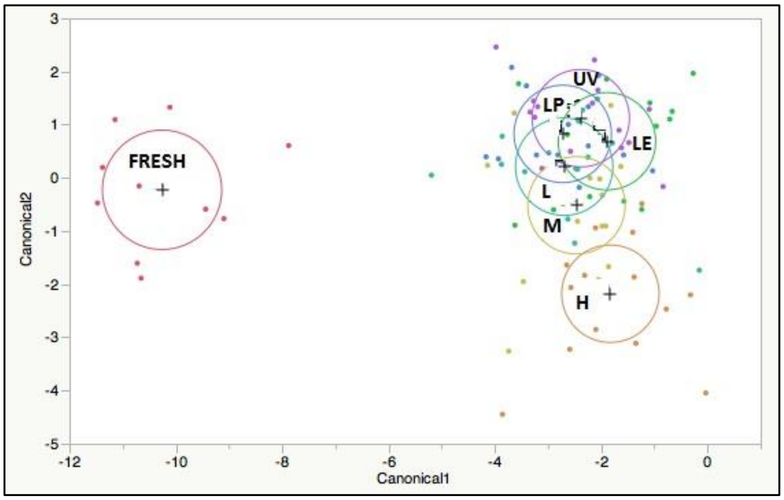

Figure 29. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of commingled PET treatments to fresh milk after 24 h of LED light exposure. LP= light-protected PET, LE= light-exposed PET, UV= UV barrier PET, L= low white PET, M= medium white PET, H= high white PET.

APPENDIX H

Analytical results for shelf-life study (Chapter IV, Study 1).

Table 14. Average ± SD TBARS (mg/L) for milk (3 replications; n=6) from 18-day shelf life study under fluorescent and LED light.

Fluorescent	4	8	24	72
LP	0.31 ± 0.18	0.22 ± 0.06	0.23 ± 0.10	0.21 ± 0.04
H	0.25 ± 0.08	0.21 ± 0.06	0.23 ± 0.15	0.27 ± 0.12
M	0.26 ± 0.09	0.20 ± 0.04	0.26 ± 0.11	0.26 ± 0.11
L	0.25 ± 0.05	0.27 ± 0.05	0.32 ± 0.17	0.27 ± 0.11
UV	0.24 ± 0.08	0.24 ± 0.05	0.27 ± 0.11	0.29 ± 0.10
LE	0.25 ± 0.12	0.23 ± 0.10	0.31 ± 0.11	0.24 ± 0.03
LED	4	8	24	72
LP	0.21 ± 0.04	0.22 ± 0.05	0.17 ± 0.01	0.21 ± 0.03
H	0.22 ± 0.04	0.20 ± 0.04	0.18 ± 0.02	0.22 ± 0.03
M	0.23 ± 0.08	0.20 ± 0.04	0.18 ± 0.02	0.22 ± 0.03
L	0.25 ± 0.03	0.25 ± 0.03	0.19 ± 0.02	0.21 ± 0.03
UV	0.21 ± 0.03	0.20 ± 0.05	0.18 ± 0.01	0.22 ± 0.03
LE	0.21 ± 0.05	0.26 ± 0.06	0.17 ± 0.02	0.24 ± 0.09

Table 15. Average ± SD Rb (mg/L) for milk (3 replications; n=6) from 18-day shelf life study under fluorescent and LED light.

Fluorescent	4	8	24	72
LP	1.22 ± 0.26	1.29 ± 0.29	1.54 ± 0.52	1.56 ± 0.22
H	1.41 ± 0.17	1.33 ± 0.05	1.42 ± 0.27	1.34 ± 0.20
M	1.45 ± 0.35	1.23 ± 0.27	1.32 ± 0.26	1.53 ± 0.41
L	1.55 ± 0.73	1.20 ± 0.14	1.42 ± 0.27	1.31 ± 0.40
UV	1.32 ± 0.33	1.13 ± 0.11	1.22 ± 0.30	1.27 ± 0.19
LE	1.20 ± 0.39	1.46 ± 0.43	1.21 ± 0.17	1.32 ± 0.38
LED	4	8	24	72
LP	1.59 ± 0.19	1.72 ± 0.53	1.33 ± 0.41	1.48 ± 0.34
H	1.33 ± 0.36	1.54 ± 0.19	1.34 ± 0.32	1.56 ± 0.23
M	1.37 ± 0.28	1.32 ± 0.15	1.34 ± 0.22	1.38 ± 0.29
L	1.34 ± 0.33	1.36 ± 0.47	1.35 ± 0.28	1.34 ± 0.19
UV	1.43 ± 0.53	1.38 ± 0.33	1.23 ± 0.25	1.40 ± 0.32
LE	1.32 ± 0.34	1.25 ± 0.31	1.24 ± 0.27	1.48 ± 0.30

