Controlling Light-Induced Flavors in 2% Milk

Kemia Nicole Amin

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Science
in
Food Science and Technology

Susan E. Duncan, Chair
Joe E. Marcy
Sean F. O'Keefe

March 24, 2016
Blacksburg, VA

Keywords: Milk, Oxidation, Packaging, Sensory Evaluation, Riboflavin

CONTROLLING LIGHT-INDUCED FLAVORS IN 2% MILK

Kemia Nicole Amin

SCIENTIFIC ABSTRACT

Energy regulations have shifted commercial retail cases from fluorescent to light emitting-diode lights (LED), however the effect of LED light on milk quality (flavor and nutritional content) has not been thoroughly studied. Packaging efficacy of light protecting additives (LPA) in high-density polyethylene (HDPE) was studied for protection against light-induced oxidation of high-temperature short-time (HTST) 2% milk under fluorescent (1882±993 lux) and LED light (915±150 lux). Milk quality measures included oxidation level, riboflavin (Rb) retention, headspace volatiles, and sensory evaluation were analyzed to determine the interaction between light source, packaging material, and storage time. HDPE packaging included translucent package (0% TiO_2) serving as control (light-exposed, light-protected: foil and plastic overwrap) and three LPA packages (low (1.3% TiO_2), high (4.9% TiO_2), yellow). Rb concentration decreased among all packages (40%-60%) after 72h for both lights. Volatile aldehydes (TBARS), increased in all packages (23%-82%) during storage over 72h at 4°C. Sensory evaluation (triangle test) revealed detectable flavor changes at a TBARS value of 0.11 mg/L; LPA packages saw this change starting at 4h and continued through 72h. The high package protected milk flavor effectively at 4h under fluorescent light; yellow package was effective for 4h under LED light. Despite detectable sensory differences, acceptability scores (9-point hedonic scale) were significantly greater for milk exposed to LED light in light-protected and high packages ($p<0.05$). We conclude that LED light may be less harmful to milk flavor vitamin content, but packaging needs to be improved to maintain milk's ideal flavor past 4h of light exposure.

Keywords: milk, oxidation, sensory, packaging

CONTROLLING LIGHT-INDUCED FLAVORS IN 2% MILK

Kemia Nicole Amin

GENERAL AUDIENCE ABSTRACT

Milk is usually packaged in translucent, unpigmented, high-density polyethylene (HDPE) plastic bottles. This type of bottle allows light to change the flavor and nutritional value of the milk. As retail case lighting changes from fluorescent to light-emitting diodes (LED) due to energy conserving initiatives, it is important to have packaging that can protect milk under both lighting conditions. Current packages on the market do not protect milk enough to give consumers the highest quality product. This study compared different types of packages with different light blocking pigments; transparent, no pigments, low (1.3% TiO_2,), high (4.9% TiO_2), and yellow in fluorescent and LED retail cases to see effects on 2% milk at (4°C) over 72h. Sensory evaluation, riboflavin (Rb) retention and oxidative measurements were used to determine success of packages. At 4h of light exposure, the high 4.9% TiO_2 package protected flavor best under fluorescent and the yellow package worked best under LED. After 4h, none of the packages effectively protected the milk from light. Rb concentration decreased in milk by 40%-60% after 72h for both lights in all packages. TBARS increased in all packages (23%-82%) during storage over 72h at 4°C. Despite detectable sensory differences, acceptability scores (9-point hedonic scale) were significantly greater for milk exposed to LED light in light-protected and high packages ($P<0.05$). We concluded that LED light may be less harmful to milk flavor and vitamin content, but packaging improvements are needed to maintain milk's ideal flavor.

Keywords: milk, oxidation, sensory, packaging

ACKNOWLEDGEMENTS

This project was funded, in part, by Dairy Research Institute (Rosemont, IL), Virginia Agriculture Experiment Station (Blacksburg, VA), Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture (Washington, DC), and by Hillphoenix (Chesterfield, VA). Thank you for the amazing opportunity to conduct this interesting research.

I would like to thank my graduate committee, Dr. Susan E. Duncan, Dr. Sean O'Keefe, and Dr. Joe Marcy for their advice, support, and expertise. Especially Dr. Duncan for her guidance, endless support, and trusting me to lead our team throughout this project. It has been a pleasure working with you and having you as a mentor. I would like to thank Melissa Wright, Dr. Wang, and Tina Plotka, Kim Waterman for their support both in and out of the lab.

I would like to thank the students of my lab group, Courtney Crist, Hayley Potts, Kayla Moberg, Alex Walsh, and Aili Wang for their invaluable support of this project. It truly does take a team to succeed in graduate school and I am glad to be a part of this team. I would like to thank the undergraduate students who also supported this project and committed many hours to helping me. Without these individuals, completion of this project would have not been possible.

Thank you Jordan Newkirk and Caroline Ryan for sticking it out through the ups and the downs. I am thankful for our friendship. Thank you Anna for being a great roommate and the first person I met at VT. Thank you Kristal and Robbie Poff for your countless prayers, random hang outs, and introducing me to my home away from home; NLCF. I love the Poff family dearly and cherish that I get to continue doing life with you all.

I would like to thank parents, Kazem and Peggy Amin, and my sister Kara Amin for their endless support. Thank you for believing in me when I did not believe in myself and lifting my spirits. Thank you for your sacrifices and everything you have done and continue to do in my life. I love you all so much! I would aso like to thank the Anders for their endless love and support; it's a gift and a huge blessing in my life. Especially John Anders; I appreciate your patience, support, spirit, and love for me more than you know. I can't wait to go through life as a team KJ! Thank you for always believing in me. I love you.

DEDICATION

This thesis is dedicated with lots of love to my father, Mohammad Kazem Amin. I admire your work ethic, drive, and care for our family more than I say. Thank you for allowing Kara and me to have opportunities to pursue our passions and dreams in life. As wonderful as it is having MS after my name, being your daughter is my favorite title.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The US dairy industry produces almost 190 billion pounds of milk annually, with an estimated $140 billion in economic output (Dairy Farming Today, 2014). Although the dairy industry does provide many households with a variety of products, there has been a decline in milk consumption and sales over the past 36 years. In 2012 19.8 gallons of fluid milk product per capita were consumed, but in 2013 fluid milk dropped to 19.2 gallons of fluid milk consumed, which is a new record low (International Dairy Foods Association, 2015). Whole fat milk consumption dropped to the lowest level in 2013 since the 1980's at 44.1 pounds per capita (International Dairy Foods Association, 2015). Reduced fat milk consumption went up 5.8 percent from 2012 after years of decline; however low fat and nonfat milk declined thereafter (International Dairy Foods Association, 2015). This decline may be from consumers' decrease in milk acceptability or competitive alternative dairy options such as coconut, almond, rice, and soy milk. Competition from alternative milk beverages may be eroding milk sales, as consumers substitute these products for fluid milk. Soy was found as a primary or secondary ingredient in about 80% of alternative milk beverages, with rice at 17%, and almonds at 10% (Food Business News, 2013).

Despite a slight decline in dairy sales over the past years, milk still holds an important role as a functional food; there are many components of milk that go beyond nutritive value to benefit health. Drinking milk has been shown to help maintain good bone health, especially when consumed during childhood and adolescence to reach peak bone mass, and among adults to prevent decrease in bone mass. Milk contains bioavailable calcium (about 30-35%) that help build strong bones and reduce chances of developing bone diseases such as osteoporosis (Dairy Farmers of Canada, 2010). Additionally, milk contains riboflavin (vitamin B_2) which has been linked to reducing the risk of osteoporotic fractures in the elderly and increasing bone mineral

density (Yazdanpanah et al., 2007). Milk and milk products also contains other nutrients such as vitamin D, vitamin A, phosphorus, magnesium, and potassium that makes it a nutritionally dense product (Dairy Council of California, 2015).

Even though other foods do contain calcium, such as spinach (115 mg calcium per serving), they contain inhibitory substances that decrease the bioavailable calcium with an estimate of about 5% of calcium truly absorbed (Dairy Farmers of Canada, 2010). For healthy individuals 4 years and older, the daily value of calcium consumption is 1,000 mg for both male and females (U.S Foods and Drug Administration, 2015). Milk contributes nearly 30% of the daily intake of calcium with just one cup (244g) of fluid milk (NDL/FNIC Food Composition Database, 2015). Studies have shown that consuming 3-4 servings of low-fat dairy a day can lower the chance of heart and blood vessel disease, diabetes, osteoporosis and other non-communal diseases (McCarron and Heaney, 2004; Schaafsma, 2009; Weaver, 2010;). Milk is not only nutritionally dense, but is the most economical and readily absorbed way to consume calcium and other essential nutrients in comparison to calcium supplements (Weaver et al., 1999; Heaney et al., 2002; Keller et al., 2002; National Dairy Council, 2005).

With dairy products being important both in revenue and public health as functional foods, it is important for products such as fluid milk to maintain integrity from on-farm milk production through processing to retail storage, consumer purchase and consumption. To maintain nutrient and sensory quality, it is important to protect fluid milk from exposure to light and oxygen through packaging (Mestdagh et al., 2005; Duncan and Webster, 2010; Johnson et al., 2015). It has been well documented that milk and dairy products are affected by light exposure (Chapman et al., 2002; Duncan and Webster, 2010; Duncan and Chang, 2012; Walsh et al., 2015; Johnson et al., 2015); current packaging needs to be improved to prevent this deterioration. Exposure to light affects the nutritional value, flavor, and odor of the milk products (Duncan and Webster, 2010; Duncan and Chang, 2012; Johnson et al., 2015). Wavelengths

below 500 nm can initiate oxidation reactions in milk and degradation in riboflavin, vitamin A, and vitamin C. (Fanelli et al., 1985; van Aardt et al., 2001). Flavors attributed to light exposure have been identified as burnt flavor, cabbage like, metallic, and cardboard flavors all of which are not true to the quality of fresh milk (Chapman et al, 2002; Moyssiadi et al., 2004). Brothersen at al. (2016), using conducted a descriptive analysis, identified milk cardboard flavor in light-exposed milk at 12 and 24h. They also concluded that cardboard flavor would most likely drive consumer liking. Oxidized flavors in light exposed milk are detectable as early as 15 to 30 minutes under fluorescent light by trained panelists and between 54 to 120 minutes by untrained panelists (Chapman et al., 2002).

There are only a few studies that have reported the effect of light-oxidized flavor in milk on consumer acceptability (Heer et al., 1995; Chapman et al., 2002; Walsh et al. 2015; Brotherson et al., 2016). Walsh et al. (2015) reported that consumer acceptability of 2% milk exposed to fluorescent light decreased over 8 hours to 168 hours from 'like slightly' to 'dislike slightly/moderately' due to light-oxidized flavors. Negative emotional terms (disgusted and worried) were used more frequently when characterizing their response to the light-exposed milk than with the light-protected milk (Walsh et al., 2015). Positive emotion terms such as content, calm, good, and happy were used when describing the light-protected milk (Walsh et al., 2015). Light exposed milk was liked significantly less than light-protected milk (Brotherson et al., 2016). The effect of LED and fluorescent light had on consumer liking was significantly different at 12h with fluorescent having lower scores than LED (Brothersen et al., 2016).These studies prove the detection of light-induced off flavors are noticeable to consumers and could adversely affect their opinion of fluid milk.

It is also documented that light-exposed milk decreases in nutritional value. One of the predominant vitamins in milk, riboflavin (vitamin B_2), has been well documented as a photosensitizer that initiates oxidation while decreasing in presence of light (Min and Boff, 2002;

Duncan and Webster, 2010). Riboflavin is important for a number of body functions including antioxidant abilities, converting carbohydrates into glucose, and helping the body convert vitamin B_6 to folate (University of Maryland Medical Center, 2015). Riboflavin is often studied as an indicator of milk quality and seen to decrease concentration in the presence of light (Duncan and Webster, 2010; Johnson et al., 2015; Brothersen et al. 2016). This problem is why optimal packaging is critical.

Historically, milk was sold in reusable glass containers but paperboard and plastic containers such as high density polyethylene (HDPE) and polyethylene terephthalate (PET) are now more commonly used. Unpigmented natural HDPE packages are not the most effective package for preserving milk quality since they transmit 58-79% of light between 350-800 nm spectral region (Robertson, 2006; Nelson and Cathcart, 1984). Johnson et al. (2015) showed an increase in oxidation, based on thiobarbituric acid reactive substances (TBARS) values, in milk packaged in unpigmented natural HDPE bottles compared to HDPE packages pigmented with titanium dioxide and completely protected from light. Likewise, Walsh et al. (2015) saw lower TBARS values in milk completely protected to light compared to unpigmented natural HDPE packages. Even though unpigmented HDPE packages are currently used in dairy industry, they are not protecting the milk quality as needed. Webster et al. (2009) reported that less than 4% light transmission caused noticeable difference in flavor when compared to light-protected milk. Similar to HDPE, unpigmented natural PET allows 75-85% of visible light transmission (Robertson, 2006). Colorants can be added to material and printing to reduce light transmission. Light protective additives like titanium dioxide (TiO_2) and carbon black can scatter and absorb light energy, which can reduce the amount of transmitted light with just packaging alone (DuPont, 2007). HDPE and PET bottles with TiO_2 inner and outer wall and a carbon black core layer provided significant protection in milk against vitamin A and riboflavin degradation in milk compared with unpigmented bottles (Moyssiadi et al., 2004). Increasing concentration of

TiO_2 (0.6%, 1.3%, and 4.3%) pigments added to HDPE bottle resin were found to have significant protection against oxidation and riboflavin deterioration in UHT-processed milk over 29 days (Johnson et al., 2015). Selecting the most appropriate type of packaging will contribute to protecting milk flavor and nutritional value during light exposure from the point of packaging through distribution, retail sales, and consumption.

The evidence that light is detrimental to milk flavor and nutrient quality is well documented, and the importance of packaging selection has been demonstrated under conditions of fluorescent light. However, recent mandates by US EPA for energy conservation is causing a rapid transition away from fluorescent lighting to light-emitting diodes (LED) in refrigerated retail dairy cases in dairy processing coolers and retail food stores. The implications of these changes are not well understood although the general assumption is that LED lighting will be less harmful than fluorescent light to fluid milk quality.

Purpose of Study

The overall purpose of this study was to evaluate how milk packaging, with and without light protective additives, interferes with light as transmitted by fluorescent and LED lighting, over 4 to 72 hrs, and influences milk quality during storage in a retail dairy case. Effects were assessed based on riboflavin degradation and oxidative stability of the fluid milk, in combination with sensory difference testing and volatile characterization to understand the sensory quality (flavor/aroma) of fluid milk. In addition, we validated the importance of packaging protection by assessing consumer acceptability of milk exposed to LED and fluorescent lighting over 4 hr. This study offers guidance and technologies for selecting effective packaging for light protection of milk from point of processing through retail sale. This study benefits dairy producers, processors, and consumers by delivering better quality milk, both in sensorial and nutritional value. Improved quality may increase fluid milk sales, increased consumption and contribute to consumer overall health.

This project is designed as three main experiments:

1. Protecting 2% fluid milk in light protective additive packaging over 4, 8, 16, 24, 48, and 72 hours in simulated retail conditions using LED and fluorescent lighting.
2. Utilizing electronic nose technology to verify volatile differences in milk.
    a. Comparing light-exposed and light-protected milk over 8 and 24 hours in fluorescent and LED simulated retail conditions.
    b. Comparing light additive packaging over 8 and 24 hours in fluorescent and LED simulated retail conditions.
    c. Comparing light additive packaging under fluorescent and LED simulated retail conditions for 4 hours.
3. Identifying consumer acceptability of 2% fluid milk in light protective additive packaging over 4 hours in simulated retail conditions using LED and fluorescent lighting.

REFERENCES

Brothersen C., D. J. McMahon, J. Legako, and S. Martini. 2016. Comparison of milk oxidation by exposure to LED and fluorescent light. J. Dairy Sci. 99:1-8.

Chapman, K. W., L. J. Whited, and K. J. Boor. 2002. Sensory threshold of light-oxidized flavor defects in milk. J. Food Sci. 67.2770-2773.

Dairy Council of California. 2015. Nutrients in milk. Available from http://www.healthyeating.org/Milk-Dairy/Nutrients-in-Milk-Cheese-Yogurt/Nutrients-in-Milk. Accessed 12/13/15.

Dairy Farmers of Canada. 2010. Calcium and bioavailability. Available from http://www.dairynutrition.ca/nutrients-in-milk-products/calcium/calcium-and-bioavailability. Accessed 10/10/14.

Dairy Farming Today. 2014. The U.S. Dairy Industry: A Vital Contributor to Economic Development. Available from http://www.dairyfarmingtoday.org/LearnMore/FactsandFigures/Pages/StateStatistics.asp. Accessed 8/21/14.

Duncan, S. E., and H. H. Chang. 2012. Implications of light energy on food quality and packaging selection. Adv. Food Nutr. Res. 67:25-73.

Duncan, S.E., and J. B. Webster. 2010. Oxidation and protection of milk and dairy products. Chapter 4. Oxidation in foods and beverages and antioxidant applications. Woodhead Publishing, Ltd., Cambridge, UK. pp. 121-155.

DuPont. 2007. Polymers, light and the science of TiO_2. DuPont™ Titanium Technologies, Wilmington, DE. Available from www.titanium.dupont.com. Accessed 9/21/14

Fanelli, A. J., J. V Burlew, and M. K. Gabriel. 1985. Protection of milk package in high density polyethylene against photodegradation by fluorescent light. J. Food Prot. 55:112-117.

Fenaille, F., V. Parisod, J. C. Tabet, and P. A. Guy. 2005. Carbonylation of milk powder proteins as a consequence of processing conditions. Proteomics. 5:3097-3104.

Food Business News. 2013. More ingredients are being used for dairy alternatives. Available from http://www.foodbusinessnews.net/articles/news_home/Consumer_Trends/2013/01/More_ingredients_are_being_use.aspx?ID={9F6B5243-0F3C-48D4-A4A5-694479F96B8C}&cck=1. Accessed 8/4/15

Heaney, R. P., M. S. Dowel, K. Rafferty, and J. Bierman. 2002. Bioavailability of the calcium in fortified soy imitation milk, with some observations on method. Amer. J. of Clinical Nutrition. 71:1166-1169.

Heer, A. K., S. E., Duncan, and D., Brochetti. 1995. Sensory detection of and consumer response to off-flavors in milk. Dairy, Food and Environmental Sanitation. 15:488–493.

International Dairy Foods Association, 2015. Milk Sales and Consumption. Available from http://www.idfa.org/news-views/media-kits/milk/milk-sales-consumption. Accessed 11/23/14

Johnson, D. S., Duncan, S. E., Bianchi, L. M., Chang, H. H., Eigel, W. N., O'Keefe, S. F. 2015. Packaging modification for protecting flavor of extended-shelf-life milk from light. J. Dairy Sci. 98: 2205-2214.

Keller, J. L., A. J. Lanou, and N. D. Barnard. 2002. The consumer cost of calcium from food and supplements. J. Amer. Dietetic Assoc. 102:1669-1671.

McCarron, D. A., R. P. Heaney. 2004. Estimated healthcare savings associated with adequate dairy food intake. Amer. J. Hypertension. 17:88-97.

Mestdagh, F., B. De Meulenaer, J. De Clippeleer, F. Devlieghere, and A. Huyghebaert. 2005. Protective influence of several packaging materials on light oxidation of milk. J. Dairy Sci. 88:499-510.

Min, D. B., and J. M Boff. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Rev. Food Sci. Food Safety 1:58-72.

Moyssiadi, T., A. Badeka, E. Kondyli, T. Vakirtzi, I. Savvaidis, and M. G. Kontominas. 2004. Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensorial aspects. Inter. Dairy J. 14:429-436.

National Dairy Council. 2005. Dairy's Role in Healthy Lifestyles. Available from http://www.nationaldairycouncil.org/SiteCollectionDocuments/education_materials/dietary_guidance/DairysRoleInAHealthyLifestyle.pdf. Accessed 8/25/15

NDL/FNIC Food Composition Database. 2011. Milk, reduced fat, fluid, 2% milkfat with added vitamin A and vitamin D. United States Department of Agriculture, Agriculture Research Service.

Nelson, K. H. and W. M Cathcart. 1984. Transmission of light through pigmented polyethylene milk bottles. J. Food Prot. 47:346-348.

Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans. 2010. Available from: http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm. Accessed 9/19/14

Robertson, G. L. 2006. Food packaging: principles and practice. 2nd edition. Boca Ranton: CRC press. Pp. 390-393.

Schaafsma, G. 2009. Health benefits of milk beyond traditional nutrition. Australian Journal of Dairy Technology, 64:113-116.

University of Maryland Medical Center. 2015. Vitamin B_2 (Riboflavin). Available from: https://umm.edu/health/medical/altmed/supplement/vitamin-b2-riboflavin. Accessed 1/6/16

U.S Foods and Drug Administration. 2015. Guidance for Industry: A food labeling guide (14. Appendix F: calculate percent daily value for appropriate nutrients). Available from: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064928.htm. Accessed 9/27/15

Walsh, A. M., S. E. Duncan, H. L. Potts, D. L. Gallagher. 2015. Comparing quality and emotional responses as related to acceptability of light-induced oxidation flavor in milk. Food Research International. 76:293-300.

Weaver, C. M. 2010. Role of dairy beverages in the diet. Physiology Behavior 2010: 100:63-6.

Weaver, C. M., W. R. Proulx, and R. Heaney. 1999. Choices for achieving adequate dietary calcium with a vegetarian diet. American Journal of Clinical Nutrition. 70:543S-548.

Webster, J. B., S. E. Duncan, J. E. Marcy, S. F. O'Keefe. 2009. Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelengths by interference. J. Food Sci. 74:390-398.
van Aardt, M., Duncan, S. E., Marcy, J. E., Long, T. E., and Hackney, C. R. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. Journal of Dairy Science, 84:1341-1347.

Yazdanpanah, N., M. C. Zillikens, F. Rivadeneria, R. de Jong, J. Lindemans, A. Uitterlinden, H. A. P. Pols, and J. B. J. van Meurs. 2007. Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: The Rotterdam Study. Bone. 41:987-994.

CHAPTER II

REVIEW OF LITERATURE

2.1 Understanding Light and Effects on Food

Light affects food in a variety of ways, causing chemical changes in pigments, lipids, proteins, and nutrients. These changes influence food quality. In order to understand these effects, an overview of light is first provided.

Light is a form of electromagnetic (EM) radiation that is part of the broader electromagnetic spectrum that also includes radio rays, gamma rays, and x-rays. The electromagnetic spectrum is fluxes of electric and magnetic fields that transport energy from one location to another through space. The fluxes of electric and magnetic fields are transmitted as waves or particles at different wavelengths and frequencies and are divided along the spectrum by decreasing wavelength and increasing energy and frequency (Ryer, 1997). Visible light is involved in these fluctuations of energy through space, but is also visible to the human eye. Visible light, which is seen by the human eye at wavelengths between 380 to 770nm (Figure 1), is between the ranges of infrared (IR; >770 nm)) and ultraviolet (UV; <400 nm). Light from the sun or artificial UV lamps includes UV, between 100-400nm, as well as visible wavelengths.

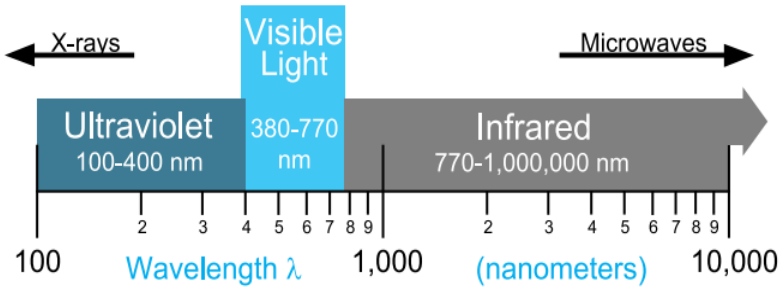

Figure 1. The optical portion of the electromagnetic spectrum (reprinted with permission from Ryer, 1997).

The sun emits light through the whole visible spectrum, but also emits UV radiation in three different bands: UV-A, UV-B, and UV-C. UV-A is in the range of 315-400nm and is not completely blocked by the ozone layer, but is the least harmful and most commonly found in UV lighting. It is often called "black light" and is relatively harmless and used to fluoresce materials (Ryer, 1997). UV-B is the most destructive form of UV light due to its ability to damage biological tissues, such as causing skin cancer. The ozone layer does block a majority of UV-B wavelengths, but a slight change in atmosphere can dramatically change the availability of this band. UV-C has shorter wavelengths that is almost completely absorbed in the air to create the ozone layer and is hardly detected in nature on its own. UV-C is also known as a germicidal light as it can change the DNA structure of bacteria, viruses, fungi, and protozoa (Shama, 1999).

UV light is well established in applications of water treatment, sanitation, and air disinfectants, but is not as widely used in foods (Koutchma et al., 2009). Majority of UV light treatment in the food industry is to decrease or inhibit microbial growth on food products such as chicken, produce, and beverages. Koutchma et al. (2009) has listed many different studies that indicate UV light as a good physical preservative. UV light may be beneficial in reduction of microbial growth, but may also deteriorate the quality of sensory quality and nutritional value of the food product. UV light altered the aroma of whole and skim milk by changing the primary structure of proteins thus causing higher levels of protein carbonyls that is characteristic of oxidized flavor milk (Scheidegger et al., 2010). Matak et al. (2007) showed that differences were detected when raw goat milk (not exposed to UV light) and UV light-exposed goat milk were compared. This study also reported that as UV dose increased the thiobarbituric acid reactive substances (TBARS) and acid degree values (ADV) increased, indicating an increase in oxidation and lipid hydrolysis (Matak et al., 2007). Guneser and Karagul (2012) reported that vitamin A, vitamin C, vitamin E, and vitamin B_2 (riboflavin) concentration in cow and goat milk decreased when exposed to UV light. As UV light intensity increased the vitamin concentration

decreased. At the lowest UV light exposure (12.6 J/ mL milk) cow's milk decreased in vitamin A, B_2, C, and E by 8–13%, 3–10%, 45–74%, and 16–33% respectively. At the highest exposure, vitamins in both cow (88.2 J/ mL milk) and goat milk (82.04 J/ mL milk) decreased significantly, with vitamin C having the most drastic decrease in concentration (Guneser and Karagul, 2012). UV light and visible light are relevant to food and beverages because the energy produced can break chemical bonds, changing the properties of products. There is more concern for fluorescent and light emitting diode (LED) lights impact on food than sunlight since products are exposed to fluorescent, LED, and other artificial light during processing, wholesale, retail stores, and consumers' homes.