APPENDIX I.
Complete results from Chapter V

Table 16. Light intensity (average, standard deviation, median) of each treatment for 4 h of light exposure under fluorescent or LED 3500K lights.

	Average ± Std. Dev	**Median**
Light-Protected PET	1461 ± 48	1465
Medium White PET	1451 ± 37	1446
High White PET	1460 ± 41	1452
Light-Exposed PET	1461 ± 52	1448
Fluorescent clear HDPE	1470 ± 40	1464
Fluorescent clear PET	1495 ± 57	1498

Table 17. Results from consumer sensory evaluation for liking questions (hedonic scale, 1=dislike extremely) and just-about-right (JAR) questions (scale 1-5; 3= JAR). Different letters represent significant differences ($p<0.05$).

	LP L	**M L**	**H L**	**LE L**	**PET F**	**HDPE F**
Overall liking	6.6^{a}	6.7^{a}	6.4^{ab}	6.6^{a}	5.7^{c}	6.1^{bc}
Appearance liking	7.2^{a}	7.1^{a}	7.1^{a}	7.2^{a}	7.0^{a}	7.1^{a}
Aroma liking	6.3^{ab}	6.3^{ab}	6.2^{ab}	6.4^{a}	6.0^{b}	6.0^{b}
Flavor liking	6.7^{a}	6.8^{a}	6.5^{a}	6.8^{a}	5.6^{c}	6.1^{b}
Mouthfeel/thickness liking	6.6^{ab}	6.8^{a}	6.6^{ab}	6.8^{a}	6.3^{c}	6.4^{bc}
Aftertaste liking	6.1^{a}	6.2^{a}	6.0^{a}	6.0^{a}	5.1^{c}	5.6^{b}
Aftertaste intensity	2.4^{b}	2.4^{b}	2.5^{b}	2.4^{b}	2.9^{a}	2.6^{b}
Freshness perception	6.5^{ab}	6.8^{a}	6.5^{ab}	6.7^{a}	5.7^{c}	6.1^{bc}
Not enough	8.3%^{b}	8.3%^{b}	12.7%^{ab}	17.8%^{a}	14.6%^{ab}	17.8%^{a}
Flavor JAR JAR	75.2%^{a}	47.1%^{c}	74.5%^{a}	52.2%^{bc}	68.8%^{ab}	62.4%^{abc}
Too much	16.6%^{bc}	44.6%^{a}	12.7%^{c}	29.9%^{ab}	16.6%^{a}	19.7%^{bc}
Not enough	11.5%^{a}	12.1%^{a}	12.1%^{a}	16.6%^{a}	12.7%^{a}	13.4%^{a}
Mouthfeel JAR JAR	75.2%^{a}	72.0%^{a}	79.6%^{a}	69.4%^{a}	76.4%^{a}	73.9%^{a}
Too much	13.4%^{ab}	15.9%^{a}	8.3%^{b}	14.0%^{ab}	10.8%^{ab}	12.7%^{ab}

LP L= light protected PET control under LED light, M L= medium white PET under LED light, H L= high white PET under LED light, LE L= light exposed (clear) PET under LED light, PET F= clear PET under fluorescent light, HDPE F= clear HDPE under fluorescent light

Table 18. Mean drops of overall acceptability scores for JAR questions for milk stored under fluorescent and LED light (n=157 consumers).