Fluorescent lights were regularly used in retail cases and therefore were the most relevant light to study, but recently LED lights have become an alternative for fluorescent lights. LED lights, unlike fluorescent lights, do not use heating filaments, but instead uses electricity that passes through chemical compounds. As industries become more energy conscience, LED lights are becoming more common due to estimated 40% in energy savings and reduce carbon emission by 670 million tons (Corrie, 2013). A large grocery store chain replaced fluorescent lights for LED lights in 7,400 vertical retail cases and saved in refrigeration and light costs of $337,000 (Peters, 2012). This change to LED lights saved the company approximately $37,000 annually from maintenance alone (Peters, 2012). LED lights can last from 50,000 to 100,000 hours or about five years (Peters, 2012).

Not only are LED lights energy efficient, but they have a long performance life and are easy to maintain. LEDs can last for up to 100,000 hours and require little maintenance. Retail cases are progressively moving away from fluorescent lights and towards LED lights (Corrie, 2013). Fluorescent light output (the amount of light reaching a product and brightness of light) correlates with the temperature and decreases as temperature decreases (Hillphoenix Learning Center, 2014). Fluorescent light peak performance is around 26.7 to 32.2°C (80 to 90°F);

performing best in warm environments. At refrigerated temperatures (4°C) fluorescent light (tubular, 2.54 cm diameter T8) light output will decrease as much as 40% (Hillphoenix Learning Center, 2014). LED light performs better at lower temperatures such as in refrigerated or freezer retail cases. Light output increases by 6% in refrigerated environments which makes LED lighting a more efficient option for lighting the retail case (Hillphoenix Learning Center, 2014). With LED lights, controlling the intensity and color of the light is flexible; this allows better control for lighting specific products in retail cases to optimize appearance and color of products. Even though fluorescent and LED lights cover the whole visible light spectrum, they have different wavelengths and spectrum bands (Figure 2). Cool white fluorescent lights (color temperature of 3,500 K or higher) have higher relative intensity in wavelengths 550-560 nm than LED with a strong peak at 610-660 nm. Fluorescent lights have sharper peaks while LED lights have a smoother peak and broader wavelength band. LED lights have higher relative intensity in wavelengths between 610-660 nm with 460-500 nm being the lowest.

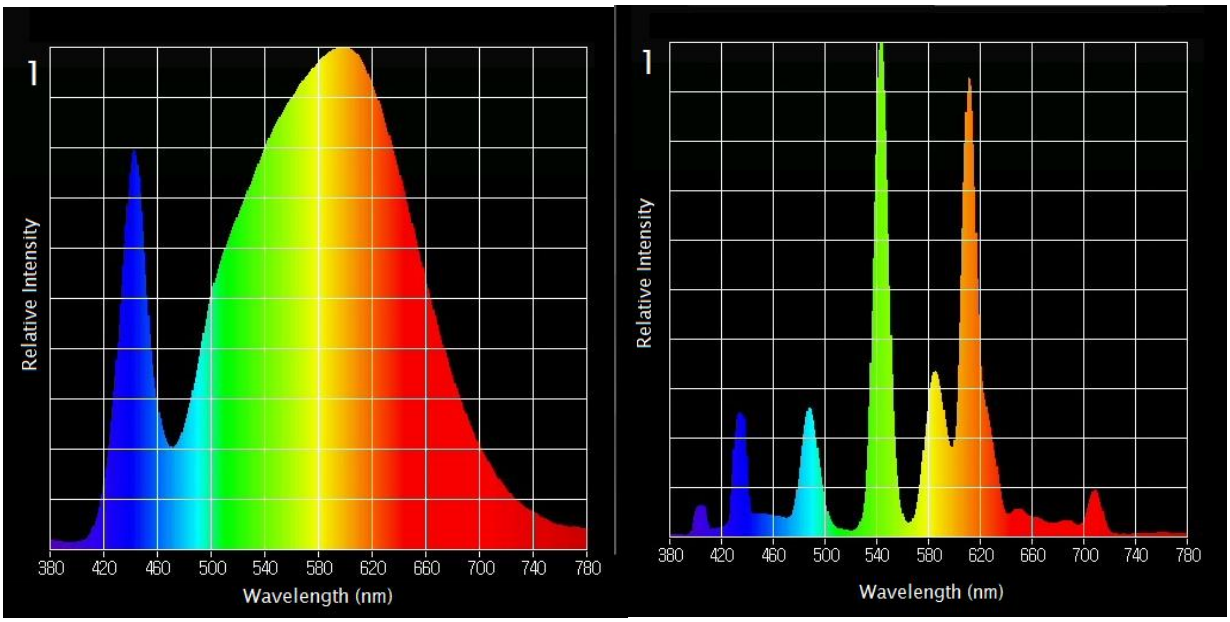

Figure 2. Relative intensity of wavelength of light emitting diode (LED; left) and fluorescent (right) lightings (MK350S UPRtek, Jhunan Township, Miao-li, Taiwan). *Imagine produced by Amin, K. 2016.*

As mentioned, milk is exposed to light many times throughout processing, distribution, and retail settings. In the past, dairy retail cases have used continuous lighting, allowing milk to be exposed to light for a few minutes to days, but now retail cases have lights that are activated by motion allowing lights to come on only when consumers are walking by or opening the retail case (Senyk and Shipe, 1981). This can reduce the amount of light the product is exposed even though overhead lighting outside the case can still reach the product. Light intensities in retail cases can vary greatly from 215 to 6,460 lux reported by Whited et al. (2002) and 750 to 6,460 lux reported by Chapman et al. (2002); whether it was fluorescent or LED retail cases was not specified. Retail cases have these differences due to the type of light, position of shelves, position of product on shelves, and type of retail cases. It is accepted to study light's impact on foods at 2,000 lux (approximate median for retail cases), but variation in light intensities found in the retail case need to be considered a variable in food deterioration.

Previous studies on light-induced oxidation in food products have been designed using fluorescent lights (Whited et al., 2002; Moyssiadi et al., 2004; Mestdagh et al., 2005; Webster et al., 2009; Johnson et al., 2015). Fluorescent lights have wavelengths across the visible light spectrum (380-770 nm) and UV spectrum (200-400 nm) in very low levels that have been known to cause flavor defects, discoloration, and nutritional deterioration in food products (U.S. Food and Drug Administration, 2014). Barbut (2004) studied the effects of incandescent light and two fluorescent lights on meat cuts' appearance acceptability, Bianchi et al. (2015) studied the oxidative, sensorial, and nutritional effect of fluorescent light on soymilk in various packages, and Walsh et al. (2015) studied the consumer emotional responses to milk exposed to fluorescent light in exposed and light blocked packages. These studies confirmed that fluorescent light has an influence on food and should be considered when designing packages and displaying food for consumer purchase. Whole, reduced fat, and nonfat milk exposed to 2,000 and 1,000 lux fluorescent light decreased vitamin A content which was directly influenced by length of

exposure and light intensity (Whited et al., 2002). As mentioned previously, Guneser and Karagul (2012) found differences in cow and goat milk vitamin concentration as UV light intensity increased. There is also a variability of wavelengths emitted by the types of fluorescent and LED lights in dairy retail cases including wavelengths in UV and visible light. Most commonly used in retail dairy cases and research studies on milk packaging is cool white fluorescent lighting. Cool white fluorescent light emits wavelengths in the whole visible light spectra, and are not ideal for retail cases of light sensitive foods (Bosset et al, 1995; Mestdagh et al., 2004). Just as fluorescent light, LED lights provide a broad range of wavelengths. Depending on the materials used to make LED white lights, intensity, efficacy, and cost can vary (Pimputkar et al, 2009). The effect LED lights on milk products has been studied by Brothersen et al. (2016), and seems to be the only discussion of LED lights impact on milk quality. It was reported that LED lights had less oxidation and higher Rb concentration than 1% milk under fluorescent light. This study is the first to look at fluorescent and LED lights impact on milk, but more research needs to be done to see how LED lights interact with packaging and how various intensity impact degradation. The complete effect of LED light on milk has yet to be determined, but LED light has seen to have potential positive effects on fresh meat cuts. Steele (2011) found that meat cuts stored under LED lights had higher acceptability for color and flavor than those exposed to fluorescent light. Additionally beef loin steaks and inside round steaks stored under LED lights showed longer shelf life than those stored under fluorescent light (Steele, 2011).

2.2 Oxidation of Milk

Lipid oxidation is major concern for food quality by giving off flavors and aromas in foods, loss of valuable nutrients and in some cases forming toxic compounds (Shahidi and Zhong, 2010). Lipid oxidation is susceptible to autoxidation, photooxidation, thermal or enzymatic oxidation that can be catalyzed by light, heat, enzymes, and metal (Shahidi and Zhong, 2010). Autoxidation and photooxidation are most common in fluid milk and both require

oxygen. Polyunsaturated fatty acids are major reactants in oxidations but riboflavin, chlorophyll, amino acids, polyphenols, and carotenoids can be susceptible to oxidation as well. Proteins can undergo oxidation and are of interest in low fat foods. Sulphur compounds have been studied as an important part of protein oxidation in fluid milk and milk products. Dimethyl disulphide has been identified as an oxidative product in milk and contributor to the light oxidized flavor in milk (Kim and Morr, 1996). Dalsgaard et al. (2010) reported that lipid oxidation products (lipid hydroperoxides) in cheeses were formed by the reaction of singlet oxygen with unsaturated lipids while dimethyl disulphide accumulated in vacuumed packed cheeses challenging the theory that dimethyl disulphide generates solely from singlet oxygen in photooxidation, but rather caused by a reaction with other relevant species. Milk's complex matrix creates opportunity for many pathways of oxidation and reactive compounds initiate and undergo oxidation.

2.2.1 Autoxidation

Autoxidation occurs in three stages:

1) Initiation: the formation of free radicals
2) Propagation: the free-radical chain reactions
3) Termination: the formation of non-radical products

Initiation is a complex process that results in a free radical that occurs when unsaturated lipids are exposed to initiators such as heat, light, metal ions. The reaction removes a hydrogen atom from a methylene group in the lipid molecule. The free radical reacts with oxygen to form peroxy radicals. During propagation, peroxy radicals react with more unsaturated lipids to form hydroperoxides, known to be the main primary products of autoxidation (Frankel, 1984; Shahidi and Zhong 2010). In the final stage, termination, radicals neutralize each other by interactions with themselves to form stable non-radical products. Hydroperxoides are extremely reactive and may initiate further reactions to form secondary products such as aldehydes and ketones which are most common in oxidized milk (Frankel, 1984; Shahidi and Zhong 2010).

2.2.2 Photooxidation

Photooxidation is another way unsaturated lipids can oxidize and occurs when photosensitive compounds and energy transfer to lipids or oxygen. This is a concern with milk since milk is exposed to light at various amounts and time throughout production, especially in retail cases. Unlike autoxidation, photooxidation involves the excited state (singlet) oxygen instead of the ground (triplet) state. Triplet oxygen is the most stable form of oxygen while singlet oxygen is 1,500 times more reactive than triplet oxygen increasing and inducing the oxidation processes (Shahidi and Zhong, 2010). Production and excitation of singlet oxygen requires photosensitive compounds such as riboflavin, and chlorophyll to absorb light energy (visible and ultraviolet) and oxidize fatty acids of the product (Shahidi and Zhong, 2010; Wold et al, 2015). When studying milk oxidation, Rb is heavily studied as a photosentizer that decreases nutritional value and changes in milk flavors when exposed to light (van Aardt et al., 2001; Wold et al., 2005; Webster et al., 2009; Duncan and Hannah, 2012; Johnson et al., 2015; Walsh et al. 2015; Wold et al., 2015). Milk contains riboflavin at an average concentration between 1.36 and 1.75 mg/L making it a predominate vitamin in milk (Dimick, 1982; Zygoura et al., 2004). It has been reported to absorb light 250, 270, 370, 400, 446, 570 nm wavelengths (Kyte, 1995). As riboflavin absorbs light it excites and transfer energy to the reactive oxygen form (Min and Boff, 2002). This induces the oxidation process causing loss of riboflavin in milk.

2.2.3 Photosensitizers and Photooxidation pathways- Type I and Type II

Photooxidation can occur in two pathways with the initiation of photosensitizers and presence of light (ultra violet and visible light). Photosensitizers such as riboflavin and chlorophyll absorb the light energy and are activated to a very unstable, excited singlet state (^{1}Sen*). The excited singlet photosensitizer returns to a stable state by excitation of lipids (Type I photooxidation) or by activation of oxygen (Type II photooxidation). Type I photooxidation

occurs when an excited molecule, such as riboflavin, and a substrate transfers an electron or hydrogen atom to another molecule, creating a free radical or free radical ions that react with oxygen (Davidson, 1979). Type II photo-oxidation occurs when low energy triplet oxygen is converted to high energy singlet oxygen by light and reacts with a substrate, such as unsaturated lipids, to form free radicals (Duncan and Hannah, 2012; Davidson, 1979). Type I and Type II photooxidation will heighten oxidation by forming reactive radical compounds or by forming singlet oxygen. The amount of oxygen present, the concentration of sensitizers, and substrate compounds will determine whether Type I or Type II oxidation occurs. Soybean oil containing chlorophyll will favor Type II pathways due to the unsaturated lipids reacting with the singlet oxygen (Min and Boff, 2002). Water based food products, such as fluid milk, favors Type I pathways due to the reduce amount of available oxygen present (Min and Boff, 2002). Type I and Type II pathways can switch from one to another depending on the oxygen and compound concentration.

2.3 Light Sensitive Compounds in Milk

Food can be altered by light and result in loss of nutrition and degradation of quality. Light sensitive compounds such as vitamins, lipids, and pigments, in food products can react with light energy to induce secondary reactions. Riboflavin is the most common photosensitizer in milk, but milk also includes protoporphyrin IX, tetrapyrroles, hemotoporphyrin, and chlorophyll (Wold et al. 2005).

2.3.1 Riboflavin as a Photosensitizer

When studying milk oxidation, riboflavin is heavily investigated and studied as a photosensitizer (van Aardt et al, 2001; Webster, 2006; Webster et al, 2009; Duncan and Hannah, 2012; Wold, 2015). Riboflavin, also known as vitamin B_2, is a water soluble vitamin found in milk and milk products that is degraded easily by light, but is heat stable. It is a flavonoid that gets its name from its sugar alcohol and from its yellow color and fluorescence under UV light. It

is also a precursor of coenzymes FMN and FAD which drive oxidation and reduction reactions. Riboflavin excites at 250, 270, 370, 400, 446, 570 nm with 430 to 460 nm causing type I photooxidation to occur has previously described (Bekbolet,1990; Kyte, 1995; Borle et al, 2001; Wold et al, 2015). This oxidation decreases the amount of riboflavin in the milk. As already stated, milk contains 1.36 and 1.75 mg/L of riboflavin (Dimick, 1982; Zygoura et al., 2004). Johnson et al. (2015) reported a 60 to 76% loss of riboflavin in 2% milk in three levels of TiO_2 loaded HDPE bottles under fluorescent light for up to 36 days. Walsh et al. (2015) had a 38% loss of riboflavin in milk exposed to fluorescent light in unpigmented HDPE bottles for 7 days. Within a shorter time of 3 days, Webster et al. (2009) reported a 52.6 to 67.5% loss of riboflavin in unpigmented and layered and colored film packaging. It was hypothesized that blocking the excitation peaks of riboflavin, would protect the quality of milk, but studies have shown other reactive compounds contribute to oxidation as well (Webster et al., 2009; Airado-Rodríguez et al., 2011).

2.3.2 Other Contributing Light Sensitive Compounds

Low fat and fat free milk are fortified with vitamin A and provides about 500 IU of vitamin A per cup (National Institute of Health, 2013). Even with this fortification, milk may not be in compliance with guidelines due to vitamin degradation from light exposure. Vitamin A has been reported to absorb light at 326 nm (Bosset et al., 1994), and has seen to be affected by fat composition, light intensity, and duration of light exposure (Whited et al., 2002). Milk exposed to fluorescent light (2,000 lux) for 2 hours had measureable vitamin A loss and increased oxidized flavor (Whited et al., 2002). As time and intensity increased, vitamin A decreased concluding these factors affect vitamin A and prove vitamin A is an impactful photosensitizer in milk. Controlling these oxidative inducers can reduce photooxidation in milk and therefore maintain the high levels of vitamins while keeping the flavor true to the fresh product.

Tertraphyrroles, fat soluble compounds, are found in milk at low levels. These include protoporphyrin IX (PpIX), hematoporphyrin, and a chlorophyll α-like compound with a complete list in Wold et al. (2005) (Airado-Rodríguez et al., 2011) Tetraphyrroles absorb between 400 to 750 nm. Chlorophyll was seen to absorb up to 700 nm in fluid milk (Airado-Rodríguez et al., 2011) and protoporhyrins absorbed at 635 to 705 nm in cheese (Wold et al., 2005). Riboflavin is not excited in these higher wavelengths such as orange light (590 to 630 nm). Airado-Rodríguez et al. (2011) reported milk exposed to orange light (590 to 630 nm) showed more sunlight and rancid flavor in milk than blue light (400 to 500 nm) associating the flavor to tertaphyrroles in milk.

These photosensitizers that use light energy to induce oxidative reactions, causing loss of nutritional value and change in sensorial qualities (Duncan and Hannah, 2012).With different photosensitizers reacting to varying wavelengths it is important to have proper packaging to block out a wide variety of wavelengths.

2.4 Packaging for Protection of Milk from Oxidation

Majority of food products are packaged in glass, plastics, or paperboard. Glass was historically used as the main beverage packaging, but now glass packaging is more common in high end beverages and specialty foods. (Duncan and Hannah, 2012). Plastics such as high density polyethylene (HDPE), low density polyethylene (LDPE), and polyethylene terephthalate (PET) are common plastics used for food packaging (Robertson, 2006). These plastics differ in chemical properties, molecular weight, density, and crystallinity which influence its gas permeability, light transmission, and visual appearance (Robertson, 2006).

Fluid milk is commonly packed in HPDE bottles in the United States, but some fluid products are packaged in PET and paperboard cartons. HDPE bottles are used because of its strength and ability to be molded under high temperatures. Natural HDPE is opaque in color and allows visibility of food product. The International Dairy Federation (IDF) recommends that

light transmittance of a package should not exceed 2% for 400 nm and 8% for 500 nm (Bosset et al., 1995). HDPE and PET transmit 62-85% of light between wavelengths of 300-700 nm which contains the spectrum that riboflavin most strongly absorbs (Webster et al. 2009). Also this range includes wavelengths that could induce excitation from other photosensitizers such as tetrapyrroles which contribute to light induces off flavors (Webster et al., 2009; Airado-Rodríguez et al., 2015).

Minimizing light transmission could be done by packaging milk in a container that blocks the excitation peaks of photosensitive compounds, but could potentially eliminate visibility of product. Consumers prefer to see their product within the package and therefore would not prefer milk packaging with dark colorants.

2.4.1 Light Protective Additives

Light protective additives (LPA) are pigments added to plastics that can protect food products from light and oxygen. Pigments added to HDPE and PET will preserve milk flavor and nutritive quality more than clear PET and HDPE. Titanium dioxide (TiO_2), a white colorant added to plastics, has shown to be an effective light barrier in protecting photooxidation. Titanium dioxide is available in two crystal structures: anatase and rutile. Rutile is favored over anatase because of its efficiency in scattering light (Dupont, 2007). Refractive index and particle size of TiO_2 determine the scattering and refracting qualities of the blend (Dupont, 2007). The greater the difference in refractive index between the light and the colorant, the more the light is bent away causing the light to scatter more. The recommended size for TiO_2 pigments is 0.2 to 0.3 microns in diameter to allow maximum light scattering at all wavelengths. When particle sizes increase or decrease, certain scattering properties of wavelengths decrease dramatically. Controlling the particle size can allow packagers to control which wavelengths they prefer to block.

Titanium dioxide and other colorants are used because they scatter wavelengths as well as absorb certain UV wavelengths (Duncan and Hannah, 2012). Carbon black is another common pigment that can be added to plastics to reduce light transmission, but consumers do not favor dark or black milk bottles (Stewart, 2015). Therefore to provide the light protection carbon black provides, it is layered with white colorants creating a multilayer package. Other pigments are used with plastics such as iron oxide, molybdate orange, ultramarine blue, and chrome green to name a few (Robertson, 2006). Yellow and green pigments in packages have shown to protect milk flavor by blocking the wavelengths riboflavin react to (400 to 500 nm) (Duncan and Hannah, 2012). Green PET bottles effectively blocked wavelengths below 500 nm and inhibited oxidation in milk better than unpigmented PET and HDPE bottles (Cladman et al., 1998). Amber PET bottles block wavelengths between 450 to 700 nm and had the least amount of oxidation compared to unpigmented HDPE, glass, and PET bottles (van Aardt et al., 2001). Intawiwat et al. (2010) studied the effect of milk packaged with different color PET films including yellow, green, amber, orange, unpigmented PET, and PET with UV-block. Red and green filters resulted in the least amount of rancid flavor compared to the unpigmented PET and PET with UV- block. HDPE with TiO_2 effectively protected milk flavor and riboflavin concentration better than unpigmented HDPE (Johnson et al., 2015). Color pigments, such as the ones mentioned above, can be added to plastics to reduce light transmission to milk thus protecting milk quality.

Different concentrations of LPAs and multiple layers of materials can be added to HDPE and PET to form a better barrier between light and milk. Johnson et al. (2015) studied the effect of light protection of milk in different concentrations of TiO_2 HDPE bottle. Increasing concentration of TiO_2 resulted in significant protection against oxidation and riboflavin deterioration. HDPE is naturally thicker than PET and may contribute to better light protection. A study from Moyssiadi et al (2004) reported that that lipid oxidation was less in pigmented HDPE bottles than in pigmented PET because HDPE has a greater thickness than PET.

Pigmented PET has been shown to have good oxygen and light barrier, but HDPE in monolayer and multilayer are at higher thickness and may contribute to better protection.

2.4.2 Packaging Impact on Consumers

Light-exposed milk directly affects consumers' acceptability of the product. Consumers associate light induced flavors with negative emotions and low acceptability. Research by Walsh et al. (2015) verified that at 8 hours of light exposure (2,000 lux) samples were less liked than complete light blocked samples. Milk at 8 hours of exposure was "liked moderately", and milk exposed at 168 hours was "disliked slightly". It is obvious in this study that as milk is exposed to light at longer periods of times, acceptability of the product decreases. This will affect consumer emotions and perceived acceptability of milk products. Oxidized milk can be described with negative emotions such as "disgusted" and acceptable milk can be associated with positive emotions like "good" and "pleased" (Walsh et al., 2015). It is important to note that teens influence household spending significantly across all food categories (Chapman et al., 2002). A product may gain the attention of teens, enough to purchase the product an initial time, but it requires acceptability of flavor to influence repeat buying. If teens do not like the flavor of a product, repeat buying will be less and acceptability later in life decreases. If teens consume milk when they are younger they are more likely to consume milk when they are older. Chapman (2002) found that 34.5% of teens could detect light oxidized flavor milk within ½ hour of exposure to fluorescent light (2,000 lux). At 3 hours of light exposure about 70% of teens noticed off flavors, nearly a 35% increase from ½ hour of exposure (Chapman, 2002). Teens' acceptability of milk decreases when oxidized flavors are noticeable. Milk can be exposed to light in dairy retail cases for minutes or days with many cases having continuous lighting (Senyk and Shipe, 1981). It is most likely that teens are drinking oxidized milk exposed to light longer than ½ and 3 hours which may correlate to decrease in milk sales and continual consumption in

adult life. The best way to decrease oxidation and therefore increase consumer liking and consumption is to limit the amount of light exposure to provide the closest taste to fresh milk.

2.5 Electronic Nose Technology Detection of Volatiles

Consumers are becoming more aware of their food's flavor, appearance, and nutritional value. As a result, there is pressure on the food industry to create highly acceptable products. Once a product is created, there is pressure to maintain the quality of that product year round. With the possibility of pathogen contamination and spoilage of food products, the food industry has implemented ways for quality discrimination in foods. Some quality control measures include swabbing equipment, tools, and food products and running chemical or microbial analysis. Other methods require sensory panelist to discriminate or describe products.

Before food enters a consumer's mouth, the consumer will analyze the product through the olfactory system and characterize the odor. Any unpleasant odor will prevent them from consuming the product and any favorable odor will encourage consumption. Volatiles are sensed by the olfactory epithelium either by sniffing a food product by the nose or by volatiles being released in the mouth. The olfactory epithelium contains sensors sensitive to different odors at different concentrations. Humans have high sensitivity to smell and can distinguish more than 10,000 different smells (Mallikarjunan, 2005). With this sensitivity, trained sensory panelists can add value to a quality program by using trained human subjects to assess products. Trained panelists need to be familiar with the testing procedures, recognize and identify sensory attributes, and continue to improve sensitivity and memory to be value (Meilgaard et al., 2007). As beneficial as trained panelists are, it can be very time consuming and require high personal motivation.

Different methods for analyzing flavors and volatiles have been developed to reflect sensory perceptions such as gas chromatography-mass spectroscopy (GC-MS), liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS), and gas

chromatography olfactory (GCO). These methods require time and costly equipment and may not provide successful correlations. The real challenge is discovering a way to correlate human experiences with smell to analytical methods.

Over the last 40 years, electronic nose technology has been used to measure gas patterns to create a "smell print" of a particular product. They are able to measure aroma compounds similar to sensory evaluation making them more representative of odor than gas chromatography (Mallikarjunan, 2005). It should be use as an objective alternative to sensory evaluation, but should not substitute sensory. This technology has been used in different areas such as medical and health care, agriculture, pharmaceuticals, and microbiological quality control of food products (Loutfi et al., 2015). It has been used in multiple food applications to detect volatiles in meat, wine, dairy, tobacco, and fish (Zou and Wu, 2002; Ampuero and Bosset, 2003; Garnder et al., 2011; Loutfi et al., 2015). A variety of sensors are used with electronic nose technology including metal oxide semiconductors, conducting polymers, and quartz crystal microbalance sensors (Loutfi et al., 2015). Metal oxide sensors are made of a ceramic base heated by wire and coated with a metal oxide semiconducting film. The metal oxide layers include zinc oxide, tin dioxide, titanium dioxide, and nickel oxide. Depending on what type of metal oxide is used, determines how the coating reacts. Sensors using negative electron coatings will respond to oxidizing compounds while those using positive coatings will respond to reducing compounds. Conducting polymer electronic noses measure based on a change in resistance of each chemical sensor (conducting polymer, counter ion, and solvent) when exposed to volatiles (Cyranose® 320, 2013). Altering the three different materials produce different sensitivities to sensors. Quartz microbalance sensors use crystals that vibrate and respond to aroma through changes in mass. When exposed to volatiles, the compounds absorb onto the gas chromatography coating of the sensors and causes the mass of the sensor to change.

Although there are different types of sensors all electronic noses are made of three main parts: 1) chemical sensors that measure volatiles, 2) electronic controls, and 3) a system that processes and recognizes information (Mallikarjunan, 2005). The electronic nose essentially gathers and analyzes complex aromas and reports data to describe aromas simply. The steps to use electronic noses are all similar: 1) generate odor; this is often time done by placing product in sealed vile to produce headspace at ambient or heated temperatures, 2) exposure sensor to aroma; such as exposure to headspace, 3) system measures changes in sensors, 4) forms patterns of aroma from sample, and 5) analyzes patterns (Mallikarjunan, 2005).

The Cyranose® 320 by Sensigent (Baldwin Park, CA), is first trained to recognize a series of volatiles by creating a digital "smell print" of each vapor by reacting with conducting polymers. Training the electronic nose produces unique smell prints that can later be compared to unknown aromas. This specific electronic nose uses conducting polymers to respond to volatiles. The Cyranose® 320has 32 sensors, but other electronic noses can range from 2 to 32 different sensors (Loutfi et al., 2015). When exposed to an unknown sample, the Cyranose® 320 compares the new smell print to the existing smell print stored in memory. The coded algorithm decides whether the new sample is a like or different (accepted or rejected) to the reference smell print. It is not designed to report specific volatile compounds, but the entire sample.

In order for an electronic nose to analyze properly, many factors must be consistent and controlled. To be creditable, the electronic nose must fulfill reproducibility, long term stability, identification capability and model robustness (Labreche, 2005). Electronic noses have a multiple of options for sample collection and analysis. The absorption draw time (sample collection) of volatiles must be standardized to ensure all volatile compounds have saturated the sensor while considering the nature of the product. Some products have strong concentrations of volatiles and may not need a long draw time compared to a product that has less concentrations. Over saturating the sensors with multiple back to back collections can lead to volatiles being

muted or unreadable by the electronic nose. Purge times and proper maintenance are important for the electronic nose to clear the sensors and maintain sensitivity. Electronic noses are statistically analyzed through multivariate analysis such as principal components analysis and multivariate discrimination (Labreche, 2005; Gardner et al. 2011; Yang et al., 2015). Canonical discrimination analysis is most commonly used with electronic nose technology. This analysis allows visualization of class in different categories. It computes unknown observation in a canonical to the mean of a known group to see if the unknown observation falls close to the mean of one group. If the unknown observation is close to a known observation it can be determined as the same material. If the unknown observation is far, it may be different or at different concentrations. This analysis is only useful if the measure of closeness can be quantified. Methods using Euclidean distance and Mahalaobois distance have been used to explain canonical plots with electronic noses (Mallikarjunan, 2005).

Fresh milk contains mixtures of acetone, hexanal, 2-butanone, toluene, limonene, heptanal, styrene, and chloroform (Ampuero and Bosset, 2003). These volatiles are found in fresh milk, but depending on heat processing, photooxidation, microbial presence the concentrations can increase or decrease changing the flavor of the product (Loutfi et al., 2015). Marsili (1999) reported heptanal and dismethyl disulfide concentrations increased in light oxidized milk under fluorescent light. These volatiles can be categorized by the electronic nose and provide valuable information on quality of milk. Labreche et al. (2005) studied the shelf life of milk with the electronic nose and microbiology. The electronic nose was predictive of bacterial growth over the storage period and can be used as one method to predict freshness. Yang et al. (2015) studied goat milk flavors and reported positive correlation with the electronic nose and sensory evaluation. Wang et al. (2010) studied the discriminative properties of the electronic nose with natural and synthetic milk flavors. The electronic nose was able to identify the differences in samples that were difficult for sensory panelists to distinguish.

Even though the electronic has proved to be a promising tool for food applications, there are still many inconsistencies. The sensors of the electronic noses can differ and often times proprietary information thus it difficult to compare different electronic noses directly to each other. Draw times, sample holding times, and headspace can differ among experimental designs also making comparison difficult. The electronic nose technology requires further research and standardization to make the technology applicable and comparable across industries. If the electronic nose is used properly, it has the potential to report data on food quality and could be an essential tool for fast, reliable results.

REFERENCES

Airado-Rodríguez, D., N. Intawiwat, J. Skaret, and J. P. Wold. 2011. Effect of naturally occurring tetrapyrroles on photooxidation in cow's milk. J. Agric. Food Chem. 59: 3905-3914.

Ampuero, S. and J. O. Bosset. 2003. The electronic nose applied to dairy products: a review. Sensors and Actuators. 94: 1-12.

Barbut, S. 2005. Effect of enhanced fluorescent light on acceptability of meat cuts. J. of Muscle Foods. 16: 77-86.

Bekbolet, M. 1990. Light effects on food. J. Food Prot. 53(5) 430-40, 571–590.

Bianchi, L. M., S. E. Duncan, J. B. Webster, D. S. Johnson, H. H. Chang, J. E. Marcy and S. F. O'Keefe. 2015. Protecting soymilk flavor and nutrients from photodegradation. Food Sci. & Nutrition, 3: 319–330.

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

Bosset, J.O., Sieber, R., Gallman, P.U. 1995. Light transmittance: Influence on the shelf-life of milk and milk products. In: International Dairy Federation, ed. Technical Guide for the Packaging of Milk and Milk Products. 3rd ed. Brussels: Int. Dairy Federation. Pg.19–39.

Brothersen C., D. J. McMahon, J. Legako, and S. Martini. 2016. Comparison of milk oxidation by exposure to LED and fluorescent light. J. Dairy Sci. 99:1-8.

Chapman, K. W., Whited, L. J., and Boor, K. J. 2002. Sensory threshold of light-oxidized flavor defects in milk. J. Food Sci. 67.2770-2773.

Cladman, W., S. Scheffer, N. Goodrich, and M. W. Griffiths. 1998. Shelf-life of milk packaged in plastic containers with and without treatment to reduce light transmission. Int. Dairy J. 8: 629-636.

Corrie, A. 2013. Shedding new light on LED lighting's benefits. Plant Engineering, Available from http://ezproxy.lib.vt.edu:8080/login?url=http://search.proquest.com/docview/1362089973?accountid=14826. Accessed on August 9, 2015.

Cyranose® 320. 2013. The Cyranose® 320 eNose® User Manual. Sensigent. Baldwin Park, CA.

Dalsgaard, T. K., J. Sørensen, M. Bakman, L. Vognsen, C. Nebel, R. Albrechtsen, and J. Nielsen. 2010. Light-induced protein and lipid oxidation in cheese: Dependence on fat content and packaging conditions. Dairy Sci. Technol. 90: 565-577.

Davidson, R. S. 1979. Mechanisms of photo-oxidation reactions. Pesticide Sci. 10: 158-170.

Dimick, P. S. 1982. Photochemical effects on flavor and nutrients of fluid milk. Can Institute Food Sci Technol J. 15: 247-256.

Duncan, S. E. and S. Hannah. 2012. Light-protective packaging material for foods and beverages. Ch. 15. In Yam, K. L. and D. S. Lee editor. Emerging food packaging technologies: principles and practice. Woodhead Publishing Ltd.

Frankel, E. N. 1984. Lipid oxidation: mechanisms, products and biological significance. J. Amer Oil Chem. Soc. 61: 1908-1917.

Gardner, D. M., B. W. Zoecklein, and K. Mallikarjunan. 2011. Electronic Nose Analysis of Cabernet Sauvignon (*Vitis vinifera* L.) Grape and Wine Volatile Differences during Cold Soak and Postfermentation. Am. J. Enol. Vitic. 62:1.

Guneser, O., and Y. Karagul. 2012. Effect of ultraviolet light on water- and fat-soluble vitamins in cow and goat milk. J. Dairy Sci. 95: 6230-6241.

Hillphenoix Learning Center. 2014. Enhance merchandising, engage customers: lighting. http://www.hillphoenixlc.com/dashboard/profile/courses/courses_video/3/

Intawiwat, N., M. K. Petterson, E. O. Rukke, M. A. Meier, A. V. Dahl, J. Skaret, D. Keller and J. P. Wold. 2010. Effect of different colored filters on photooxidation in pasteurized milk. J. Dairy Sci. 93:1372-1382.

Johnson, D. S., S. E. Duncan, L. M. Bianchi, H. H. Chang, W. N. Eigel, and S. F. O'Keefe. 2015. Packaging modification for protecting flavor of extended-shelf-life milk from light. J. Dairy Sci. 98: 2205-2214.

Kim, Y. D., and C. V. Morr. 1996. Dynamic headspace analysis of light activated flavor in milk, Intern Dairy J. 6: 185-193.

Koutchma, T., L. Forney, C. Moranu. 2009. Ultraviolet Light in Food Technology: Principles and Applications.

Kyte, J. 1995. Mechanisms in Protein Chemistry. Garland Publishing, Inc., New York, NY.

Labreche, S., S. Bazzo, S. Cade, and E. Chanie. 2005. Shelf life determination by electronic nose: application to milk. Sensors and Actuators B. 106:199-206.

Loutfi, A., S. Coradeschi, G. K. Mani, P. Shankar, and J. B. B. Rayappan. 2015. Electronic noses for food quality: a review. J. Food Engineering. 144: 103-111.

Mallikarjunan, P. 2005. Electronic nose applications in the food industry. In Nondestructive testing of food quality. Chapter 10 in Nondestructive Testing of Food Quality. J. Irudayaraj et al, eds. Wiley-Blackwell. Hoboken, NJ.

Marsili, R. T. 1999. Comparison of Solid-Phase Microextraction and Dynamic Headspace Methods of the Gas Chromatographic-Mass Spectrometric Analysis of Light-Induced Lipid Oxidation Products in Milk. J. Chromatographic Sci. 37:17.

Matak, K.E., S. S. Sumner, S. E. Duncan, E. Hovingh, R. W. Worobo, C. R. Hackney, and M. D. Pierson. 2007. Effects of Ultraviolet Irradiation on Chemical and Sensory Properties of Goat Milk. J. Dairy Sci. 90: 3178-3186.

Mestdagh, F., B. De Meulenaer, J. De Clippeleer, F. Devlieghere, and A. Huyghebaert. 2004. Protective Influence of Several Packaging Materials on Light Oxidation of Milk. J. Dairy Sci. 88: 499-510.

Meilgaard, M. C., G. V. Civille, and B. T. Carr. 2007. Sensory Evaluation Techniques. 4th ed. CRC Press, New York.

Min, D. B., and J. M. Boff. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Rev. Food Sci. Food Safety 1:58-72.

Moyssiadi, T., A. Badeka, E. Kondyli, T. Vakirtzi, I. Savvaidis, and M. G. Kontominas. 2004. Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensorial aspects. Int. Dairy J. 14: 429-436

National Institute of Health. 2013. Vitamin A: fact sheet for health professional. Available from https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#h3. Accessed January 8, 2016.

Peters, L. 2012. LEDs Magazine. LED lighting fits perfectly in refrigerated display cases. Available from http://www.ledsmagazine.com/articles/print/volume-9/issue-10/features/led-lighting-fits-perfectly-in-refrigerated-display-cases-magazine.html. Accessed December 16, 2015.

Pimputkar, S., J. S. Speck, S. P. Denbaars, and S. Nakamura. 2009. Prospects for LED lighting. Nature Photonics. 3: 180-182.

Robertson, G. L. 2006. Food packaging: principles and practice. 2nd edition. Boca Ranton: CRC press. 390-393.

Ryer, A. 1997. The light measurement handbook. International Light Technologies. Peabody, MA.

Scheidegger, D., R. P. Pecora, P. M. Radici, S. C. Kivatinitz. 2010. Protein oxidative changes in whole and skim milk after ultraviolet or fluorescent light exposure. J. Dairy Sci. 93: 5101-5109.

Senyk, G. F., and W.F. Shipe. 1981. Protecting your milk from nutrient losses. Dairy Field, March. 164: 81, 84-85.

Shahidi, F, and Y. Zhong. 2010. Lipid oxidation and improving the oxidative stability. Chem. Soc. Rev. 39: 4067-4079.

Shama, G. 1999. Ultraviolet light. In Robinson, R. K., Batt, C. and Patel P. (Eds). Encyclopedia of Food Microbiology, p. 2208. London: Academic Press.

Steele, K. S. 2011. Shelf life of five meat products displayed under light emitting diode or fluorescent lighting. Master's thesis. Kansas State University. Manhattan, KS.

Stewart, M. 2015. Consumer appetite sours for all black milk. Business Day. Available from http://www.stuff.co.nz/business/72368664/consumers-appetite-sours-for-all-black-milk. Accessed January 1, 2016.

U.S. Food and Drug Administration. 2014. Compact Fluorescent Lamps (CFLs) Fact Sheet. Available from http://www.fda.gov/Radiation-EmittingProducts/RadiationEmittingProductsandProcedures/HomeBusinessandEntertainment/ucm116400.htm#2. Accessed February 29, 2016.

van Aardt, M., S. E. Duncan, J. E. Marcy, T. E. Long, and C. R. Hackney, C. R. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. J. of Dairy Sci. 84: 1341-1347.

Wang, B., X. Shiying, and D. Sun. 2010. Application of the electronic nose to the identification of different milk flavorings. Food Research Int. 43:255–262.

Walsh, A. M., S. E. Duncan, H. L. Potts, and D. L. Gallagher. 2015. Comparing quality and emotional responses as related to acceptability of light-induced oxidation flavor in milk. Food Research Int. 76:293-300.

Webster, J. B. 2006. Changes in Aromatic Chemistry and Sensory Quality of Milk Due to Light Wavelength. Thesis (Ph.D.) Virginia Polytechnic Institute and State University. Blacksburg, VA. 6-21,29-39

Webster, J.B., S. E. Duncan, J. E. Marcy, and S. F. O'Keefe. 2009. Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelength by interference. J Food Sci. 74:390-398.

Whited, L. J., B. H. Hammond, K. W. Chapman, and K. J. Boor. 2002. Vitamin A degradation and light-oxidized flavor defects in milk. J. Dairy Sci. 85:351–354

Wold, J. P., J. Skaret, and T. K. Dalsgaard. 2015. Assessment of action spectrum of photooxidation in full fat bovine. Food Chem. 179:68-75.

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

Zou, X. B., and S. Y. Wu. 2002 Evaluating the quality of cigarettes by an electronic nose system. J.of Testing and Evaluation. 30:6.

Yang, C. J., W. Ding, L. J. Ma, and R. Jia. 2015. Discrimination and characterization of different intensities of goaty flavor in goat milk by means of an electronic nose. J. of Dairy Sci. 98:55–67.

Zygoura, P, T. Moyssiadi, A. Badeka, E. Kondyli, I. Savvaidis, and M. G. Kontominas. 2004. Shelf-life of whole pasteurized milk in Greece: effect of packaging material. Food Chem. 87:1-9.

CHAPTER III

PROTECTING 2% FLUID MILK IN LIGHT PROTECTIVE ADDITIVE PACKAGING IN FLUORESCENT AND LED LIGHTING RETAIL CONDITIONS

ABSTRACT

The effect of light-emitting diode (LED) and fluorescent lighting sources on milk flavor were studied under conditions of retail storage. Packaging efficacy, as altered by light protecting additives (LPA) in high-density polyethylene (HDPE) for protection against light-induced oxidation of high temperature short time (HTST) milk (2% fat), was evaluated under both lighting systems. Our objective was to determine how light source, packaging, and time influenced on riboflavin retention, volatiles, and sensory quality. Changes in milk were assessed by sensory evaluation (triangle tests, untrained panel), thiobarbituric reactive substances (TBARS), riboflavin (Rb) concentration, and electronic nose (ENose) technology. Packaging (HDPE) included two commercial packages (low 1.3% TiO_2 and Yellow) with opaque appearance and a high package (4.9% TiO_2). Translucent HDPE (0% TiO_2) was used for the light-exposed control; a light-protected control used the translucent HDPE package with foil and white plastic wrap. The high package protected milk sensory quality as well as the light-protected package up to 4 h under fluorescent light (1882 ± 993 lux), while both high and yellow packaging protected as well as light-protected package for 4 h under LED light (915 ± 150 lux); other packages caused change in flavor by 4 h. By 24 h of light exposure, all packages failed to sufficiently protect milk. Riboflavin concentration decreased among all packages (40% to 60%) by 72 h for all light and package combination. Volatile aldehydes, as measured by TBARS, increased in all treatments during storage.

Key Words: oxidation, sensory, packaging, milk, riboflavin

3.1 Introduction

It has been well documented that milk and dairy products are negatively affected by light exposure and effective packaging is needed to protect milk quality (Chapman et al., 2002; Duncan and Webster, 2010; Duncan and Chang, 2012; Walsh et al., 2015; Johnson et al., 2015). Exposure to sunlight and artificial light affects nutritional value, flavor, and odor of milk products (Duncan and Webster, 2010; Duncan and Chang, 2012; Johnson et al., 2015). Flavors attributed to light exposure have been identified as burnt protein, cabbage-like, metallic, and cardboard flavors, all of which are not true to the quality of fresh milk (Alvarez, 2009; Chapman et al., 2002; Moyssiadi et al., 2004). Untrained consumers can identify light oxidized flavors in 2% milk samples under fluorescent light as early as 1h (Chapman et al., 2002). Brothersen et al. (2016) reported detection of off flavors by consumers under fluorescent light (2,200 lux) at 12h and 24 h of exposure under LED light (4,000 lux) in a light box. Consumer acceptability of 2% milk exposed to fluorescent light (1,738 lux) in a light box decreased over 8h to 168h; on a 9-point hedonic scale (1=dislike extremely; 9=like extremely), acceptability scores decreased from 5.9 ("like slightly") to 3.5 ("dislike moderately to "dislike slightly") due to light oxidized flavors (Walsh et al., 2015). Negative emotional terms (*disgusted* and *worried*) were used more frequently to describe light-exposed milk than light-protected milk, while positive terms (*content*, *calm*, *good*, and *pleased*) were used when describing milk protected from light (Walsh et al., 2015). These studies demonstrate that detection of light-induced off-flavors is noticeable to consumers and could adversely affect their opinion on the product, which might contribute to decreased sales and consumption.

Immediately after processing and packaging, milk is transferred into the processing plant cooler; typically milk is moved within a few hours, but may be over 24 hours, into distribution. During this time milk can be exposed from hours to days to artificial (fluorescent and light emitting diode (LED)) light during cooler/warehouse holding, distribution, and in retail cases

(Senyk and Shipe, 1981). Traditionally, fluorescent lights have been used in dairy retail cases, but recently there has been an increased use in LED lights due to its energy savings, reduced carbon emissions, and easy maintenance (Corrie, 2013). Retail refrigerated cases can use almost 40% of total energy use, contributing to majority of a grocery store's utility costs (U.S. Department of Energy, 2014a). Refrigeration systems need to be on at all times to prevent foods from perishing which causes high energy use. The Department of Energy (DOE) is requiring these systems to be 30% more efficient compared to current standards from 2009 (U.S. Department of Energy, 2014a). In addition to making the cooling systems more efficient, manufacturers and retail stores are turning to LED lights to reduce energy use and cost for lighting in retail case doors and shelves. A typical T8 fluorescent light bulb is between 30 to 40 watts while LED lights are about 4 watts. Fluorescent lights perform at peak performance between 26 to 32°C, while LED performs 6% better under refrigerated environments (3°C), making LED light more ideal for refrigerated retail cases (Hillphoenix Learning Center, 2014). According to Hillphoenix Learning Center (2014), reach-in retail cases (the standard dairy retail case) are using LED lights for almost all applications for this model of retail case. It is expected that by 2020 that global commercial sales of LED lights will increase by 20%, influencing retail cases to be predominantly lit by LED lights (U.S. Department of Energy, 2014b).

The specific effects of LED lights on milk are not well documented, but it is known that fluorescent and LED lights emit wavelengths in the visible light range that cause excitation to photosensitive compounds such as riboflavin (Rb). Fluorescent light has wavelengths in the UV spectrum while LED typically does not. There are new applications using UV LED, but are not common in retail cases (Shih, 2015). Photosensitive compounds such as vitamins, lipids, and pigments in food products can react with light energy to induce secondary reactions. Riboflavin (vitamin B_2) is a water soluble vitamin found in milk and milk products that degrades under light. Rb is well studied as a photosensitizer; when milk is exposed to light, Rb excitation occurs,

initiating a series of reactions that leads to a decrease in nutritional value and changes in flavors (Min and Boff, 2002; Choe and Min, 2007). It also has been reported that photosensitizers in milk other than Rb cause oxidation. Wold et al. (2006) reported photosensitizers in milk, other than riboflavin, include riboflavin, protoporphyrin IX, tetrapyrroles, hemotoporphyrin, and chlorophyll. Riboflavin excites at 250, 270, 370, 400, 446, 570 nm wavelengths (Bekbolet,1990; Kyte, 1995), absorbing in blue and violet light (<500 nm) (Wold et al., 2015; Airado-Rodríguez et al., 2015). Fluorescent and LED lighting emit a broad range of visible wavelengths, including Rb excitation wavelengths, but at different relative intensities. Cool white fluorescent lights have sharper relative intensities between 550 to 560 and 610 to 629 nm while cool LED has a normal distributed curve from 510 to 710 nm with its highest peak at 450, 610 to 660 nm (Figure 2). To limit oxidation, appropriate packaging needs to be used to minimize transmission of wavelengths that can excite Rb and other photosensitizers in order to limit initiation of oxidation reactions.

Milk is commonly packaged in HDPE or polyethylene terephthalate (PET) as seen in retail stores in the United States. Translucent HDPE and clear PET transmit 62-85% of light between wavelengths of 300-700 nm, which includes the wavelengths (400 to 500 nm) that riboflavin most strongly absorbs (Webster et al., 2009). This range also includes wavelengths that could induce excitation from photosensitizers other than riboflavin that contribute to light-induced off-flavors (Wold et al., 2005; Webster et al., 2009; Airado-Rodríguez et al., 2011).

Pigments added to PET and HDPE may preserve milk flavor and nutritive quality more than clear PET and HDPE but visual opacity of packaging does not guarantee efficacy. Titanium dioxide is the most commonly used white pigment in coatings and in rutile form is very effective at scattering light while providing durability and stability (DuPont, 2007). Blending is an inexpensive way to add light protection to plastics. It combines light protective additives into plastics or by adding them as a coat or laminate (Duncan and Hannah, 2012). Titanium dioxide (TiO_2) blends in milk packaging provide light barrier properties, providing some protection of

milk from photooxidation (Moyssiadi et al., 2004; Johnson et al., 2015). Titanium dioxide and other colorants are used because they scatter light as well as absorb certain UV wavelengths (DuPont, 2007; Duncan and Hannah, 2012). Johnson et al. (2015) compared various TiO_2 concentrations in HDPE to show the protective differences of visually white packages. UHT-processed milk packaged in HDPE bottles with 4.2% TiO_2 showed less oxidation and higher riboflavin retention when exposed to light fluorescent light (2,200 lux) for up to 36 days than bottles with 1.3% and 0.6% TiO_2 (Johnson et al., 2015). Different concentrations of colorants can be added to HDPE and PET to form a more effective barrier between light and milk. HTST-processed milk packaged in PET bottles loaded with TiO_2 and carbon black, showed sufficient oxygen and light barriers, but pigmented HDPE in monolayer and multilayer may contribute to better protection due to greater wall thickness (Moyssiadi et al. 2004). Moyssiadi et al. (2004) also proved that lipid oxidation is lower in pigmented HDPE bottles than pigmented PET because HDPE has a greater wall thickness than PET.

The overall purpose of this research was to determine efficacy of HDPE packaging modifications with light protective additives (LPA) in protecting sensory quality and vitamin (Rb) concentration, and limiting oxidation of 2% milk under fluorescent and LED lights under refrigerated retail conditions.

Objective. To determine changes in sensory quality, vitamin retention, and oxidation of 2% milk packaged in HDPE bottles with different LPA added to bottle resin up to 72 hours of refrigerated (3°C) storage under fluorescent and LED light simulating retail conditions.