	Flavor JAR		Mouthfeel JAR	
	Not enough	Too much	Not enough	Too much
HDPE-F	1.2	2.3	0.2	1.2
PET-F	1.3	2.5	0.0	0.2
LE-L	1.2	2.5	0.7	1.3
LP-L	1.5	2.6	0.6	1.6
H-L	1.4	2.2	0.9	1.7
M-L	1.1	2.5	0.4	1.4

LP-L= light protected PET control under LED light, M-L= medium white PET under LED light, H-L= high white PET under LED light, LE-L= light exposed (clear) PET under LED light, PET-F= clear PET under fluorescent light, HDPE-F= clear HDPE under fluorescent light

Table 19. Analytical measurements of oxidation for each treatment package (average ± standard deviation).

	Dissolved Oxygen (ppm)	**TBARS (mg/L)**	**Riboflavin (mg/L)**
Fresh Milk	11.27 ± 0.06	0.07 ± 0.00	1.44 ± 0.01
Light-Protected PET	10.78 ± 0.42	0.16 ± 0.06	1.46 ± 0.01
Medium White PET	10.78 ± 0.18	0.14 ± 0.06	1.45 ± 0.03
High White PET	10.76 ± 0.16	0.17 ± 0.09	1.44 ± 0.04
Light-Exposed PET	10.54 ± 0.26	0.20 ± 0.10	1.44 ± 0.02
Fluorescent clear HDPE	10.24 ± 0.26	0.20 ± 0.06	1.45 ± 0.03
Fluorescent clear PET	9.42 ± 0.45	0.22 ± 0.14	1.44 ± 0.02

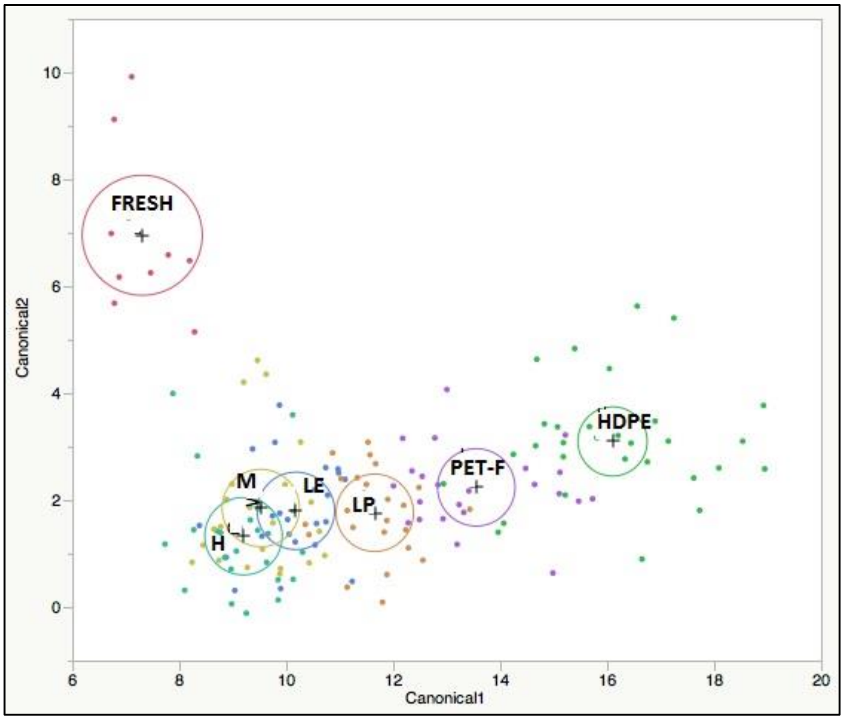

Figure 30. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles to fresh milk after 4 h of fluorescent and LED light exposure. HDPE-F= HDPE under fluorescent light, LE-F= light-exposed PET under fluorescent light, LP= light-protected PET under LED light, LE-L= light-exposed PET under LED light, M= medium white PET under LED light, H= high white PET under LED light.