3.2 Materials and Methods

Packaging

Packages varied in light protection by containing four levels of commercially available LPA within the bottle resin. The four levels of LPA packages (total of five package treatments)

were unpigmented HDPE bottles (0% TiO_2), which served as the controls (light-exposed (LE) with no barrier; light-protected (LP): 0% TiO_2 HDPE with foil and plastic overwrap, commercially available yellow pigment (Y) (masterbatch with yellow colorant), low (1.3% TiO_2), and high (4.9% TiO_2) (TiPure®, DuPont™ Titanium Technologies, Delaware). Control bottles were translucent and product was easily visible in light-exposed treatments. Yellow, low, and high experimental packages were opaque and did not allow visibility of milk although fill volume could be seen with close inspection. The four levels of LPA were blow-molded into half-gallon HDPE packages (Consolidated Container Co., Atlanta, GA). Bottle dimensions were 9.875" height x 3.875" and a volume of 1,892 mL. Wall thickness measured 15 cm at center of face wall. Package color readings were taken by Konica Minolta CR-300 Chroma Meter (Tokyo, Japan). Lab color space was recorded as L*, a*, and b color values, respectively, for packages as follow: Unpigmented HDPE: 73.115, -0.635, -3.06: yellow: 89.91, -17.43, 12.27; low: 93.27, -0.06, -1.65; and high: 97.08, -0.26, -0.48.

Packages were analyzed for transmission and absorption by UV-visible spectrophotometer (Cary 300 UV-Vis, Agilent Technologies, Santa Clara, CA). Packages were cut into a 2×2 inch squares and placed into UV-vis spectrophotometer to test for transmission and absorption. Packages (0% natural HDPE, low (1.3% TiO_2), high (4.9% TiO_2), yellow) were measured in UV and visible ranges (200-700 nm). Light-protected control was analyzed but not represented on the figure. The UV-vis shines light to measure absorbance and transmission and due to reflective nature of aluminum foil data was not accurately recorded; it is assumed aluminum foil does not allow light transmission.

Milk Processing

HDPE resin and additives (Ampacet, Tarrytown, NY) were blow-molded into half-gallon packages (Consolidated Container Co., Atlanta, GA). Homogenized, high temperature short time

(HTST; 77.7 ºC for 15 seconds) pasteurized, vitamin A and D added, 2% milk was processed at a Kroger Co. dairy processing plant (Westover Dairy, Lynchburg, VA). Milk was bottled and capped on the same processing line and then moved into a refrigerated warehouse. Bottled milk was stored approximately 20 to 60 min in a lighted cooler (100 lux) before loaded on a refrigerated truck (3.33°C ± 1). Milk was not exposed to light during transportation to Virginia Tech Food Science and Technology Department. Upon arrival, bottled fresh milk (n = 480) was placed in a dark walk-in cooler (4°C) until the filling process began.

The filling process included transferring fresh delivered milk into experimental packages (yellow, low, high) and control packages (light-protected and light-exposed) with a headspace of 40 ± 15 mL. Before filling, transported milk bottles were wiped with sanitizer (Santi-Bac Quat, St. Paul, MN) around neck and cap to reduce contamination of milk. Fresh milk was clean-filled into LPA and control packages and sealed with screw caps under positive laminar flow hood (Atmos-Tech Industries, Ocean, NJ) to prevent contamination. Filled LPA and control bottles (half gallon) were wiped with sanitizer, and immediately transported in coolers to limit light exposure into the dark walk-in cooler.

Filling occurred on two different days with two different filling schedules for replication 1 and replication 2 and 3 (Appendix D). Due to the schedule, replication 1 required the first four days of panel (16h, 24h, 4h, and 72h) to be filled first (n=320) and the last two days (48h and 8h) to be filled second (n=160). Replication 2 and 3 required the first three days of panel (16h, 4h, and 72h) to be fill first (n=240) and the last three days (48h, 8h, and 24h) to be filled second (n=240). Each replication had a total of 480 bottles.

Storage Conditions

Refrigerated dairy case lighting in retail stores. To gain a better insight on retail case conditions of milk in stores, we took light readings with a hand held meter (Model SD400, Extech Instruments, FLIR Commercial Systems, Inc., Nashua, NH) from local grocery and convenient

stores. Light readings were taken from six to twelve locations (depending on retail case availability) within two doors of dairy retail cases from 20 various grocery and convenience stores. Light readings were taken from a left, middle, and right position of the middle shelves within the retail case (Appendix Q).

Lighting conditions. In the experimental studies, samples were stored in closed door refrigerated beverage case to model retail conditions (Model ONRB4, Hillphoenix, Chesterfield, VA). The retail case cooler was glass fronted with four doors (158.4 cm height x 76.2 cm width), five shelves (55.9 cm length), and with two lights on the sides of each door that ran vertically of the retail door (163.5 cm). A total of eight 3,500K light bulbs were in the retail case; four cool white fluorescent bulbs on the two door sections and four warm LED bulbs on two door sections. The retail case was divided by a vertical metal barrier inside the case to separate the retail case into two sections: a fluorescent-simulated retail case and a LED-simulated retail case. A ceiling high black tarp was used to block light from windows and reduce ambient lighting. This ensured the light projected onto the bottles were only from the retail case.

Packaging arrangement. Each retail case door contained five shelves, but only the four upper shelves were used to accommodate the amount of bottles tested. In each light treatment, five package treatment bottles (light-exposed control, light-protected control, yellow, low, and high) and two half gallon bottles filled with water, for a total of 7 bottles, were placed on each shelf (n=5) within each door section (2 doors for LED; 2 doors for fluorescent) of the retail case. Only the front row on each shelf was filled with package treatments; water bottles filled the remaining rows (behind the front row) on each shelf to simulate a fully stocked retail case. Each shelf had a water bottle in the center position, which was furthest from the vertical lights, on each shelf. A total of 56 bottles were in the front position of each section (fluorescent, LED) of the retail case (8 of each package treatment and 16 water bottles). Bottle placement was randomized by JMP 10.0 Statistical Discovery Software (SAS Institute, Cary, NC) within each shelf so that all

treatments were distributed randomly within the dairy case to reduce the effects of different lighting intensity within the case.

Temperature and light intensity within the case were measured routinely. Temperature was monitored (3°C) with an installed thermometer in the retail case after the case was filled for each time interval. Light intensity (lux) was measured using a handheld light meter (Model SD400, Extech Instruments, FLIR Commercial Systems, Inc., Nashua, NH) after packages for each time interval were arranged in the retail case. Light intensities were measured at each bottle location. The average light intensity for all three replications for all positions within the retail case were 1,958 ± 1007 lux for fluorescent light and 926 ± 164 lux for LED light.

Light intensity and wavelength spectrum was assessed using a light meter (MK350S UPRtek, Jhunan Township, Miao-li, Taiwan). Readings were taken from inside LPA packages to represent light exposure and spectrum on milk; the light reader was placed in the bottle and recorded where milk would be to gain a better idea of what light intensity and spectrum was reaching the milk within the package. Packages were placed in retail case under fluorescent and LED light at positions with 1,000 to 1,025 lux. Packages were cut 4×4 inches to allow light meter to sit on the inside of the bottle with about 0.5 inch of space between the light meter sensor and the inside of the bottle. Bottles were placed on retail case shelf with light meter facing towards the glass door looking from the inside of the case to the outside of the case.

Light readings (Model SD400, Extech Instruments, FLIR Commercial Systems, Inc., Nashua, NH) and thermal image photo was prepared by Hillphoenix representatives. Readings were recorded approximately 3 inches from each door opening and one in the middle of the shelf. Retail cases were filled with half gallon water bottles for thermal imaging. Cool areas of the retail case are represented with blue/green colors while warmer spots of the retail case are yellow to red. This allowed us to see the “hot spots” (areas closer to light bulbs) and “cool spots” (spots further away from the light bulbs) of the retail case.

Duration (time) of light exposure. Milk from each packaging treatment (n=8 bottles each) was stored under continuous light exposure for 7 time intervals (0, 4, 8, 16, 24, 48, 72 h). Due to the design of the retail case, only one time period (all packages) could be tested at a time, with all time periods tested within nine days of product processing. Six bottles of each package treatment, targeted for sensory evaluation, were removed from light and stored in a dark walk-in cooler (Model 300, Hillphoenix, Chesterfield, VA), for no more than 24 hours until preparation for sensory testing. The remaining two bottles from each packaging treatment were removed from the lighted case and subsequently stored in a dark walk-in cooler until the total time from first light exposure was 18 days. The purpose was to model a consumer's purchase of milk that was exposed to light for a period of time in the retail dairy case and then placed in a dark refrigerator (at home) until code date.

For replications 1 and 2, samples (10 ± 2 mL for TBARS analysis and 40 ± 5 mL for Rb analysis) were poured into labeled test tubes and capped from each individual bottle after each time treatment. Individual samples from each bottle were not collected on replication 3. Samples were stored in polypropylene tubes (Thermo Fisher Scientific, Waltham, MA) in a blast freezer (-20°C, Harris Environmental Systems, Andover, MA) and shielded from light. Samples (10 ± 2 mL for TBARS analysis and 40 ± 5 mL for Rb analysis) of commingled milk (six bottles per package treatment × time for sensory testing) were collected on each day of sensory testing in the same manner.

Analytical Analysis

Microbiological quality. Microbial analysis of fresh milk (n=3 bottles per replication) and milk stored for 18d was completed by standard methods (Marshall, 1992) to verify that milk was properly pasteurized and maintained microbial quality over storage period. Milk samples were evaluated by standard plate count of aerobic organisms using Petrifilm™ Aerobic Count Plates and Petrifilm™ Coliform Count Plates (3M™, St. Paul, MN). Aerobic count plates were

incubated at 32° ±1°C for 48 ± 3h. Coliform Count Plates were be incubated at 37°±1°C for 24h. Microbial testing ensured that fresh milk did not exceed the standard plate count limit for pasteurized milk (20,000 SPC/mL) and the coliform plate count limit (10 CFU/mL) (Jay et al., 2005). Bottles were also tested on 18 days post-processing to confirm flavors and vitamin retention were due to light exposure and not microbial presence (Appendix E).

Milk composition. Milk composition was analyzed by Foss MilkoScan™ with a fourier transform infrared spectrophotometer (Hillerød, Denmark) by the United Federation of Dairy Herd Improvement Association (Radford, VA). Average composition of the milk was 1.97% fat, 2.99% protein, 8.67% solid nonfat, and 4.83% lactose.

***Degradation of Rb by fluorometric analysis*.** Frozen samples representing each time × package treatment (n=6 samples per replication 1 and 2; n=3 samples for commingled milk from all three replications) were thawed in the dark in an ice water bath. Rb concentration was analyzed using the fluorometric analysis method (AOAC method 970.65) and measured on Shimadzu RF-1501 Spectrofluorophotometer (Shimadzu Scientific Instrument, Inc., Columbia, Md., U.S.A.) (Bradley, 2000; Webster et al., 2009). Samples were prepared by adding 0.150 mL (10 N) hydrochloric acid to milk (10 mL) to pH 5.0-6.0. An additional 0.1 mL 10 N hydrochloric acid was added to milk samples. These samples were then autoclaved under pressure at 17.0 psi (117.2 kPa) for 30 minutes at 121-123°C. Samples were cooled and pH was adjusted to 6.0-6.5 using sodium hydroxide (0.05 mL 1 N). Hydrochloric acid (0.075 10 N) was immediately added to stop precipitation around 4.5 pH. Each sample was centrifuged (4,000 rpm) and filtered using 25 mm syringe with 0.2 μm filter (Grace, Deerfield, IL) and 10 mL Norm-Jet syringe (Henke Sass Wolf Inc. Tuttlingen, Germany). Three bottles per package treatment were tested for each time interval and each light condition (pkg × time × light) for each replication (n=6 total packages per package × time). Bottles were chosen based on light intensity (within one standard deviation of average intensity for that light and time treatment in each replication). Riboflavin

concentration of commingled milk were tested for all replications (n=3 samples for each pkg × time × light). Riboflavin concentration was evaluated in shelf-life bottles (n=2 for each pkg × time × light) for all replications. All analysis was done with minimal light exposure; overhead lights turned off with minimum light exposure from outdoor light.

Formation of secondary oxidation by-products by thiobarbituric acid reactive substances analysis (TBARS). TBARS method was used to measure the formation of the secondary products of oxidation, including malondialdehyde (MDA) and other secondary oxidation aldehyde products. The TBARS method we used was modified from Spanier and Taylor (1991). Frozen samples were thawed in an ice water bath in the dark until completely melted. Samples (1 mL) were pipetted into 15 mL disposable centrifuge tubes with 4 mL of sodium dodecyl sulfate solution and 0.1 mL EDTA solution. Samples were vortexed for 20s and incubated in water bath (95°C) for 60 min. Tubes were cooled in an ice bath to room temperature (approximately 23°C) and then mixed with pyridine and butanol solution. Tubes then were centrifuged at 4,000 rpm, room temperature for 25-30 min. The top organic layer (2.5 mL) was transferred into a 1.5 mL polystyrene cuvette. Oxidative products reacted with thiobarbituric acid to create a reddish/pink color, which was measured at 532 nm using a spectrophotometer (Milton Roy: Spectronic 21D, Ivyland, PA).

Three samples were tested per pkg × time × light for replication 1 and 2. Bottles were chosen based on light intensity (within one standard deviation of average intensity for that light and time treatment in each replication). Riboflavin concentration of commingled milk were tested for all replications (n=3 samples for each pkg × time × light). Riboflavin concentration was evaluated in shelf-life bottles (n=2 for each pkg × time × light) for all replications.

Sensory Analyses

Experimental design. Sensory analysis was performed by triangle tests (Meilgaard et al., 2007) for each time interval, comparing each experimental package against each control (LE, LP). Six

milk bottles (pkg × time × light) were commingled to provide sufficient volume of the treatment to complete sensory testing. For each light condition, milk in each experimental package was compared to the LP control (foil wrapped) to determine if the experimental package provided equivalent and adequate protection (similarity) as the light-protected control condition; the statistical parameters for similarity were pre-established, based on an estimated proportion of discriminators (p_d) = 20%, as $\alpha = 0.20$ and $\beta = 0.05$ (n=86) when analyses from all three replications were combined. In addition, milk (commingled) from each experimental package (and light treatment) was compared to the light-exposed control milk to determine if a difference could be detected. The statistical parameters, based on an estimated $p_d = 20\%$ were $\alpha = 0.05$; $\beta = 0.20$ (n=87 observations) based on Meilgaard et al. (2007). The p_d of 20% was estimated based on previous work in our laboratory (Johnson et al., 2015). There were 16 comparisons total tested per day. Significant differences were determined as described in Meilgaard et al. (2007) for the total number of observations for each comparison. To minimize Type I error and Type II error, LPA packages were tested for differences against the translucent control and tested for similarity against foil wrapped control, respectively. The minimum number of panelist needed to reach power was 67, but 94 to 108 panelists were tested over all replications providing a high power to validate the sensory results.

On each day of sensory evaluation, only one time interval was tested. Each experimental pkg × light treatment was compared against the respective light controls (LE, LP) for a total of 6 comparisons for LED and 6 comparisons for fluorescent light treatments. In addition, fluorescent control samples (LE, LP) were compared to their respective LED controls. Therefore, 14 comparisons (triangle test sets) were completed on each day of evaluation.

An incomplete block design was used, with each participant receiving 5 triangle sets. A minimum of 40 sensory observations of each comparison was targeted in each replication for a total of 94-108 observations for each comparison after all replications.

Preparation of samples. Milk from each pkg × light treatment (6 packages) was commingled into cleaned and sanitized generic insulated coolers (5 gal) with spigots. Plastic serving cups (2 oz), identified with 3-digit codes representing each triangle test set comparison, were filled with approximately 30 mL milk, capped, and stacked in aluminum pans (11 ¾ in x 9 ¼ in x 2 ½ in) identified with the packaging treatment (n=14) and stored in a two-door refrigerator at 39 ± 2°C (Model T-35G, True Manufacturing Co., O'Fallon, MO). For each experimental pkg × light treatment, sixty 3-digit codes were needed to allow for comparisons with each control, requiring a total of 120 total cups to be filled for each pkg × light treatment. A total of 600 cups were needed for each control pkg × light treatment. All cups were filled and stored in the refrigerator at least 30 minutes prior to testing to chill the samples to the targeted temperature range (2.2 ± 2°C).

All comparisons (n=5) for each participant were placed on a tray in random order prior to serving. To facilitate this task, filled sample cups were temporarily transferred from the refrigerator to an aluminum pan (identified with the proper 3-digit codes) nested on ice and covered with aluminum foil to protect samples from light; each pan contained four sample codes for the targeted comparison. Samples (5 triangle test sets) for each participant were organized in order on a tray and placed in a miniature refrigerator (2.7°C ± 1.5) to keep samples cold until presentation to the panelist. Trays were withdrawn from this storage unit immediately prior to serving to the panelist. This process continued throughout the sensory testing period, which typically took 4-6 h until the number of participants to complete the replicate was completed.

Comparisons were planned in a random balanced incomplete block designed, where all panelists received five triangle tests (not all comparisons were tasted by one panelist) in random order. All comparisons were designed to be equally tested with 8 complete blocks per comparison evaluated in each replication.

Sensory testing conditions. Virginia Tech Institutional Review Board (11-477) approved the study before testing. (Appendix A). Sensory testing was conducted in HABB1 Sensory Laboratory (room 205). Participants reviewed and provided informed consent on each day of testing prior to receiving samples (Appendix B).

Panelists were in partitioned booths, under white LED light (Gotham Architectural Downlighting Conyers, GA) using a touch screen monitor to record responses. One triangle test was provided at a time by passing the samples on a tray through the hatch to the participant. Participants were asked to verify the sample codes on the presented samples to the codes on the electronic ballot, then evaluate all samples on the tray from left to right and identify the different sample. Unsalted oyster crackers and water were provided for cleansing the palate between each triangle test. After completing the triangle test, the panelist was guided to return the tray and wait for the next sample set. Sensory Information Management Systems by Sensory Computer Management SIMS 2000 (Morristown, NJ) was used for preparing the electronic ballots and recording responses. The responses from each replication were combined and analyzed as described in Meilgaard et al., (2007).

Participants were rewarded daily with a selection of snacks. For each day of participation, panelists also accumulated 'points' toward a $10 gift card. Typically, 2 points were offered per day, so a regular participant could readily earn a $10 gift card for each replication.

Headspace Analysis Using Conducting Polymer Electronic Nose

A conducting polymer electronic nose (Cyranose® 320, Sensigent, Baldwin, CA) was used in this experiment. Settings for the ENose were establish in a preliminary study assessing the draw and purge time of the instrument (Table 1).

Table 1. Cyranose® 320 electronic nose 2% milk evaluation parameters.

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

The Cyranose® 320 measurement is based on change in resistance of each chemical sensor in the 32 sensor NoseChip® when exposed to volatiles. Inside the Cyranose® 320 a valve and pump are used to pull the baseline purge and sample volatiles over the sensors. The purge and draws allow the volatiles to reach the 32 sensor NoseChip® and properly refresh the sensor between tests.

Volatiles in fresh milk (0 h of light exposure) and pkg × time × light treatments (8 and 24 h) were tested using the ENose method for replication 3. For fresh milk, 5 bottles were pulled for duplicate testing. This served as a baseline for what "acceptable" and "good" milk was. After the experimental and control packages were exposed to fluorescent and LED light at 8 h and 24 h, the bottles were removed and samples (8 mL) collected for subsequent electronic nose sampling. For each of the five package treatments, one sample (8 mL) was collected from each individual bottle per package treatment for both fluorescent and LED lights (total of 8 samples for each pkg × light × time treatment). Additionally, samples (n=8) for ENose evaluation were collected from commingled milk for each pkg × light × time (8 h and 24 h) during the sensory preparation to represent sensory tested milk. Milk (8 mL) was poured into lean amber glass vials (40 mL), fitted

with rubber septum and cap, to allow 80% headspace and 20% sample in each vial. Samples were stored in a dark walk-in cooler (4°C) for a minimum of 30 minutes to allow equilibrium of volatiles to occur within the headspace. Samples were kept at refrigerated temperatures during data collection to mimic temperatures that consumers would consume milk. Samples were collected by piercing the airtight rubber septum with the ENose needle and running ENose to collect the headspace volatiles. One ENose reading was collected for each vial. Data collection occurred within 6 h of bottle removal from the retail case.

Statistical Analyses

Three-way analysis of variance (3-way ANOVA) was used to determine changes in oxidation reported in TBARS and riboflavin concentration data. The main effects were time (n = 6), package treatment (n=5), and light treatment (n=2), and interactions (pkg × time, pkg × light, pkg × time × light) for two replications. Statistical analyses were completed using JMP 10.0.0 Statistical Discovery Software (SAS Institute, Cary, NC). When significant 3-way interactions were observed, 2-way ANOVA for all pkg × time treatments within light type were completed. Statistical significance was determined based on a pre-established α=0.05. When significant differences were identified, mean separations were completed using Tukey's HSD. Data for Rb and TBARS of commingled milk (3 replications) were also analyzed (α=0.05) in the same manner.

Since light intensity between LED and fluorescent conditions was different, analysis of covariance (ANCOVA) (α = 0.05) also was used to determine light intensity impact on Rb and TBARS. Correlation of Rb and TBARS was used to test the relationship with light exposure using Pearson correlation coefficients (SAS Institute, Cary, NC). Dixon's Q-test was completed to determine outliers (extreme observations) as mentioned in Snedecor and Cochran (1980). Shelf life study analysis (n=6) was also performed with 3-way ANOVA as mentioned above.

Sensory analysis was completed on all three replications. Sensory data was analyzed based upon statistical parameters mentioned above and equations outlined in Meilgaard et al. (2007) by replication as well as for commingled data. The actual proportion of discriminators in this study was determined for each triangle test comparison (Meilgaard et al., 2007). To aid in understanding the application of sensory difference and similarity testing used in this study to package efficacy in protecting milk quality, package effectiveness definitions were identified. These definitions were used to assess package protection based on the statistical parameters used (Appendix F). The table describes the language used in this paper to describe the best and worst case scenarios for the packages as well as the intermediate zones of protection in relation to the sensory outcomes.

ENose data was analyzed by canonical discriminant analysis from Euclidean distances (CDA) using JMP version 7 software (SAS Institute, Cary, NC) and a logistic regression with elastic net penalty using SAS/STAT® Software to identify the important sensors for milk volatiles (SAS Institute, Cary, NC). Multivariate analysis of variance (MANOVA) analyzed the treatments for each important sensor to show how packages compared to fresh milk.

3.3 Results and Discussion

Our goal was to determine how effective packaging with different light protective additives might be in protecting fluid milk from light-induced oxidation under the conditions of lighted retail storage. Previous studies used lights typical for retail storage in a light box, but did not use the exact replica of a retail case. In our study we used a commercially manufactured retail case provided by a major supplier (Hillphoenix, Chesterfield, VA). The packages that were selected represented commonly utilized HDPE packaging (no LPA, natural HDPE); low (1.3% TiO_2; yellow pigmenting) for fluid milk packaging and a high package (4.9% TiO_2) targeted to protect riboflavin from photoexcitation and reduce oxidation. It is valuable to consider packaging

efficacy under both the traditional fluorescent lighting condition as well as LED lighting, which is rapidly increasing in retail settings.

The results of this project are described first based on the analyses of individual bottles within the retail case (Study 1, based on replications 1 and 2) to provide an understanding of the degree of variability associated with the storage conditions within the retail case on the pkg × time × light treatments and the interaction of light type and intensity with packaging on the oxidation of milk. This study included the analytical analyses only (no sensory interpretation) and would be indicative of the quality of milk as purchased from the retail store. In the second section (Study 2), the implications of the test conditions on the sensory quality of milk, based on commingled milk and eliminating the variability from light intensity within the case; this study included analytical analyses (3 replications) as well and can be used to ascertain if any packaging treatment effectively protected fluid milk flavor quality during the light exposure and if light type (LED vs fluorescent) had greater effect on milk quality. In addition, a summary of the analytical analyses (TBARS, Rb) of milk at 18d, after light exposure and subsequent storage in the dark, provided an indication of changes in oxidation during the typical consumer use period. We conclude with a description of the relative changes in volatile chemistry, as measured by ENose, to determine if the volatile 'smell print' is altered by the interaction of pkg*light.

Study 1. Interaction of light and LPA in packaging in contributing to oxidation of milk under various positions within retail storage conditions.

Storage conditions. Lighting intensities vary in retail cases due to lighting, size of retail case and shelves, and model of retail case. Hillphoenix Learning Center (2014) states shelf size makes a large difference in light intensity on reach-in door cases because lights are vertical in orientation and project light differently across the retail case. Retail cases with fluorescent lights have been reported to have intensity ranging from 215 to 6,460 lux (Whited et al., 2002) or 750 to 6,460 lux (Chapman et al., 2002). We confirmed light intensities differ within retail cases from study 1 and

from a survey of retail cases in the greater Blacksburg, VA area and in Knoxville, TN. Our preliminary screening documented that variation not only occurred from store to store, but also within the retail case (shelf, position (Appendix Q). Product in a retail case will receive different amount of light intensity based on the location within the retail case. Lighting in the retail case used in our study falls within observed ranges in retail case settings. Appendix O shows the different intensity for each position in our retail case. Not only do retail cases vary in light intensity based on position, but also emitted energy. Figure 3 shows thermal images of the fluorescent and LED lighted retail cases used for this project. The fluorescent case in this study not only has higher light intensity (lux), but also has warmer thermal energy than LED lights. Thermal imaging shows the radiated energy emitted from an object; more energy is emitted with increasing temperature. Fluorescent light gives off heat due to the heating filament in the bulb. This heat is transferred from the light to food products and is known to increase meat surface temperature by 1°F as light intensity increases by 108 lux (Kropf, 1998). This causes discoloration from oxidation and even microbial growth in meat products (Kropf, 1998). The temperature emitted from fluorescent light could impact milk as well. LED light does not contain heating filaments, but can still emit warmer thermal energy with increased light intensity. Not all LED retail cases exhibit lower lux readings when compared to fluorescent lights like this project. As seen in Potts' (2016) retail case study, different retail cases with LED lights exhibited large variation with intensity (ranging from 900 to 5,400 lux). Depending on the retail case, light intensity can greatly vary. Each individual package in a dairy retail case is exposed to different intensities of light, dependent on the position of the case as shown in Figure 3. Studying the effects of light intensity variation can provide more information on how to design and improve retail cases. This also provides information for designing improved packaging with effective light barrier properties.

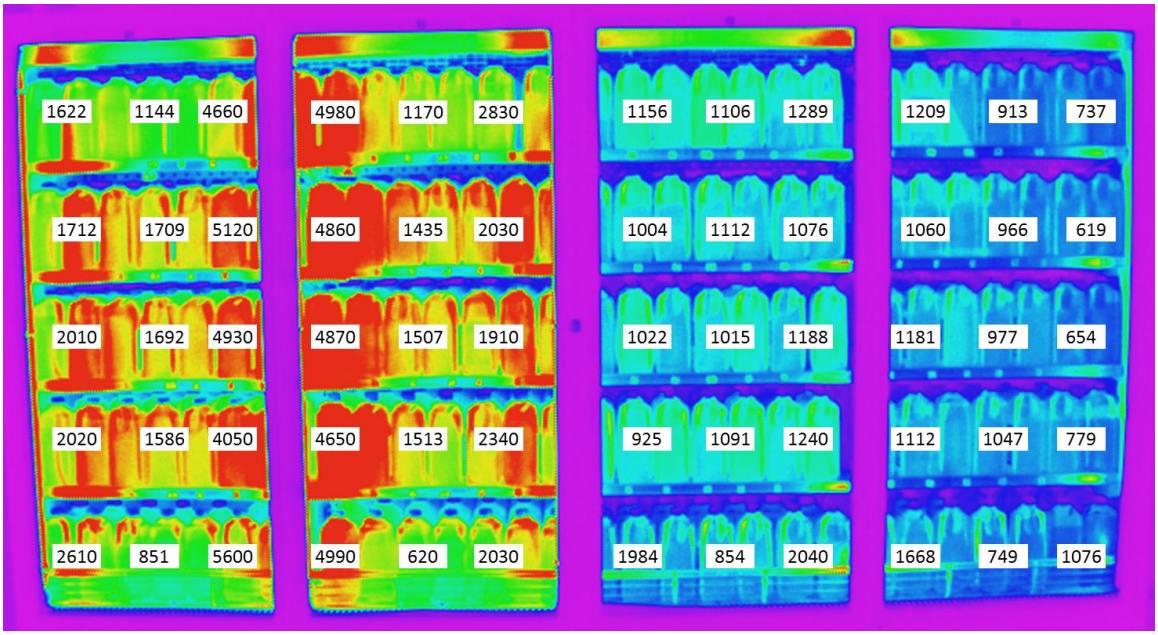

Figure 3. Thermal imaging of fluorescent and LED retail cases with average light intensities in three locations (left, middle, right) on each shelf. *Image produced by Jack Sjogren of Hillphoenix, Chesterfield, VA.*

Lights not only have different intensities, they also have different color temperatures. Color temperature describes the color characteristics of the light by identifying the color emitted as warm, neutral, or cool in degrees Kelvin (K) and range from 2,400K to 7,000K (Hillphoenix Learning Center, 2014). Lights with low Kelvin values emit a warm, yellow tones such as a candle, and lights with higher values emit a cool blue, natural light tone. Depending on the ambience desired, different lights and color temperature can be used. The color temperature of a light not only determines the visual appearance of the product, but can also impact the wavelength spectrum of the light. "Cool" lights are in the range of 3,500K and above and "warm" lights are 3,000K and below (Hillphoenix Learning Center, 2014). As mentioned, cool lights give off more blue tones and is ideal for showcasing produce, milk, and packaged cheese while warm lights are more yellow in color and provides the best display lighting for meats and seafood products. Figure 4 shows the difference in cool and warm wavelength spectrum for LED light. Cool LED light has higher relative intensity in the 425 to 450 nm range. This could impact the sensitivity of Rb and other photosensitive compounds that react to the blue/violet region of

the light spectrum. Cornell University (1998) recommends using warm fluorescent light over cool fluorescent light because of its lower degradative energy. This may also be true for LED lights, but no published work was found identifying the color temperature of LED lights effect on foods. Lighting in retail cases and grocery stores are more focused on aesthetics and display of products without awareness of the destructive qualities light has on food products. Because cool white lights best display dairy products, they are used most often in grocery stores for fluid milk, packaged cheese, and other packaged dairy products. Cool white light was used in this study to stimulate current lighting in retail cases.

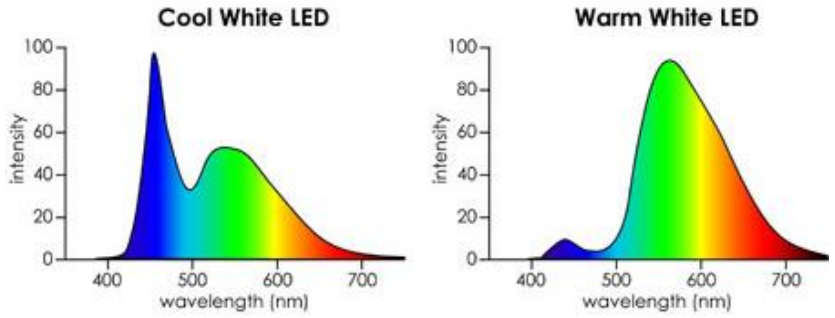

Figure 4. Wavelengths of cool and warm LED lights (reprinted with permission from Floroiu, V. A, 2015).

In addition to collecting survey information about the light intensities of various retail cases in the area in regional stores, we also conducted an informal interviews to gain information about managers' and employees' awareness and knowledge of light impact on milk quality. A total of 20 stores participated in our survey in conjunction to recording the light intensities within retail cases. Majority (18 out of 20) said there was no specific training about light effects on milk and lighting was decided by corporate based on energy efficiency. Out of 20 stores, 7 stores continuously kept lights on in the retail cases while the other 13 stores either turned off lights after store hours or had motion activated sensors. The motivation for lighting used in the retail cases is not to protect milk quality, but to effectively display products in the cases in the most visually effective and most cost efficient way. Even though there are new mandates to reduce the

energy output of retail cases, this may not reduce degradation in milk quality and nutritional value. Other protective measures must be taken to protect milk in order to ensure protection of food quality.

With the understanding that retail cases differ in light intensity throughout the case, shelves, and between models, it is necessary to understand how packages interact with these environments. Light protective additives like titanium dioxide (TiO_2) can scatter and absorb light energy and reduce the amount of transmitted light with just packaging alone (DuPont, 2007). Yellow colorants added to dairy packaging has also been used to prevent light induced flavors in milk (Cornell University, 1998). Light transmission and absorption was tested to see packages protective qualities (Appendix P1-2). All LPA packages had similar pattern of absorbance and transmission with the unpigmented HDPE with the most transmission and least absorption as shown in Appendix P1-2. Light readings were taken from inside the bottles to mimic light exposure of milk using the light meters used through the study (Appendix H1-2). The natural (no LPA) HDPE allowed the full wavelength spectrum to penetrate the bottle providing no apparent protection. The high package showed lower peaks of 540 nm for fluorescent and 420 to 460 nm for LED light while the low package appeared to have similar peaks to the unpigmented HDPE. The yellow package reduced the blue/violet region, which matches published reports (Duncan and Hannah, 2012). As much as lighting changes and varies, the way packages interact with light also has differences. Study 1 will help explain how individual bottles interact with these differences to give a better idea of how to control milk quality through light and packaging

For this study, samples representing three bottles each from rep 1 and rep 2 and within one standard deviation of the mean light intensity for each light condition, were randomly selected for riboflavin and TBARS analyses. The average light intensity to which the samples were exposed was 1,617 ± 505 lux for fluorescent and 929 ± 97 lux for LED.

Excitation of photosensitive molecules under retail lighting conditions. Milk contains the photosensitizing molecule, riboflavin, at an average concentration between 1.36 and 1.75 mg/L (Dimick, 1982; Zygoura et al., 2004). In this study, the concentration of Rb in fresh milk was 1.46 mg/L falling within the normal range.

ANOVA was used to analyze the overall main effects (time, package, light). Time (P= <0.001) and package (P= 0.002) were significant effects while light source (LED and fluorescent; P= 0.971) showed no significant differences in Rb concentration. Having significant differences in time and package were expected; it has been reported that time and different concentrations of LPA affected Rb in UHT milk (Johnson et al., 2015). No significant differences by light source was detected which was unexpected because fluorescent and LED lights had different intensities (lux) and different wavelength spectrums. Using Analysis of covariance (ANCOVA), no significant difference (P= 0.183) was found between fluorescent and LED light intensity impact on Rb concentration. ANCOVA is used when two treatments are continuous data; time and light intensity were set as continuous data making ANCOVA appropriate to run. Intensity of fluorescent and LED light was not seen as a main effect on milk quality in Brothersen et al. (2016) study even though the fluorescent light has a lower intensity (2,200 lux) than LED lights (4,000 lux). However, fluorescent light at its lower light intensity resulted in more vitamin A loss (30%) and higher hexanal, heptanal, and other aldehydes than did LED light. Brothersen et al. (2016) hypothesizes that wavelength spectrum is a main influence on milk quality under fluorescent and LED light, not just light intensity. It is obvious from Figure 2 that there are differences between fluorescent and LED light spectra. Regardless of light intensity, these wavelengths will still be present and perhaps be just as effective at degrading Rb at low and high light intensities.

Interactions were tested with two-way and three-way ANOVA (time × light, time × pkg, light × pkg, time × light × pkg). Differences in Rb concentration were observed for light and

package interaction (P= 0.042) and time × light × pkg interactions (P= 0.012). No differences were observed for fluorescent and LED LE packages for light*pkg interactions (P> 0.05); the natural HDPE was not efficient at blocking out light and affected the milk similarly (Rb 1.04-1.07 mg/L). LP under fluorescent and yellow under LED demonstrated similarities in light protection with the highest levels of Rb out of the packages. All other packages shared qualities of LP control and LE control (P> 0.05 LP control, P> 0.05 LE control; partially protected package). Differences were not observed with time × pkg interactions (P= 0.140) and time and light interactions (P=0.508); these interactions were not the main two-way interactions effecting the three-way interaction.

Rb concentration was analyzed by two-way ANOVA and Tukey's HSD analysis for differences in time and packages for each light source. Under LED light, package (P=0.002) and time (P= <0.001) were different. This was expected because Rb concentration decreased over time and packages performed differently (Figure 5). The LE control performed less effectively, as predicted, and the yellow and low package performed most effectively under LED light. We observed differences in time between 4h and 48-72h, with 4h having the highest Rb concentration and 72h having the lowest Rb concentration across packages (Figure 5).

Under fluorescent light, time (P= <0.0001), package (P= <0.0001), and time × package interaction (P= 0.03) were significant. The 4h light exposure retained higher Rb concentration (1.38 mg/L) while 72h had lowest (0.84 mg/L) across packages. We observed differences (P < 0.05) between the LP control and the other packages. The LP control was able to protect the Rb in milk most efficiently under fluorescent light. There was a time × package interaction, unlike the LED light. This suggests fluorescent light is interacting with packages differently than LED light does, perhaps because of the differences in wavelength spectra, and the interaction is influencing the Rb concentration in milk. Fluorescent light may have greater impact on Rb in milk than LED light as suggested by Brothersen et al. (2016).

We reported Rb in % retention to show the loss of Rb over time under the conditions of our study (Figure 5). The concentration of Rb as a function of package, time and light is provided in Appendix K.

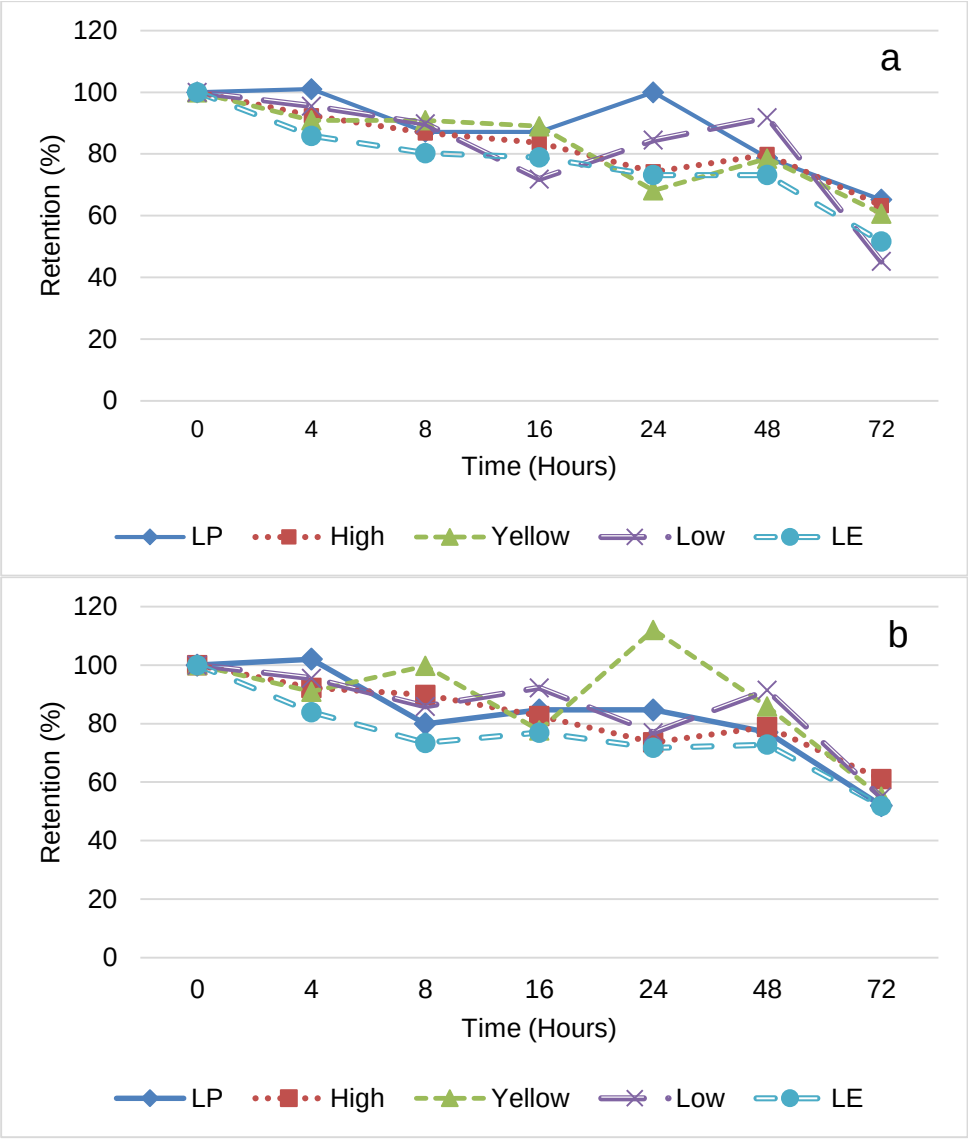

Figure 5. Riboflavin retention (%) in individual bottles of 2% milk packaged in high-density polyethylene (HDPE) with different light protective additives (yellow, low (1.3% TiO_2), high (4.9% TiO_2)) compared with controls (light-exposed (LE); light-protected (LP)) over 72 h of (a) fluorescent light (1,617 ± 505 lux) and (b) light emitting diode (LED) (929 ± 97 lux) exposure.

The key excitation wavelengths for Rb are under 570 nm. However, other photosensitizing molecules absorb light at longer wavelengths and may also contribute, probably to a lesser degree, to oxidation of milk. Under fluorescence analysis chlorophyll absorbs light up

to 700 nm and protoporphyrin IX degrades under white light (the visible spectrum; 380 to 770 nm) (Airado-Rodríguez et al., 2011). Fluorescent and LED lights contain wavelengths across the whole visible light spectrum including those that degrade chlorophyll and protoporphyrin IX (Figure 2). Airado-Rodríguez et al. (2011) characterized milk under different color films reporting more sunlight and rancid flavor in milk under orange light (520 to 750 nm) than blue light (300 to 580 nm) demonstrating harmful effects of the orange light spectrum. Rb excitation wavelengths in the blue and violet regions are well studied, but there is indication that other photosensitive compounds are causing oxidation that contribute to sensory changes in our study 2 results.

The packages used in this experiment have different levels of LPA added, but still allow some amount of light to reach the milk product resulting in Rb loss. The LP control even had a decrease in Rb concentration. According to Fanelli et al. (1985), milk storage with no light exposure decreased by 7% over 72h with the initial degradation occurring within 16h. Even though our light-protected control degraded more than 7% over 72h, both studies indicate even with full protection Rb concentration does decrease in the dark, but not enough to cause significant changes in concentration and sensorial quality. However, it is known that Rb is not the only photosensitive compound reacting to light and these compounds may be contributing to the overall flavor changes in milk (Airado-Rodíguez et al., 2011).

The overall purpose of studying individual packages within retail cases is to understand that spaces within a retail case vary in intensity and how each individual bottle may be receiving different exposure unique to that position. Also this study proves that packages interact differently with fluorescent and LED light due to package characteristics and wavelength spectrum of light. From this study it appears that light intensity did not influence Rb degradation as expected. With fluorescent and LED light having different wavelength spectra, these differences could be influencing how the package interacts with the light more than light

intensity thus affecting the milk's nutritional composition. Packaging needs to be designed to efficiently block excitation wavelengths of Rb and other photosensitive compounds regardless of light source spectra or intensity.

Estimate of volatiles related to related to lipid oxidation. Deterioration in food flavor is often due to lipid oxidation in foods. Malondialdehyde (MDA), a secondary oxidation product of lipids, and other aldehydes are measured by TBARS assay. TBARS is most commonly used for evaluating oxidation in meat products (e.g. Campo et al., 2006) and has been reported as an indication of light-induced oxidation in fluid milk (van Aardt et al., 2005; Matak et al., 2007). Johnson et al. (2015) reported TBARS of 1.3 mg/L to be associated with sensory evaluation differences, but values from Study 1 (0.11 mg/L) were much lower than that.

TBARS was analyzed first by ANOVA to determine any significant main effects, two-way, and three-way interactions. Time (P= <0.0001), package (P= 0.004), and time × light interaction (P= 0.027) were significantly different. Main effects of time and package were expected; 4 and 8h TBARS values were significantly lower than 48h and 72h. The LP control and high packages performed more effectively maintaining low amounts of TBARS (mg/L) values, as shown in Figure 8. The LE control and low package had the highest oxidation level based on increased TBARS value, but the extent of oxidation is still very low (0.17-0.20 mg/L) compared to Johnson et al. (2015). The milk used in this study was HTST processed in a commercial dairy processing plant milk; the milk evaluated by Johnson et al. (2015) was UHT processed in a pilot plant setting with separate processes for raw milk separation, standardization, and using a pilot plant scale tubular heat exchanger for pasteurization. In addition, the milk processed for the Johnson et al. (2015) study did not include Vitamins A and D, as did the commercially processed milk used in our study. The different process, inclusion of added vitamins, and different environments probably contributed to the differences in volatiles.

Two-way ANOVA was used to analyze effects of time and package under each light source on TBARS values. For both fluorescent and LED light, time was a significant effect (P= <0.0001) while package and time × package interaction were not. For fluorescent and LED light, 4 and 8h were different from 48h and 72h as shown in Figure 8. Milk in light-protected control packages showed the least oxidation by 72h in both fluorescent and LED lighting (Figure 6).

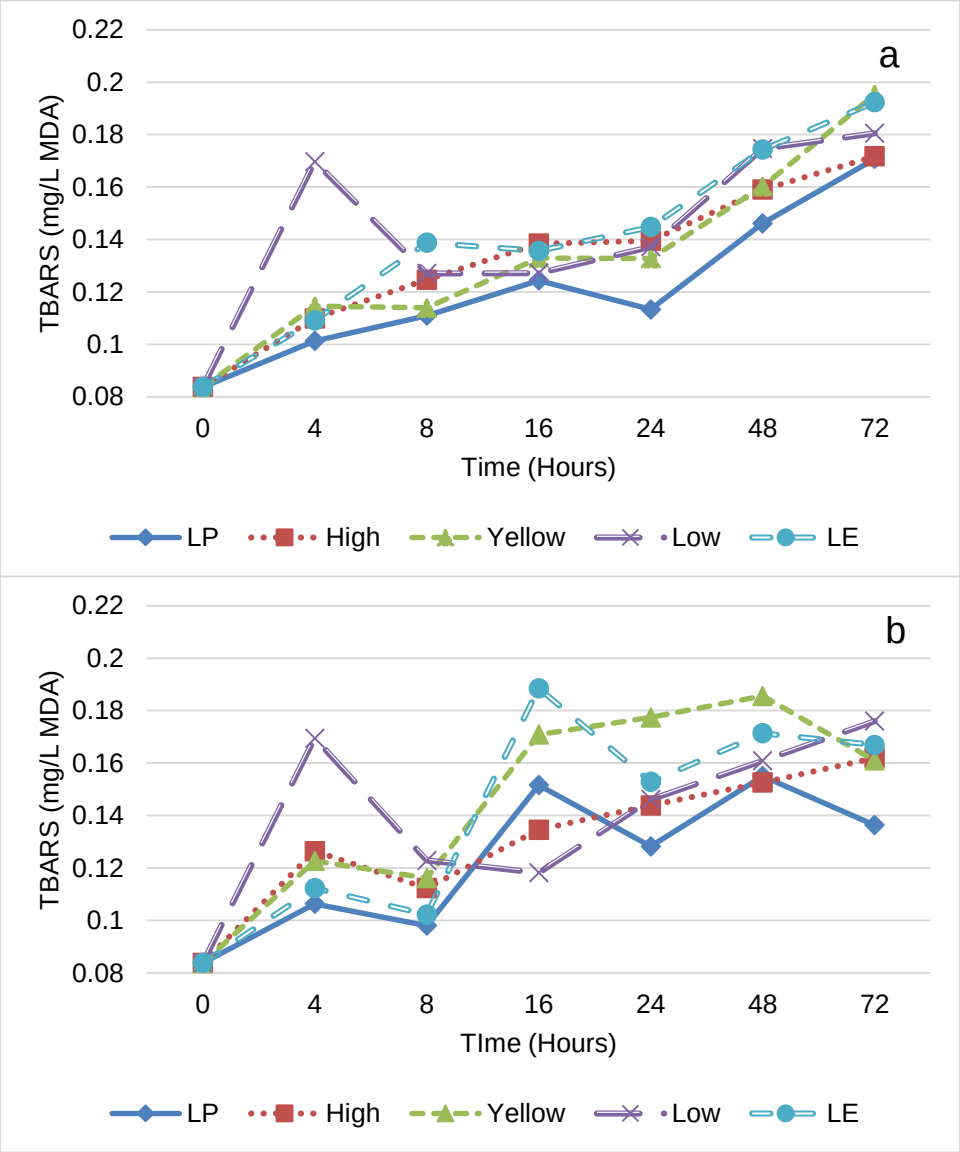

Figure 6. Aldehyde concentration, as measured by thiobarbituric acid reactive substances (TBARS) in individual bottles of 2% milk packaged in high-density polyethylene (HDPE; n=6 packages) with different light protective additive levels (yellow, low (1.3% TiO_2), high (4.9% TiO_2)) compared with controls (light-exposed, LE); light-protected (LP) over 72 h of (a)fluorescent light (1,617 ± 505 lux) and (b) light emitting diode (LED) (929 ± 97 lux) exposure.

TBARS assay estimates low molecular weight aldehydes, based on MDA, produced at the end of autoxidation reactions. However, other volatiles also are produced during photooxidation and can be assessed by different methods. Lipid oxidation is studied predominantly with milk, but protein oxidation due to UV and visible light is also an important contributor to changes in flavor. Jung et al. (1998) studied the effect of sunlight and fluorescent light on cysteine and methionine, common amino acids in milk, in the presence and absence of Rb. Protein oxidation occurred under both sunlight and fluorescent light in the presence of Rb, causing light-induced flavors in the milk. Some proteins with aromatic amino acids such as tryptophan, tyrosine, and phenylalanine can absorb wavelengths below 300 nm (Bartosz, 2014). These amino acids can produce volatile compounds contributing to off-flavor that consumers may be able to detect but TBARS cannot. Volatiles identified by several studies as indicators of oxidation are n-pentanal, n-hexanal, heptanal, acetaldehyde, methyl sulfide, dimethyl disulfide, propanal, nonanal, 3-methyl butanol, 2-metyl propanal, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-nonanone (van Aardt et al., 2001; Mestdagh et al., 2005; Webster et al., 2009; Intawiwat et al., 2010; Webster et al., 2011; Johnson et al., 2015; Wold et al., 2015). Therefore, TBARS may not be sensitive to the total volatiles produced under the conditions of this study. Other testing methods should be used when validating oxidation in milk.

Correlation analysis between TBARS and Rb, based on Pearson's Correlation Coefficients (α=0.05) reported strong correlation between TBARS and Rb results for all treatments; as TBARS values increased, Rb values decreased (-0.718). Light intensity on TBARS and Rb was analyzed based on ANCOVA, with no significant differences in light intensity on Rb (P=0.142) and TBARS (P=0.183) results. The light intensity differences between fluorescent and LED lights did not significantly impact Rb and TBARS results between the two light treatments.

We expected intensity would have a greater impact on milk oxidation based on results from Guneser (2012) and Kropf (1998). When looking at Brothersen et al. (2016), we see that light intensity (lux) did not have much influence. Instead, the type of light impacted milk quality more than light intensity. This conclusion strengthens the argument that fluorescent and LED light create differences in milk quality; not with light intensity alone, but perhaps because of the different light spectrum emitted.