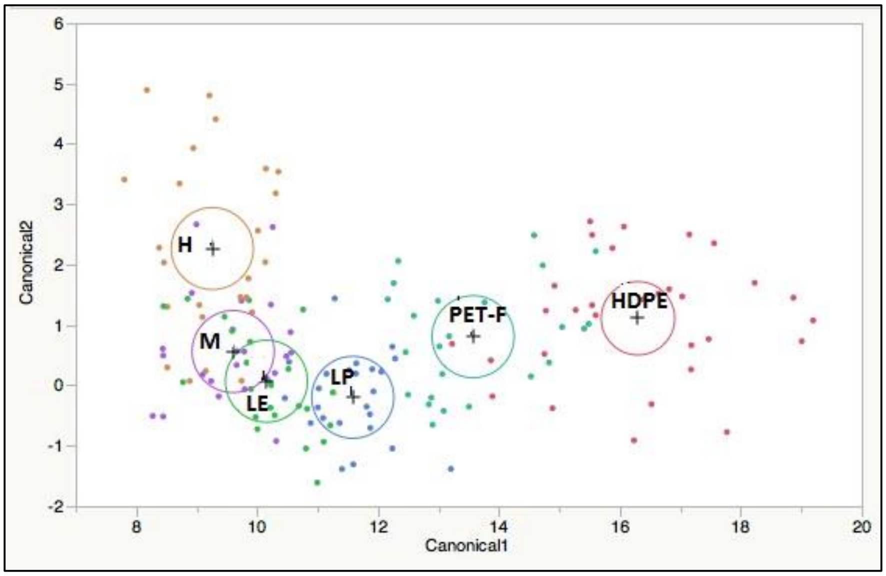

Figure 31. Canonical discrimination analysis (CDA) comparing 2% milk volatile profiles of PET and HDPE packages after 4 h of fluorescent and LED light exposure. HDPE-F= HDPE under fluorescent light, PET-F= light-exposed PET under fluorescent light, LP= light-protected PET under LED light, LE= light-exposed PET under LED light, M= medium white PET under LED light, H= high white PET under LED light.

APPENDIX J. Sensory Evaluation Informed Consent and Scorecard for Consumer Study

North Carolina State University
Informed Consent for Participants in Research Projects Involving Human Subjects (Sensory Evaluation)

Milk Consumer Taste Test Ballot
Sensory Service Center

SUBJECT CONSENT TO SENSORY EVALUATION

February 24, 2016

I AGREE TO PARTICIPATE IN SENSORY EVALUATION OF **MILK** FOR THE DEPARTMENT OF FOOD SCIENCE AT NORTH CAROLINA STATE UNIVERSITY. I UNDERSTAND THAT PARTICIPATION IS VOLUNTARY AND THAT I MAY WITHDRAW MY PARTICIPATION AT ANY TIME. I ALSO UNDERSTAND THAT INFORMATION I PROVIDE IS CONFIDENTIAL AND THAT RESULTS WILL NOT BE ASSOCIATED WITH MY NAME.

By printing your name below, you are providing consent to participate in today's evaluation and you are confirming that you do not have any allergies or intolerances that would prohibit you from participating in this test.

Signature:____

Directions:

Thank you for participating today! Today you will evaluate six different milks. You will evaluate each milk one at a time and answer questions about your experience with the milk. When you are presented with a sample of milk, you will first answer a question about your overall liking of the sample before proceeding onto the appearance, aroma, and flavor questions.

You may withdraw your participation in this study at any time. Failure to complete the study will result in forfeiture of your compensation. If you are ready to begin please let your server know.

Please ***take a sip*** of sample _____ and answer the following question:

Considering everything, how much do you like this sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

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We want you to consider the aroma and the appearance. Please answer the following questions about this sample.
Considering everything about this product, what is your overall impression of the APPEARANCE of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

How much do you like the AROMA of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Please ***take several sips*** of sample _____ and answer the following questions:

How much do you like the FLAVOR of this sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

How do you feel about the FLAVOR of this sample?

Not nearly enough flavor	Not enough flavor	Just About Right	Too much flavor	Much too much flavor

How much do you like the MOUTHFEEL/THICKNESS/VISCOSITY of this sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

How do you feel about the MOUTHFEEL/THICKNESS/VISCOSITY of this sample?

Not nearly thick enough	Not thick enough	Just About Right	Too Thick	Much too thick

How much do you like the AFTERTASTE of this sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Which statement best describes your impression of the AFTERTASTE INTENSITY of this sample?

Much Too Mild	Too Mild	Just About Right	Too Strong	Much Too Strong

What is your perceived FRESHNESS of this sample?

Not At All Fresh				Moderately Fresh				Extremely Fresh
☐	☐	☐	☐	☐	☐	☐	☐	☐