Study 2. Interaction of light and LPA packages in contributing to light-induced off flavors of milk within retail storage conditions. The goal of this experiment one was to determine if the LPA packages provided comparable protection to the ideal light protective package (foil wrapped, no light exposure) and protected better than the natural HDPE (translucent control) package based on sensory evaluation, oxidation analysis (TBARS), and Rb retention. Unlike study 1, this study eliminates the different effects of a single bottle in retail case storage. With commingling 6 bottles, each panelist received the same pooled sample for each treatment under fluorescent (1,882 ± 993 lux) and LED light (915 ± 150 lux).

Protection of sensory quality of milk in LPA packages under simulated retail cases of different lights. Effectiveness of packaging for protecting sensory quality was determined when the sensory outcomes were similar (not significantly different; high statistical power) between the treatment package compared to the complete light-blocking protective package (foil wrapped, no light exposure) and protected better than (were statistically different from) the natural HDPE (translucent control) package. This is represented by p-values for each package comparison at each exposure time (Table 2). It is assumed that LP control provided the most effective light protection and the natural translucent HDPE (LE control) was the most ineffective package treatment.

Differences between the milk packaged in LP control and the milk packaged in natural HDPE (light-exposed control) were obvious at 4h, thus demonstrating that the tested population

was sensitive to the difference. Chapman et al. (2002) reported off-flavors were detectable in milk at 30 minutes of fluorescent light exposure (2,000 lux). Heer et al. (1995) identified the geometric mean of detection threshold for light oxidized milk occurred at 2h 40 min under fluorescent light of 1,100-1,300 lux; however, a high proportion of the tested population in that study were not able to discriminate between samples even after 48h of exposure. Little is known about the effect of LED light on milk flavor. In our study, LP control milk was different in flavor from milk in natural HDPE package (LE control) ($P < 0.001$) at 4h and consistently throughout 72h under both lighting conditions, with the exception of 8h under LED light ($P = 0.288$). The unexpected results at 8h under LED light possibly associated with a disproportionate number of inexperienced/low discriminating participants among the tested population ($P_d = 4\%$; preset 20%). All other LP and LE comparisons had proportion of discriminators 20-47%. Proportion of discriminators of 20% was chosen based on previous studies within our lab group. The sensory work was completed on milk that was commingled from 6 different packages for each treatment over three replications.

Table 2. Summary of statistical significance of sensory testing (triangle test) for 2% milk packages in high-density polyethylene (HDPE) with different light protective additive levels (yellow, low, high) compared with controls (light-exposed, LE); light-protected (LP) over 72 h of fluorescent light (1,882 ± 993 lux) and light-emitting diode (LED) (915 ± 150) exposure[1,2].

	Storage Time					
Fluorescent	4h	8h	16h	24h	48h	72h
Difference P_d= 20%; α=0.05; β=0.30						
LE vs. Yellow	<0.001*	0.610	0.049*	0.033*	<0.001*	0.110
LE vs. Low	0.288	0.687	0.973	0.335	0.184	0.010*
LE vs. High	**0.026***	0.945	0.197	0.003*	0.016*	0.057
LP vs. LE	0.002*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Similarity P_d= 20%; α=0.30; β=0.05						
LP vs. Yellow	0.041*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LP vs. Low	0.064	0.007*	<0.001*	<0.001*	<0.001*	<0.001*
LP vs. High	**0.118**	0.045*	<0.001*	<0.001*	<0.001*	<0.001*
LED	4h	8h	16h	24h	48h	72h
Difference P_d= 20%; α=0.05; β=0.30						
LE vs. Yellow	**0.019***	0.006*	**0.013***	0.308	0.071	0.708
LE vs. Low	0.555	0.472	0.258	0.087	0.635	0.708

LE vs. High	0.064	0.289	0.022*	0.557	0.094	0.608
LP vs. LE	<0.001*	0.288	<0.001*	<0.001*	<0.001*	<0.001*
Similarity P_d= 20%; α=0.30; β=0.05						
LP vs. Yellow	**0.064**	<0.001*	**0.237**	0.012*	0.005*	<0.001*
LP vs. Low	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LP vs. High	0.391	0.010*	<0.001*	<0.001*	0.002*	<0.001*
Controls: Fluorescent vs LED						
LP (F) vs. LP (L)[a]	0.506	0.472	0.615	0.557	0.500	0.554
LE (F) vs. LE (L)	<0.001*	0.046*	0.615	0.008*	0.029*	0.267
Panelist/ test	103-105	101-104	94-99	98-100	100-105	104-108

[1]Dosage of LPA-HDPE packaging [yellow (commercially available), low (1.3% TiO_2), high (4.9% TiO_2)]. Controls: light-protected (HDPE bottle with foil overwrap); light-exposed (HDPE bottle with no LPA; translucent). p_d = proportion of discriminators. * $P < 0.05$.
[a]Fluorescent light (F); LED (L).
[2]Colored font corresponds to package efficacy: green=fully effective package; purple=partially effective; blue=partially ineffective; red=ineffective.

We describe packaging efficacy based on how well the package protected sensory quality against the LP and the LE control package conditions (Appendix F). Treatment packages that protected milk as well as LP control ($P < 0.05$ compared to LE control and $P > 0.05$ compared to LP control) were defined as an effective package condition (ideal condition) in green font (Table 2). The high package under fluorescent light at 4 h and yellow under LED at 4 and 16 h met this criteria.

Packages that did not fully meet the effective packaging criteria, but still demonstrated similarities to LP packages were considered partially effective. Some packages provided partial protection that improved over the natural HDPE package that is traditionally used. Partially-protected conditions were defined as intermediate sensory protection (not different ($P > 0.05$) compared to LE control but evidence of similarity ($P > 0.05$) compared to LP control (purple font; Table 2); the low package under fluorescent and high package under LED at 4 h provided partial protection.

Another intermediate condition is described as a 'partially-ineffective' package. We described LPA packages as partially-ineffective package when LPA packages were significantly

different than LP and LE (LP ($P< 0.05$) and LE control ($P < 0.05$)). This condition was considered less protective than the partially-effective conditions due to significant differences from LP. This protective condition, even though an intermediate protection condition, is not providing the most protection to maintain an ideal flavor of milk. Treatments that were partially-ineffective are represented in blue in Table 2. Yellow and high packages fell into this performance category but differences were observed under fluorescent (more occurrences, especially later in the testing period) compared to LED light.

The worst case scenario is represented by the ineffective package condition (red, Table 2) defined by its inability to protect milk more effectively than the standard translucent (0% LPA) ($P > 0.05$ compared to light-exposed control and $P < 0.05$ compared to light-protected control). The low package performed ineffectively more often than the other LPA packages indicating low light protection. All packages failed under LED light by 24h whereas only the low package illustrated this type of performance (exception at 72h) under fluorescent light.

Differences in milk flavor were noticed as early as 4h under fluorescent and LED lights. At 4h under fluorescent light, the high package protected most effectively and would be a recommended package for these conditions. The low package did not perform like the ideal packaging condition under fluorescent light therefore not protecting milk flavor under fluorescent light for 4h. The yellow package may interfere with Rb excitation but a uniquely different flavor may develop if longer wavelengths are exciting other photosensitive compounds and initiating oxidation in a different manner. Under LED light at 4h, the yellow package performed most effectively and better than the other LPA packages. The yellow package would be a recommended barrier for these conditions. The high package partially protected, but was still not as effective as the yellow package. The low package ineffectively protected milk from LED light. With increasing concentration of TiO_2 between the low (1.3%) and high (4.9%)

packages, the high package performed better than low as expect. But even with an increase in LPA, there is still opportunity for light to change the flavor of milk.

At 8h all LPA packages ineffectively protected milk under fluorescent lighting. With most testing, the high package may contain protective qualities similar to the LP control. The high package had a significance level near $P > 0.05$ ($P = 0.064$) when compared to LP; with only 12% of the true population able to find a difference according to the calculated proportion of discriminators on that data. This was lower than the preset proportion of discriminators (20%) and may indicate the participating population was not discriminating enough for this comparison. The low proportion of discriminators suggests that a larger number of observations, as many as 250, would be needed for providing sufficient power for this comparison. Our preset p_d of 20% was sufficient for most of the comparisons.

Yellow pigments added to packaging can protect milk flavor by blocking wavelengths that excite riboflavin (Duncan and Hannah, 2012). The yellow package in this study blocked wavelengths 300-500 nm more efficiently under LED and fluorescent light than the other treatments (Appendix H). By blocking this spectrum of light, the yellow package could control the initiation of oxidation in the milk for a few hours. However, the longer wavelengths were not sufficiently blocked. This may have allowed other photosensitive compounds that are excited by longer wavelengths, such as tetrapyrroles, to absorb sufficient energy to initiate oxidation (Airado-Rodríguez, 2011). Tetrapyrroles absorb light energy between 400 to 750 nm. The high package had higher concentration of TiO_2 (4.9%), which has been designed to scatter and absorb light. Increasing concentration of TiO_2 (0.6%, 1.3%, and 4.3%) pigments added to HDPE bottle resin were found to have significant protection against oxidation and riboflavin deterioration in UHT-processed milk over 29 days (Johnson et al., 2015). We agree that increased concentration of TiO_2 does provide better protection based on sensory data.

Even though complete block of riboflavin excitation wavelengths was not executed, decrease in Rb exposure may demonstrate the efficacy of the yellow and high package protection through early light exposure (< 4h). The high and yellow packages protected most effectively under fluorescent and LED, respectively. The high package performed well at 4h; thereafter this package may have created a unique flavor profile over the next 48h based on the 'partially ineffective' categorization based on sensory testing. Initial protection is important to reduce initiation of oxidation, as seen in our analytics. However, partially ineffective packaging may be creating flavors that influence consumer acceptability. Ideally, such packaging would need to be designed to provide better protection. Natural and low packages in this study are not recommended as milk packaging as they provide no protection against light. Providing the highest quality milk is the main concern for dairy packaging. LPA packages that are not fully effective would not be recommended for use without further testing.

Vitamin retention of sensory evaluated LPA packaged milk. In this study the concentration of Rb in fresh milk was 1.459 mg/L, falling within the normal range of 1.36-1.75 mg/mL (Dimick, 1982; Zygoura et al., 2004). We reported Rb in % retention to show the loss of Rb over time.

Light intensity for commingled milk was 1,882 ± 993 lux for fluorescent and 915 ± 150 lux for LED. Main effects and interactions were determined using ANOVA. We observed a difference with time (P= <0.0001) and package (P= 0.0003) which is similarly to Study 1 However, no two-way or three-way interactions were significant. As expected, light-protected control retained the most Rb throughout the 72h under both light sources, but still decreased more rapidly than expected. Muñoz et al. (1994) found loss of Rb (20.1 to 23.4%) in packages kept in the dark up to 6 d with changes in Rb within 1d. Even though Rb degraded more rapidly in our study, there is evidence that Rb does decrease concentration without light exposure. The light-exposed milk had the greatest loss of Rb over 72h for both lights (48% decrease), as

expected (Figure 7). The LPA-containing packages (yellow, low, high) under fluorescent light retained Rb at 87-89% up to 8h.

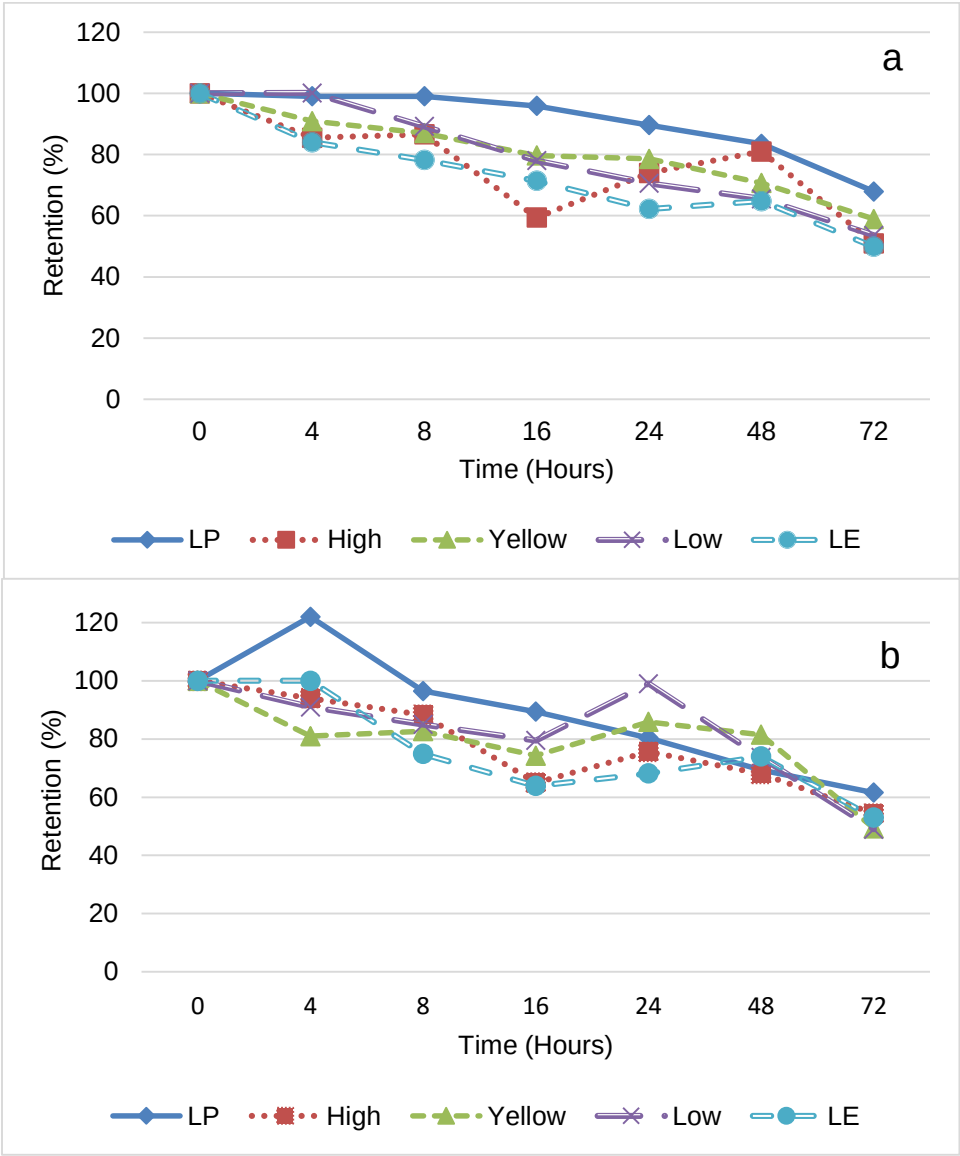

Figure 7. Riboflavin retention (%) in commingled 2% milk packaged in high-density polyethylene (HDPE) with different light protective additive (yellow (Y), low (1.3% TiO_2), high (4.9% TiO_2) compared with controls (light-exposed (LE); light-protected (LP)) over 72 h of (a) fluorescent light (1,882 ± 993 lux) and (b) light emitting diode (LED) (915 ± 150).

Two-way ANOVA and Tukey's HSD was used to analyze Rb under fluorescent and LED light. Under fluorescent light, time (P= <0.0001) and package (P= 0.0017) were significant

based on two-way ANOVA. Time followed an expected pattern with 4, 8, and 16h different than 72h; as time increased, Rb concentration decreased for all packages. For packaging, the LP and high packages retained the most Rb whereas the low and LE control retained the least amount of Rb. For LED light, time was the only main effect that was significance (P= <0.0001). Similar to fluorescent light, 4h and 8h were significantly different than 72h. As time increased the Rb concentration decreased for all packages. Differences were observed in Study 1 for packages under LED light unlike Study 2. Since specific individual samples were tested for Study 1 (based on light intensity of individual bottle), differences may be observed over the pooled, commingled samples. Both Study 1 and 2 have a decrease in Rb concentration for all packages over time.

Sensory data from this study does not relate directly to the Rb observations, but may lead to inferences about other flavor changes occurring from oxidation of other photosensitive compounds noted in previous studies. It has been proven that Rb, even though predominant in milk, is not the only compound in milk that responds to light as a photosensitizer (Wold et al., 2006; Intawiwat et al., 2010; Airado-Rodríguez et al., 2011). Wold et al. (2006) reported photosensitizers in milk, including protoporphyrin IX, tetrapyrroles, hemotoporphyrin, and chlorophyll. As mentioned in Study 1, each photosensitive compound absorbs energy at different wavelengths (Airado-Rodríguez et al., 2011). Blocking only the excitation wavelengths of Rb, does not exclusively maintain milk's fresh flavor. Although Rb retention varied in this experiment, it is known that other compounds may oxidize and change the flavor profiles in milk. These changes could be responsible for differences noticed in the sensory test that were not observed in the Rb analytical test.

Oxidation control in LPA packages of sensory evaluated milk. As mentioned in Study 1, lipid oxidation often causes deterioration in flavor in fat-containing foods. TBARS is used to measure secondary oxidation products of lipids (MDA) and other aldehydes. TBARS value of 1.3 mg/L was reported to be associated with noticeable sensory differences of light-induced UHT milk

processed in a pilot plant setting (Johnson et al., 2015), but results from our study in commercially processed milk were well below that level (Figure 8). No definite threshold has been defined for milk acceptability, but has been estimated for beef (2.28 mg/ L) (Campo et al., 2006).

We observed the main effect of package as the only significant effect (P= 0.0004); there were no other main effects or three-way and two-way interactions. The LP control was different than all other packages for all times and light. Under LED light, package showed significant differences (P= 0.0066). The LP and yellow package were similar and had the lowest TBARS values. Additionally, the yellow package was also similar to the LE and LPA packages. No differences were observed under fluorescent light when evaluated by two-way ANOVA.

We observed a dramatic increase from 0 to 4 h with TBARS values nearly doubling for certain packages under both light sources (Figure 8). This shows that when milk is exposed to light for only 4h in a retail setting there is a sudden increase in oxidation resulting in detectable sensory changes as seen in our sensory study. This could mean that lower levels of MDA (0.16 to 0.18 mg/L) may be detectable by consumers.

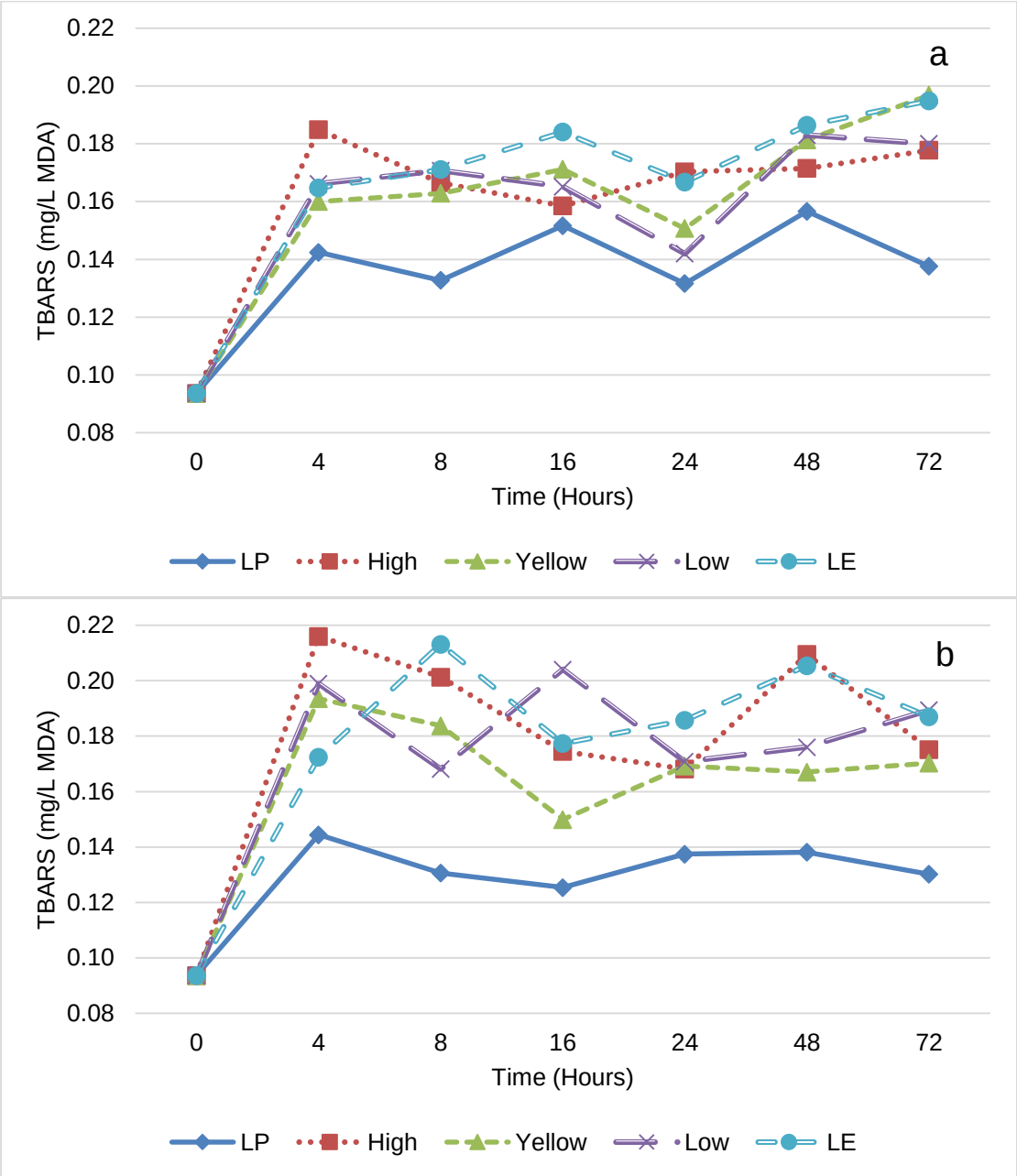

Figure 8. Malondialdehyde concentration in commingled 2% milk packaged in high-density polyethylene (HDPE) with different light protective additive levels (yellow (Y), low (1.3% TiO_2), high (4.9% TiO_2) compared with controls (light-exposed, LE); light-protected (LP) over 72 h of [a]fluorescent light (1,882 ± 993 lux) and [b]light emitting diode (LED) (915 ± 150) exposure.

Oxidation detected by the electronic nose. Fresh milk tends to have a low concentrations of volatile compounds, but increased concentrations of hexanal, pentanal, and dimethyl disulphide can serve as good indicators for light-induced flavors in milk (Marsili, 1999). Utilizing the

ENose to identify these compounds as light-induced off flavors can serve as quick, convenient, and accurate way to validate if a milk product is acceptable or not. The Cyranose® 320 ENose tool was used to verify a simple quality control method for evaluating milk flavor and quality in different levels of HDPE LPA modifications under fluorescent and LED light.

The canonical distribution of volatile changes during milk storage showed 100% separation of volatile compounds under fluorescent and LED light compared to fresh milk (0 h of exposure) (Figure 9). Each circle surrounding data clusters represent the multivariate mean for that group at a 95% significance level. Significant differences are seen with nonintersecting circles. With only 8 hours of exposure, all packages are significantly different ($P < 0.05$) than fresh milk under fluorescent and LED light.

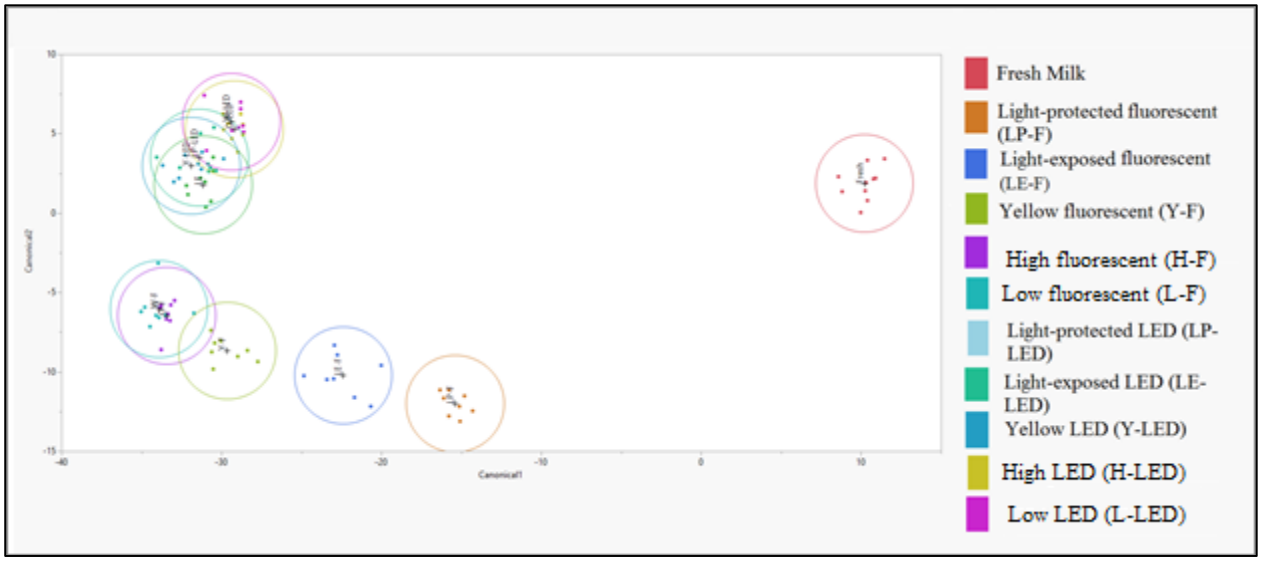

Figure 9. Canonical distribution of differences detected by ENose analysis of individual LPA HDPE bottles (light-exposed (LE), light-protected (LP), yellow, high (4.9% TiO_2), and low (1.3% TiO_2) for 8h under fluorescent (F) and light emitting diode (LED) light and of fresh milk (0h light exposure). Significance differences at α=0.05 level indicated by nonintersecting circles.

Within 8 h of light exposure significant volatile changes occur causing the exposed milk to have different aroma profiles than fresh milk. LE and LP milk under fluorescent light showed 100% separation demonstrating significant differences in aroma profiles in milk. The yellow,

low, and high package demonstrate similarities in volatile chemistry under fluorescent light indicated by the canonical overlap. Under LED light, milk from all packages, including LE and LP, all demonstrated similar volatile compounds and concentrations.

At 24h of fluorescent light exposure, LP and LE milk showed 100% separation from fresh milk, from each other, and from all packaging treatments (Figure 10). The canonical circles are more closely aligned then at 8h of light exposure. This could mean the time is influencing volatile profile across packages, with a higher concentration of similar volatiles. Fluorescent light may have a different impact to LE packages than LPA packages under fluorescent and LED light; LED light may not impact light-exposed milk as much as fluorescent based on nonintersecting circles. These figures give perspective on how fresh and 8h and 24h exposed milk under fluorescent and LED light relate in aroma to each other.

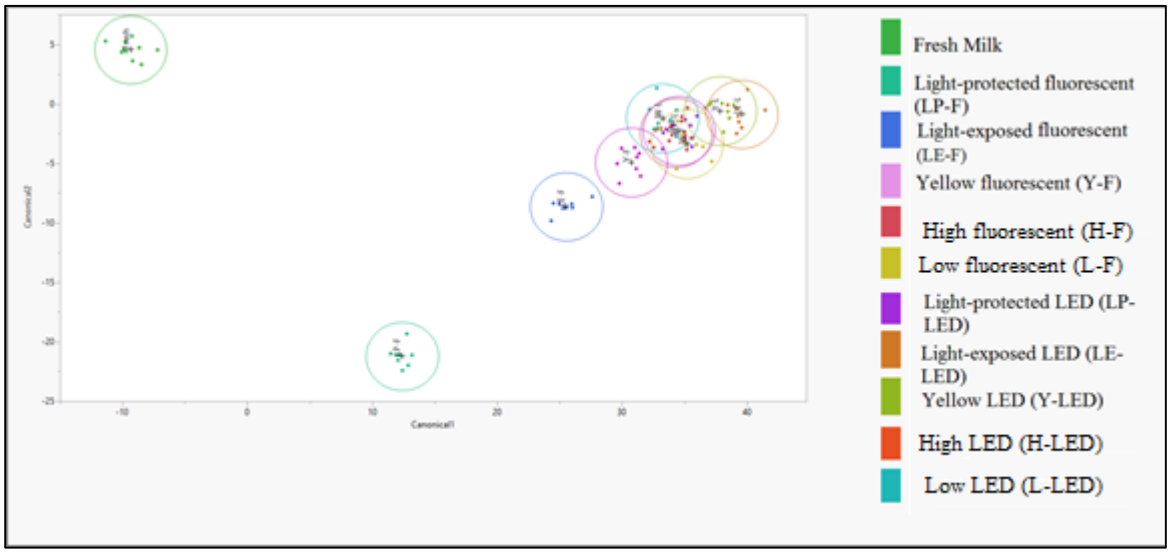

Figure 10. Canonical distribution of differences detected by ENose analysis of individual LPA HDPE bottles (light-exposed (LE), light-protected (LP), yellow (Y), high (H), and low (L)) for 24h under fluorescent (F) and light emitting diode (L) light and of fresh milk (0h light exposure). Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Using sensory tested milk (commingled) and comparing the results to ENose data provides additional interpretation on ENose discrimination. Each LPA package was compared to LP and LE control using discrimination triangle testing (Table 3).

Table 3. Summary of statistical significance of sensory testing (triangle test) for 2% milk packages in high-density polyethylene (HDPE) with different light protective additive levels (yellow, low 1.3% TiO_2, high 4.9% TiO_2) compared with controls (light-exposed, LE); light-protected (LP) at 8 and 24 hours of fluorescent light (2,204 ± 1,154 lux) and light emitting diode (LED) (914 ± 120) exposure[1]

Item		8h	24h
Difference			
LE vs. Yellow	Fluorescent	0.425	<0.001*
LE vs. Low	Fluorescent	0.342	0.144
LE vs. High	Fluorescent	0.992	0.009*
LP vs. LE	Fluorescent	0.044*	0.066
Similarity			
LP vs. Yellow	Fluorescent	<0.001*	0.021*
LP vs. Low	Fluorescent	<0.001*	0.001*
LP vs. High	Fluorescent	0.025*	0.004*
Difference			
LE vs. Yellow	LED	0.342	0.829
LE vs. Low	LED	0.829	0.560
LE vs. High	LED	0.829	0.690
LP vs. LE	LED	<0.001*	0.044*
Similarity			
LP vs. Yellow	LED	0.021*	0.004*
LP vs. Low	LED	0.001*	<0.001*
LP vs. High	LED	0.044*	0.021*
Controls: Fluorescent vs LED			
LP (F) vs. LP (L)[a]		0.903	0.974
LE (F) vs. LE (L)		0.083	0.004*
Panelist/ test		38-40	39-40

[1]Dosage of LPA-HDPE packaging [yellow (commercially available), low (1.3% TiO_2), high (4.9% TiO_2)]. Controls: light-protected (HDPE bottle with foil overwrap); light-exposed (HDPE bottle with no LPA; translucent). p_d = proportion of discriminators. * $P < 0.05$.
[a]Fluorescent light (F); LED (L).

ENose canonical analysis was based on these comparisons to see if ENose discrimination was similar to sensory evaluation. The rationale behind this approach is that using sensory evaluation represents consumers' flavor threshold. If ENose relates to the sensory data, then

ENose may be a sufficient additional method for milk discrimination when sensory tests are unable to be performed. Under 8 h of fluorescent and LED light the yellow, high, and low package were ineffective, having no significant difference to light-exposed control ($P > 0.05$) in sensory evaluation (Table 3). Figure 11 and 12 shows the 3-D canonical distribution of ENose for fluorescent and LED light. ENose showed 100% separation of milk volatile for both lights and controls. The ENose provided information that sensory evaluation may not have been sensitive to detect acute differences. The sensory evaluation data shows that under both lights all packages were similar in protection to LE control, but the ENose shows significant differences between LPA packages and LE and LP control. The ENose data shows the LPA packages different than both controls under both lights possibly saying packages could be partially protecting the milk. The ENose data does not support the sensory evaluation data saying that packages are ineffective (not significantly different ($P < 0.05$) to LE and significantly different to LP ($P > 0.05$). The ENose canonical plots for fluorescent light show the yellow package having a closer position to LE than LP while the high and low package being further away from the light-exposed. For the high and low package the LE and LP controls where closer in distance than expected. It would be expected to have LE and LP milk further apart since literature has shown differences in HTST 2% milk flavor, oxidation, and vitamin concentration at 8 h of exposure (Walsh et al., 2015).

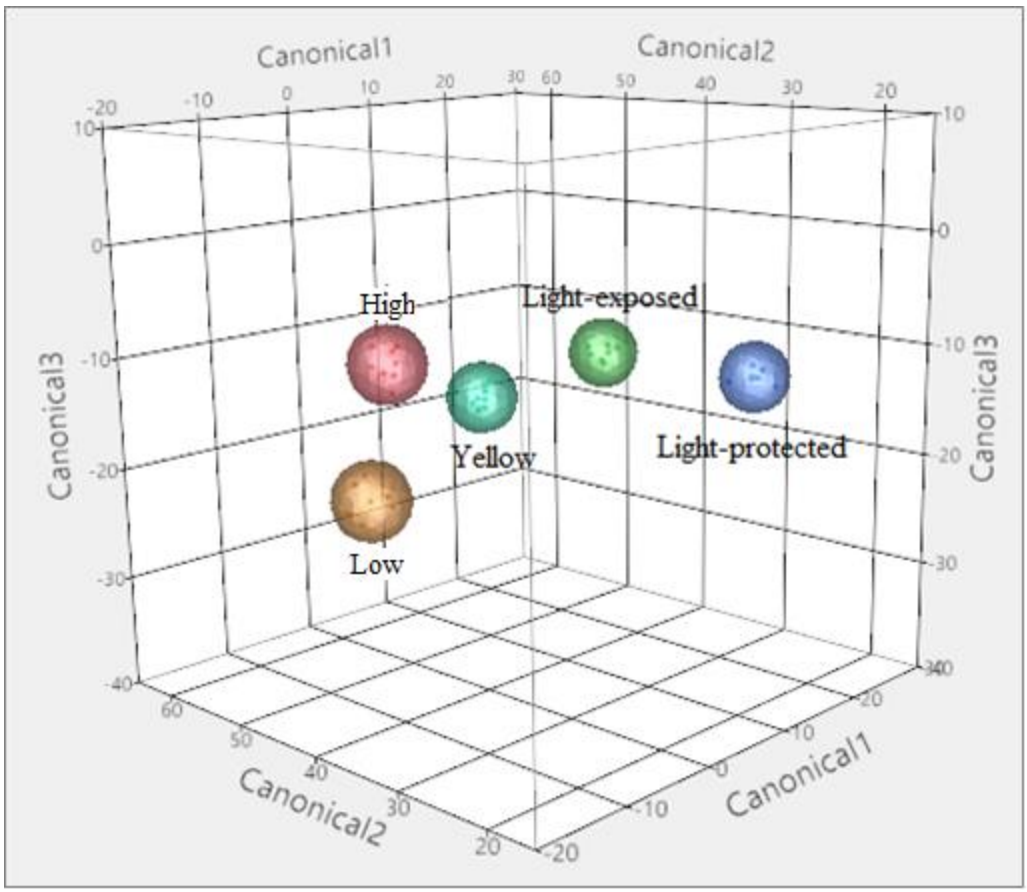

Figure 11. 3-D canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, yellow, high 4.9% TiO_2, and low 1.3% TiO_2 HDPE packages for 8h under fluorescent light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles; no circles were intersecting in this plot.

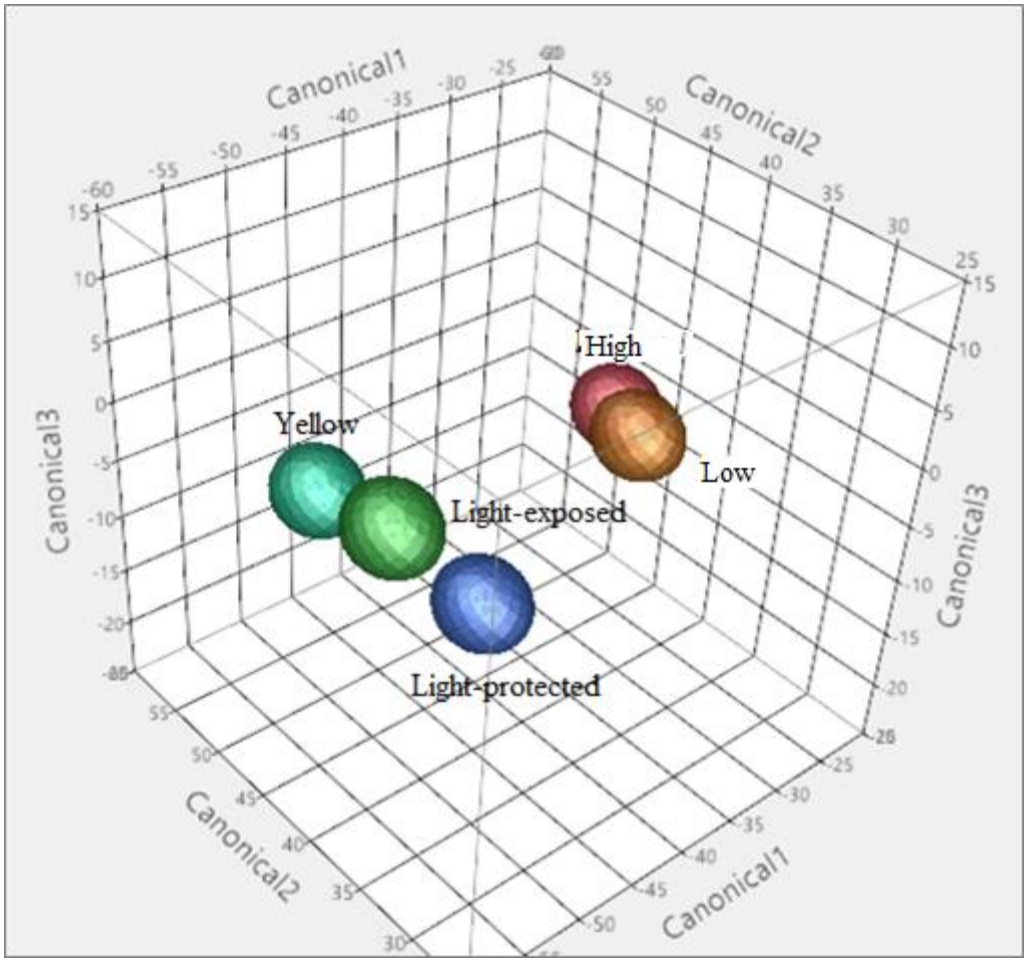

Figure 12. 3-D canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, yellow, high 4.9% TiO_2, and low 1.3% TiO_2 HDPE packages for 8h under LED light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles; no circles were intersecting in this plot.

Based on sensory evaluation, the low package was ineffective while yellow and high were partially ineffective at 24h of light exposure under fluorescent. Under LED light at 24h all packages were ineffectively protecting milk from oxidation. Figure 13 and 14 shows 3-D canonical plots of LPA packages compared to LE and LP controls. Based on sensory evaluation data it would be expected to have all LPA packages overlapping with light-exposed control to represent no significant difference. The ENose shows significant differences between the LPA packages and controls based on a 100% separation.

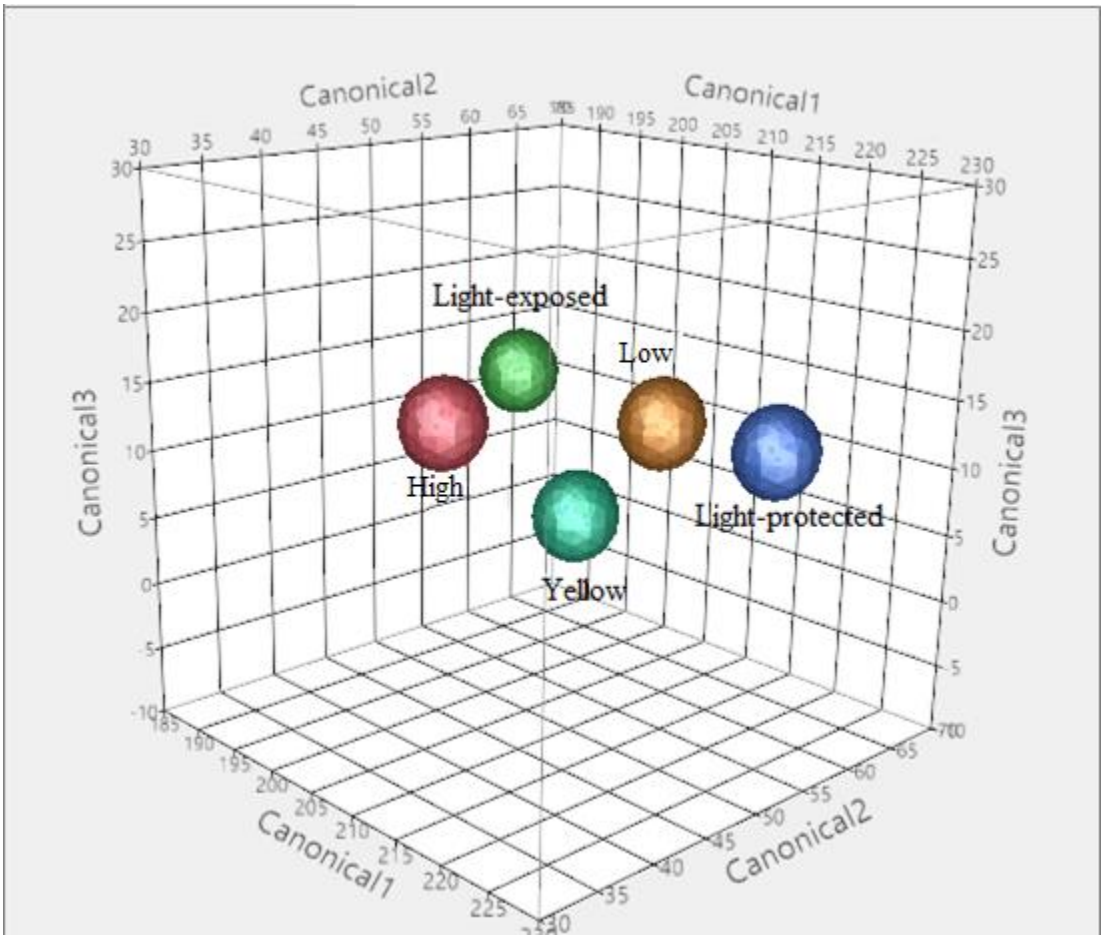

Figure 13. 3-D canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, yellow, high 4.9% TiO_2, and low 1.3% TiO_2 HDPE packages for 24h under fluorescent light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles; no circles were intersecting in this plot.

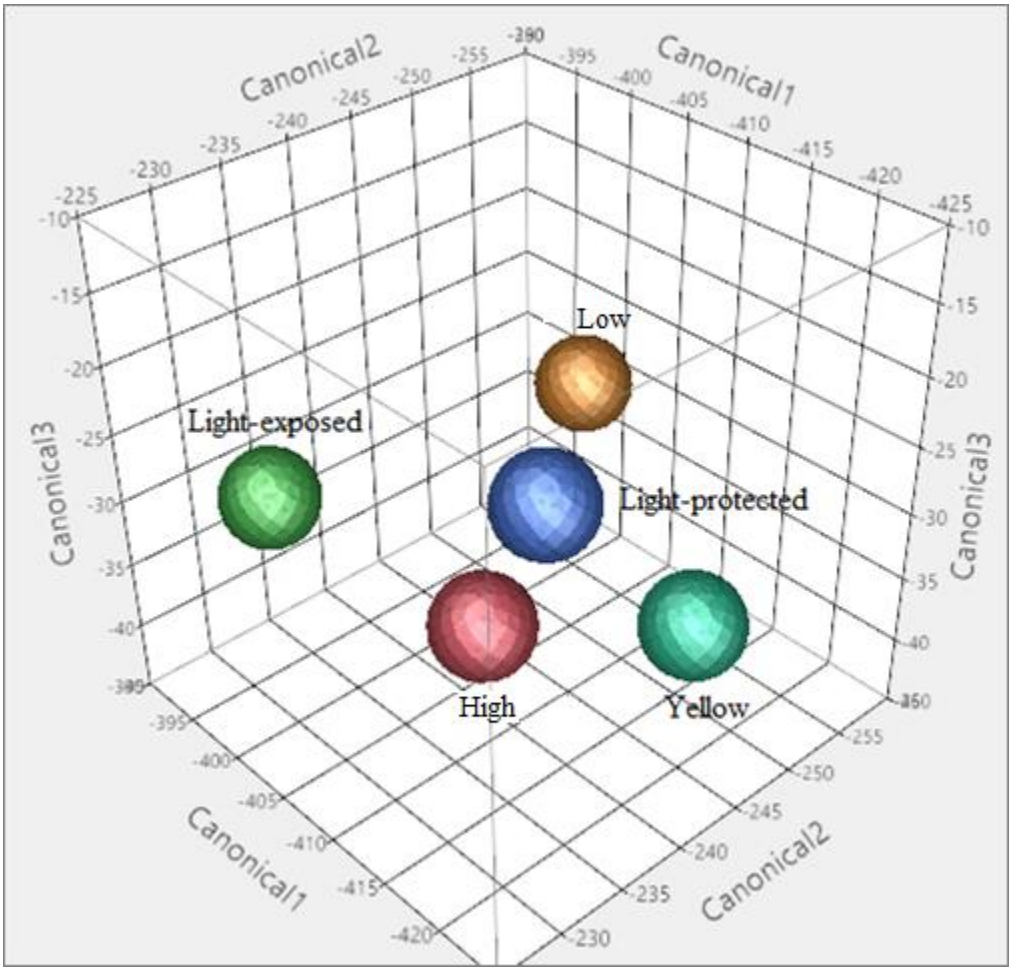

Figure 14. 3-D canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, yellow, high 4.9% TiO_2, and low 1.3% TiO_2 HDPE packages for 24h under LED light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles; no circles were intersecting in this plot.

Based on milk's aqueous composition, nearly 90% water (Park and Haenlein, 2013), certain sensors may need to be deselected to increase discrimination (Cyranose® 320, 2013). Ambient humidity for aqueous based applications may saturate sensors 05, 06, 23, and 31, causing lower discriminating abilities for the Cyranose® 320 (Cyranose® 320, 2013). These sensors are sensitive to polar compounds such as water. Removing these sensors from discriminant analysis may show better discriminating results, but also removes information of polar compounds that could be in the fluid milk.

In addition to canonical discriminant analysis, elastic net analysis was used to identify which sensors in the Cyranose® 320 appeared to be most important for different light exposure. Elastic net is able to find predictors relevant to response variables that random forest and LOSSO are unable to find. With highly correlated variables, those that have a linear relationship, Elastic Net can be used. Elastic Net has two tuning parameters (lambda1 and lambda2) and need to be specified. Tuning parameters, lambda1=0.9 used for all data sets and lambda2 based on the result of 10-fold cross-validation for each dataset, were used in this study. Prominent sensors for volatile detection differed from time and light.

Table 4. Cyranose® 320 sensors of importance for discrimination of 2% milk in LPA packages under fluorescent and LED light for 8h and 24h based on elastic net analysis.

Sensors	8h Fluorescent	8h LED	24h Fluorescent	24h LED
1		X		
2		X		
3		X		
4		X		
5				X
6	X		X	X
7	X	X		X
8				
9		X		X
10	X	X		X
11		X		X
12		X		X
13		X		X
14		X		X
15		X		X
16		X		X
17		X		X
18				X
19	X	X		
20			X	X
21	X	X	X	X
22	X	X	X	X
23	X		X	X
24	X		X	X
25	X		X	
26	X		X	X
27	X	X	X	X
28	X	X	X	X
29	X		X	X
30	X	X	X	X
31			X	
32	X	X	X	X

*highlighted cells indicate important sensors for all time and light treatments

Based on the Elastic Net analysis sensors 21, 22, 27, 28, 30, and 32 are most important for volatile discrimination for all light and time treatments (Table 4). Based on the light and time treatment certain sensors may be more important in the detection of volatiles than other sensors. Based on this analysis, the sensors sensitive to humidity (sensors 5, 6, 23, and 31) may be more

important to keep during analysis due to elastic net output. These sensors are still needed for discrimination and are important to volatile detection.

MANOVA was used to analyze the treatments to each important sensor (21, 22, 27, 28, 30, and 32). We observed significant differences (P= <0.0001) among treatments under the different important sensors. Figure 15 shows the means for each treatment for each sensor. As seen, the fresh milk is significantly different than the other treatments. A positive value would indicate a "good" quality milk similar to fresh milk. Those with negative values do not share similarities with fresh milk. All samples were different than fresh milk.

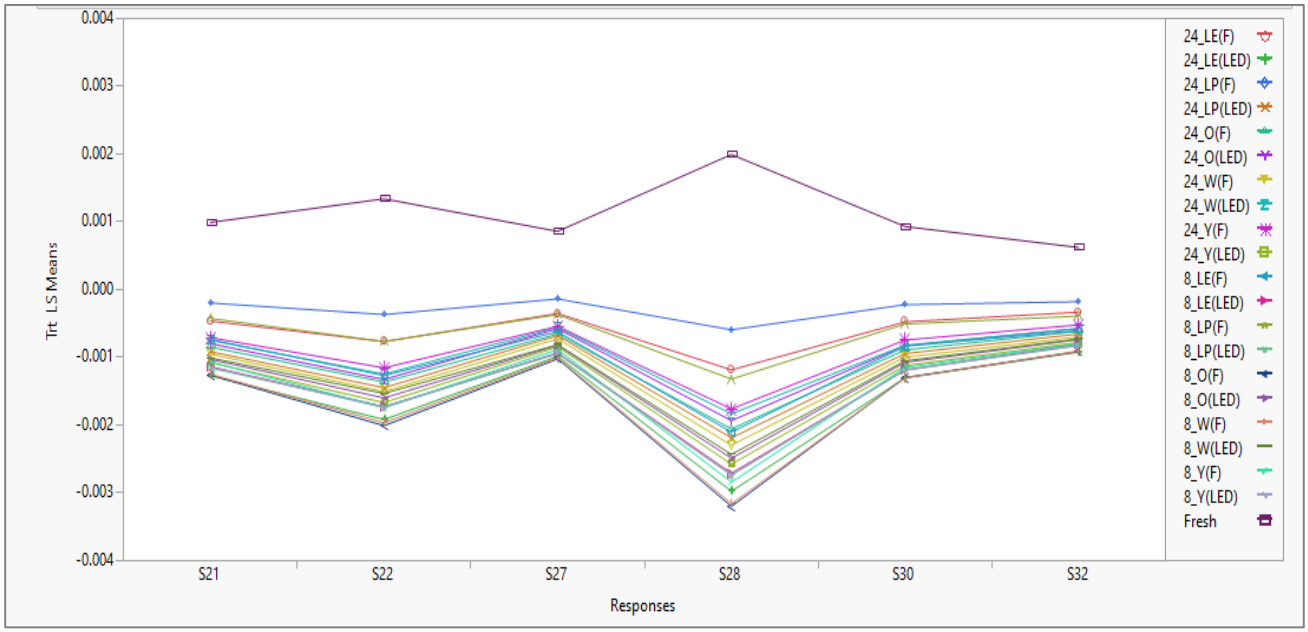

Figure 15. MANOVA means plot for treatments at important sensors for milk quality (21, 22, 27, 28, 30, 32) in HDPE bottles. Fresh milk and LPA packaged milk with different light protective additive levels (yellow (Y), low (W), high (D)) and controls (light-exposed, LE); light-protected (LP), time (8 and 24h), and light (fluorescent (F) and LED) were combined in combinations to form treatment names.

Boxplot of each treatment under each important sensor are seen in Appendix V1-6. Just like the LS mean plot, "good" milk quality was based on fresh milk. Those with similar qualities to fresh would have positive values and those not similar would have negative values.

Appendix W shows the MANOVA plot means without fresh milk to see how volatiles of

milk under the same conditions of time and lighting related. Analysis without fresh milk would be useful when setting another treatment of milk of the standard of acceptable or unacceptable. Because oxidative changes occurred in the light-protected control milk from TBARS and Rb concentration data, fresh milk was decided to be the standard to which milk was either accepted or rejected. As mentioned in Chapter 2, ENose is only capable of identifying what products are acceptable or good. Based on our data we can see the ENose discriminating between fresh and treatment milk. In order for the ENose to discriminate with multiple "good" standards, the ENose would need to have intensive training that was not carried out with this study. The ENose can be used as a tool for milk quality, but time would need to be focused on the training aspect of the ENose and identifying which sensors would identify the volatiles more effectively.

We recognize study limitations that can be improved for later studies. Targeting a similar light intensity range for fluorescent and LED light will help to make direct comparison of packages without introducing light intensity as a variable. This will help identify if the light wavelength spectrum influenced oxidation alone. We also recognize that the schedule of sensory panels may have contributed to 8h sensory results to differ from 4h and 16h. The milk tested for the 8h light exposure was packaged on a separate day, approximately 3-4 days after the 4h and 16h milk was packaged. It was not feasible to package all milk and complete all treatments simultaneously because of the complexity of the project and size of retail case. Additionally, extensive ENose training would be best to establish to utilize the ENose in the most appropriate manner.

CONCLUSION

Our study showed that regardless if HDPE packages with light protective additives in resin, it does not necessarily mean 2% milk is sufficiently protected from light. As retail cases make a transition from fluorescent lights to LED lights, package performance needs to be

considered to protect milk under those unique conditions. Yellow packages, often utilized in limited, specific geographical regions, may provide the desired targeted protection, performing better than the widely used translucent and white packages (TiO_2 loaded) under LED lights up to 4h. The high package, with 4.9% TiO_2, provided targeted protection under fluorescent light up to 4 h. The packages used were not able to provide targeted protection for either light source for 8h or more. Improved packages need to be evaluated using different formulations of LPA to achieve the desired performance for all potential lighting conditions. Rb is the main photosensitizer of concern when studying photooxidation, but other photosensitive molecules need to be considered and studied when deciding the best protection from light for milk. Our study showed differences in oxidation for packages in both experiments and resulted in relatively low values for TBARS. TBARS of 0.16 to 0.18 mg/ L can be detected by consumers and can be used as a reference for sensory quality of milk.

Electronic nose technology has been seen in different studies to detect the differences in food products. In this study the electronic nose was unable to mimic sensory evaluation results. The ENose may be more sensitive than human senses, and could be used as a predictor of light oxidized flavors, but may also be too discriminative of volatiles that otherwise are not detected by consumers. More training and research needs to be conducted with the ENose to make it more applicable to the dairy industry. The dairy industry can use this information to improve packaging under different lights of 2% fat fluid milk in HDPE bottles.

ACKNOWLEDGEMENTS

This project was funded, in part, by Dairy Research Institute (Rosemont, IL), Virginia Agriculture Experiment Station (Blacksburg, VA), Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture (Washington, DC), and by Hillphoenix (Chesterfield, VA).

REFERENCES

Airado-Rodríguez, D., N. Intawiwat, J. Skaret, J. P. Wold. 2011. Effect of naturally occurring tetrapyrroles on photooxidation in cow's milk. J. Agric. Food Chem. 59: 3905-3914.

Alvarez, V. B. 2009. Fluid milk and cream products. Chapter 5. The sensory evaluation of dairy products. 2nd Springer Science and Business Media, New York. Pp. 73-134.

Bartosz, G. 2014. Protein oxidation in foods and its prevention. Pages 120-125 in Food Oxidant and Antioxidants: Chemical, Biological, and Functional Properties. CRC Press. Boca Raton, FL.

Bekbolet, M. 1990. Light effects on food. J Food Prot. 53: 430-440.

Bradley, R. L. 2000. Dairy Products. 1-83 in Official Methods of Analysis of AOAC Intl. Vol. II. 17th ed. William Horwitz W, ed. AOAC Int., Gaithersburg, Md.

Brothersen C., D.J. McMahon, J. Legako, and S. Martini. 2016. Comparison of milk oxidation by exposure to LED and fluorescent light. J. Dairy Sci. 99:1-8.

Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

Chapman, K. 2002. New study validates light blocking efforts: Teens taste light-oxidization in milk and don't like it. Dairy Foods 103:40-42.

Chapman, K. W., L. J. Whited, and K. J. Boor. 2002. Sensory threshold of light-oxidized flavor defects in milk. J. Food Sci. 67:2770-2773.

Choe, E., and D. B. Min. 2007. Chemistry and reactions of reactive oxygen species in foods. Critical review in Food Science and Nutrition. 46:1-23.

Cornell University. 1998. Light-oxidized flavor defect of milk. Dairy Science Fact Sheet. Department of Food Science, Ithaca, NY.

Corrie, A. 2013. Shedding new light on LED lighting's benefits. Plant Engineering. Accessed on May 22, 2015. http://ezproxy.lib.vt.edu:8080/login?url=http://search.proquest.com/docview/1362089973?accountid=14826.

Cyranose® 320. 2013. The Cyranose® 320 eNose® User Manual. Sensigent. Baldwin Park, CA.

Dimick, P. S. 1982. Photochemical effects on flavor and nutrients of fluid milk. Can. Inst. Food Sci. Technol. J. 15:247-256.

Duncan, S. E. and H. H. Chang. 2012. Implications of light energy on food quality and packaging selection. Adv. Food Nutr. Res. 67:25-73.

Duncan, S. E. and S. Hannah. 2012. Light-protective packaging materials for foods and beverages. Chapter 15 in Emerging Food Packaging Technologies: Principles and Practice. K. L. Yam and D. S. Lee, ed. Woodhead Publishing Ltd. Philadelphia, PA.

Duncan, S. E. and J. B. Webster. 2010. Oxidation and protection of milk and dairy products. Chapter 4 in Oxidation in Foods and Beverages and Antioxidant Applications: Management in Different Industry Sectors (Volume 2). E. Decker, R. Elias, and D. J. McClements, ed. Woodhead Publishing Ltd., Philadelphia, PA.

DuPont. 2007. DuPont™ Ti-Pure® titanium dioxide: titanium dioxide for coatings. Wilmington, DE.

Fanelli, A. J., J. V. Burlew and M. K. Gabriel. 1985. Protection of milk package in high density polyethylene against photodegradation by fluorescent light. J. Food Prot. 55:112-117.

Floroui, V. A. 2015. The ABCs of truly energy efficient LED lighting. Accessed on February 23, 2016. https://www.linkedin.com/pulse/abcs-truly-energy-efficient-led-lighting-victor-adrian-floroiu.

Guneser, O., and Y. Karagul. 2012. Effect of ultraviolet light on water- and fat-soluble vitamins in cow and goat milk. J. Dairy Sci. 95: 6230-6241.

Heer, A. K., S. E., Duncan, and D., Brochetti. 1995. Sensory detection of and consumer response to off-flavors in milk. Dairy, Food and Environmental Sanitation. 15:488–493.

Hillphoenix Learning Center. 2014. Enhance merchandising, engage customers: lighting. Accessed Nov. 18, 2015. http://www.hillphoenixlc.com/dashboard/profile/courses/courses_video/3/.

Intawiwat, N., M. K. Petterson, E. O. Rukke, M. A. Meier, A. V. Dahl, J. Skaret, D. Keller and J. P. Wold. 2010. Effect of different colored filters on photooxidation in pasteurized milk. J. Dairy Sci. 93:1372-1382.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Page 511 in Modern Food Microbiology. Springer Sciences. New York, NY.

Johnson, D. S., S.E. Duncan, L. M. Bianchi, H. H.Chang, W. N. Eigel, and S. F. O'Keefe. 2015. Packaging modification for protecting flavor of extended-shelf-life milk from light. J. Dairy Sci. 98: 2205-2214.

Jung, M. Y., S. H. Yoon, H. O. Lee, and D. B. Min. 1998. Singlet oxygen and ascorbic acid effects on dimethyl disulfide and off-flavors in skim milk exposed to light. J. Food. Sci. 63:408-412.

Kropf, D. 1998. Meat Display Lighting. American Meat Science Association Fact Sheet. Accessed February 20, 2016. http://old.pork.org/filelibrary/factsheets/pigfactsheets/newfactsheets/12-05-03g.pdf.

Kyte, J. 1995. Mechanisms in Protein Chemistry. Garland Publishing, Inc., New York, NY.

Marshall, R. 1992. Standard methods for the examination of dairy products. 16th ed. American Public Health Association. Washington, DC.

Marsili, R. T. 1999. Comparison of Solid-Phase Microextraction and Dynamic Headspace Methods of the Gas Chromatographic-Mass Spectrometric Analysis of Light-Induced Lipid Oxidation Products in Milk. J. Chromatographic Sci. 37:17.

Matak, K. E., S. S. Sumner, S. E. Duncan, E. Hovingh, R. W. Worobo, C. R. Hackney, and M. D Pierson. 2007. Effects of ultraviolet irradiation on chemical and sensory properties of goat milk. J. Dairy Sci. 90:3179-3186.

Meilgaard, M. C., G. V. Civille, and B. T. Carr. 2007. Sensory Evaluation Techniques. 4th ed. CRC Press, New York.

Mestdagh, F. F., B. Meulenaer, J. Clippeleer, F. F. Devlieghere, and A. A. Huyghebaert. 2005. Protective influence of several packaging materials on light oxidation of milk. J. Dairy Sci. 88:499-510.

Min, D.B., and J.M Boff. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Rev. Food Sci. Food Safety 1:58-72.

Moysssiadi, T., A. Badeka, E. Kondyli, T. Vakirtzi, I. Savvaidis, and G.K. Kontominas. 2004. Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensorial aspects. Int. Dairy J. 14:429-436.

Muñoz, A., R. Ortiz, and M. A. Murica. 1994. Determination by HPLC of changes in riboflavin levels in milk and nondairy imitation milk during refrigerated storage. Food Chem. 49:203-206.

Park, Y. W., and G. F. W., Haenlein. 2013. Milk and Dairy Products in Human Nutrition. 1st edition. West Sussex, UK: Wiley-Blackwell. 8-11.

Potts, H. L. 2016. Oxidation barrier and light protective packaging properties for controlling light induced oxidation in milk. Master's thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA. Print.

Senyk, G. F., and W. F. Shipe. 1981. Protecting your milk from nutrient losses. Dairy Field, March. 164: 81, 84-85.

Shih, M. 2015. Emerging applications for UV LEDs drive broad interest. LED Magazine. Accessed Febuary 11, 2016. http://www.ledsmagazine.com/articles/print/volume-12/issue-11/features/strategically-speaking/emerging-applications-for-uv-leds-drive-broad-interest.html.

Spanier, A. M. and R. D. Taylor. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscles foods. J. Muscle Foods 2:165-176.

Snedecor, G. W. and W. G. Cochran. 1980. Failures in assumptions. Pages 279-280 in Statistical Methods. 7th ed. Iowa State University Press.

U.S. Department of Energy. 2014a. New Engery Effciency Standards for Commerical Refrigeration Equipment to Cut Businesses' Energy Bills and Carbon Poulltion. Accessed Feb. 20, 2016. http://energy.gov/articles/new-energy-efficiency-standards-commercial-refrigeration-equipment-cut-businesses-energy.

U.S. Department of Energy. 2014b. Solid-State Lighting Research and Development: Multi-Year Program Plan. Accessed Oct. 12, 2014. http://apps1.eere.energy.gov/buildings/publications/pdfs/ssl/ssl_mypp2014_web.pdf

van Aardt, M., S. E. Duncan, J. E. Marcy, T. E. Long, and C. R. Hackney. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. J. Dairy Sci. 84:1341-1347.

van Aardt, M., S. E. Duncan, J. E. Marcy, T. E. Long, S. F. O'Keefe, and S. R. Nielsen-Sims. 2005. Effect of antioxidant (α-tocopherol and ascorbic acid) fortification on light-induced flavor of milk. J. Dairy Sci. 88:872-880.

Webster, J.B., S. E. Duncan, J. E. Marcy, and S. F. O'Keefe. 2009. Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelengths by interference. J. Food Sci. 74:390-398.

Webster, J. B., S. E. Duncan, J. E. Marcy and S. F. O'Keefe. 2011. Effect of narrow wavelength bands of light on the production of volatile and aroma-active compounds in UHT milk. Int. Dairy J. 21:305-311.

Whited, L. J., B. H. Hammond, K. W. Chapman, and K. J. Boor. 2002. Vitamin A degradation and light-oxidized flavor defects in milk. J. Dairy Sci. 85:351–354

Wold, J. P., R. Bro, A. Veberg, F. Lundby, A. N. Nilsen, and J. Moan. 2006. Active photosensitizers in butter detected by fluorescence spectroscopy and multivariate curve resolution. J. Agric. Food Chem. 54:10197-10204.

Wold, J. P., J. Skaret, and T. K. Dalsgaard. 2015. Assessment of the action spectrum for photooxidation in full fat bovine milk. Food Chem. 179:68-75.

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

Walsh, A. M., S. E. Duncan, H. L. Potts, D. L. Gallagher. 2015. Comparing quality and emotional responses as related to acceptability of light-induced oxidation flavor in milk. Food Research Int. 76:293-300.

Zygoura, P., T. Moyssiadi, A. Badeka, E. Kondoyli, I. Savvaidis, and M. G. Kontominas. 2004. Shelf-life of whole pasteurized milk in Greece: Effect of packaging material. Food Chem. 87:1-9.

CHAPTER IV

CONSUMER ACCEPTBILITY OF 2% MILK IN LIGHT PROTECTIVE ADDITIVE PACKAGING

ABSTRACT

Little is known about milk acceptability under retail case condition lighting, especially for light emitting diode (LED) light. It is known that under light, milk develops light-induced off-flavors. The objective of this project was to assess consumer acceptability of 2% HTST milk under fluorescent and LED light for 4h in various LPA packages (Low; 1.3% TiO_2, Yellow, High; 4.9% TiO_2, and translucent HDPE (0% TiO_2) was used for the light-exposed control; a light-protected control). Impact of light was studied by consumer liking of light-exposed controls under fluorescent and LED light. Impact of packaging protectiveness was evaluated by hedonic (9-point) and just-about-right (JARS) sensory evaluation test, riboflavin retention, and thiobarbituric reactive substance (TBARS) for LPA packages and controls under LED light for 4h. Sensory evaluation showed light-exposed package under fluorescent light (953±54 lux) scored lower on overall liking and flavor liking than light-exposed under LED light (976±56 lux). The high package scored highest on all attributes except aftertaste liking. We did not observe significant differences in Rb concentration, but amounts were highest in light-protected, yellow, and high (1.36 mg/L) and lowest in light-exposed fluorescent (1.03 mg/L). This trend is also found for TBARS with light-exposed package under fluorescent light having higher values (0.19 mg/L) than LED light-exposed and LPA packages. Acceptability in milk quality declines with light exposure, regardless of light source, but fluorescent lighting creates a significance decrease in acceptability. Having a higher concentration of TiO_2 may be a recommended package to have higher liking and JAR scores.

Keywords: acceptability, milk, oxidation, packaging

4.1 Introduction

There has been a decline in milk consumption and sales over the last 30 years. Over the last 4 years fluid milk has decreased about 3% of fluid milk product per capita (International Dairy Foods Association, 2015). Whole fat milk has had the biggest decline while reduced fat milk has had a slight increase in consumption, but still not approaching per capita consumption rates reported in the 1980's (International Dairy Foods Association, 2015). This decline may be from competitive alternative dairy options such as coconut, almond, and soy milk. Soymilk is the predominate competitive "milk" product, representing about 80% of alternative milk beverages, and continues to increase in consumption (Food Business News, 2013).

Another reason for declining sales and consumption of bovine milk may be a decrease in consumer acceptability. Fresh milk has a sweet, bland flavor while light-exposed milk has been described to have a burnt-protein and cardboard flavor (Heer et al., 1995; Alvarez, 2009). Light exposure of milk is well known to cause off-flavors and decreased vitamin content in milk (as reviewed in Duncan and Webster, 2010; Duncan and Chang, 2012). Many studies have looked at the effect of fluorescent light on this deterioration because of its use in retail cases. However, light emitting diodes (LED) lights are being used more frequently in retail cases but the full effects of this light have yet to be determined in fluid milk. Brothersen et al. (2016), recently studied the effects of fluorescent and LED lights on 1% HTST milk and found that at lower intensities (2,200 lux), fluorescent light was more detrimental to milk than higher intensity LED light (4,000 lux). The specific reasons are still unknown, but differences in wavelength spectrum may be a contributing factor. Fluorescent and LED light encompass the total visible light spectrum (380-770 nm), but each light has unique characteristics (Chapter 3). Unlike LED light, fluorescent light also has wavelengths within the UV spectrum (100-380 nm) that influence milk flavor and photosensitive nutrients. UV light altered the aroma of whole and skim milk by changing the primary structure of proteins thus causing higher levels of protein carbonyls that is

characteristic of oxidized flavor milk (Scheidegger et al., 2010). Light causes oxidative changes in milk fat, proteins, and vitamins leading to off flavors (Duncan and Webster, 2010). Riboflavin, a predominant vitamin, as well as other photosensitive compounds, in milk will excite in presence of light and cause a chain of oxidation reaction. Both light sources contain the wavelength spectrum to which Rb will react.

Packaging has been a primary tool to combat light induced flavors in milk. Even with the variety of packaging materials and light protective additive (LPA) blends, photooxidation is still a problem for the dairy industry. Natural, unpigmented high density polyethylene (HDPE) and polyethylene terephthalate (PET) packages do not sufficiently protect milk flavor and nutritional quality under retail case conditions, but yet are used often in milk packaging (Johnson et al., 2015). Different light barrier additives have been added to HDPE and PET packages, but still allow a sufficient amount of light to cause changes (Johnson et al., 2015; Potts, 2016). Additionally, oxygen permeability is another characteristic of packaging that needs to be considered. van Aardt et al. (2001) reported PET bottles have significantly lower oxygen transmission level than HDPE bottles. Plastic transmission rates are discussed in Robertson (2006). Oxygen is required for lipid oxidation and high transmission rates of oxygen can cause spontaneous reactions of lipids through a chained reaction of free radicals; which later go on to breakdown to secondary oxidation products that result in flavor changes (Shahidi and Zhong, 2009). The headspace of milk can also allow additional oxygen, but generally not as much of an impact has atmospheric oxygen.

Decrease in milk quality has been studied extensively, but there is still no clear relationship with light-oxidized flavors in milk and consumer acceptability. Trained panelists are used to identify off-flavors in milk and have high sensitivity and discrimination of these flavors as defined by the American Dairy Science Association (ADSA) (Heer et al., 1995). Having trained panelists identify the different off flavors that form from processing, microbiological

growth, light exposure, and oxygen exposure, were intended to be used as an indicator of consumer acceptability, but may be misleading. It has been seen that trained panelists description and intensity of off flavors are not always the same as untrained, consumer panelists (Heer et al., 1995). Heer et al. (1995) studied the acceptability of milk off-flavors in children and college aged adults. Children were more critical of milk flavor, but college students were able to discriminate between samples. College-aged adults ranked light-exposed milk as "neither like nor dislike" in hedonic testing while light-protected milk was "like slightly". This was not significantly different, but may indicate light-protected milk is preferred over light-exposed milk. Chapman (2002) found that 34.5% of teens could detect light oxidized flavor milk within ½ hour of exposure to fluorescent light (2,000 lux). At 3 hours of light exposure about 70% of teens noticed off flavors, nearly a 35% increase from ½ hour of exposure (Chapman, 2002). Teens' acceptability of milk decreases when oxidized flavors are noticeable. Walsh et al. (2015) studied emotional responses to light-exposed and light-protected 2% HTST milk under fluorescent light. The study reported that light-exposed milk had more withdrawal responses (disgust and worried) while light-protected milk had higher responses of approach emotions (happy and content). Trained panelists were reported to detect flavor differences in 2% milk as little as 15 to 30 minutes under fluorescent light (2,000 lux) and within an hour for consumer panels (Chapman et al., 2002). Additionally, Brothersen et al. (2016) studied consumer panels for liking on light-exposed and light-protected 1% HTST milk under fluorescent (2,200 lux) and LED light (4,000 lux) for 12 and 24h. The milk protected from light was significantly liked more (overall liking and flavor liking) than the light-exposed milk. Flavor liking decreased significantly at 12h for light-exposed milk under both light sources, but fluorescent light had a greater and faster decrease in liking than LED light. These studies may be indicating that light-exposed milk is less favorably perceived by consumers than light-protected milk. Many studies have identified that consumers can tell differences in light-exposed milk compared to light-protected milk, but there

has yet to be a define relationship between acceptability and light exposure under retail case lighting conditions.

Having accurate analysis of milk can provide faster results for milk quality than consumer panels. As mentioned trained panelists for descriptive sensory evaluation may not directly relate to consumers' perception of milk quality. Consumer panels are often used to gain insight to a population's perception of a food product, but requires a large number of participants to accurately predict a larger population's liking (Drake, 2007). This requires time and financial investment for a company to gather a representative model of the population. Chemical analysis for milk quality are sought after for quick, accurate information that can be associated with sensory evaluation. Different methods of measuring Rb concentration, oxidative products, dissolved oxygen amount, and volatile chemistry can be used. Johnson et al. (2015) attempted to quantify TBARS amount in milk to relate to differences perceived by sensory evaluation, but our studies prove otherwise.

The objective of this project was to assess consumer acceptability of 2% HTST milk under fluorescent and LED light for 4 hours in various LPA packages. Impact of light was studied by consumer liking of light-exposed controls under fluorescent and LED light. Impact of packaging protectiveness was evaluated by hedonic and just-about-right (JAR) sensory evaluation tests for LPA packages and controls (light-exposed, light-protected, high, low, and yellow) under LED light for 4 hours. Rb concentration, TBARS, dissolved oxygen, and electronic nose technology was used to support sensory evaluation data.

4.2 Materials and Methods

Packaging. Three LPA HDPE bottles were evaluated against three controls. The experimental packages had three different levels of LPA added to the bottle resin: commercially available yellow colorant (Y), low (L) (1.3% TiO_2), and high (H) (4.9% TiO_2) (TiPure®, DuPont™

Titanium Technologies, Delaware). Unpigmented HDPE overwrapped with foil and white plastic under LED light was utilized as light-protected control. Unpigmented HDPE under fluorescent and LED light was utilized as light-exposed control. The four levels of LPA were blow-molded into half-gallon HDPE packages (Consolidated Container Co., Atlanta, GA). Bottle dimensions were 9.875" height × 3.875" and a volume of 1,892 mL.

Milk Processing. HDPE resin and additives (Ampacet, Tarrytown, NY) were blow-molded into half-gallon packages (Consolidated Container Co., Atlanta, GA). Homogenized, high temperature short time (HTST; 77.7 ºC for 15 seconds) pasteurized vitamin A and D added 2% milk was processed at a Kroger Co. dairy processing plant (Westover Dairy, Lynchburg, VA). Milk was bottled and capped on the same processing line and then moved in a refrigerated warehouse. Bottled milk was held in a lighted (100 lux, LED light) refrigerated warehouse for about 8 h before loaded into coolers with ice. Milk was not exposed to light during travel transportation to Virginia Tech Food Science and Technology Department. Upon arrival bottled fresh milk (n = 90) was placed in a dark walk-in cooler until filling process began. Filling process included transferring fresh delivered milk into experimental packages (yellow, low, high) and control packages (light-protected and light-exposed) with a headspace of 40 mL ± 20. Before filling began, transported bottles were wiped with sanitizer (Santi-Bac Quat, St. Paul, MN) around neck and cap to reduce contamination of milk. Fresh milk was clean-filled under positive laminar flow hood (Atmos-Tech Industries, Ocean, NJ) in LPA and control packages to prevent contamination and capped (Portola DBJ/358, Silgan). Filled LPA and control bottles (half gallon) were wiped with sanitizer and transported in coolers to limit light exposure. A total of 15 packages were in each of the six package treatments (n=90).

Storage Conditions. Samples were stored in closed-door refrigerated beverage case to model retail conditions (Model ONRB4, Hillphoenix, Chesterfield, VA). The retail case cooler was glass fronted with four doors (158.4 cm height x 76.2 cm width), five shelves (55.9 cm length),

and with two lights on the sides of each door that ran vertically of the retail door (163.5 cm). A total of eight light bulbs were in the retail case (3,500K); four cool white fluorescent bulbs and four cool LED bulbs. The retail case was divided by a vertical shelf inside the case to separate the retail case into two sections; a fluorescent simulated retail case and a LED simulated retail case. A ceiling high black tarp was used to block light from windows and reduce ambient lighting. This ensured the light projected onto the bottles were only from the retail case. Placement of bottles were decided on preliminary data about retail case light intensity. A targeted 950 ± 100 lux were used to place packages in appropriate spots. Due to constraints of retail case design, 5 replications were needed to expose 90 bottles to light treatments. Replications 1 and 2 were only using the LED retail case with 3 packages for each treatment (yellow, low, high, light-protected control, and light-exposed control) (n=15). Replications 3, 4 and 5 used fluorescent and LED light with 5 light-exposed packages under fluorescent and 3 packages for each treatment (n=15) for LED lights. All replications were completed within 48h. The remaining spaces in the retail case were filled with half gallon water bottles to simulate a full retail case.

Analytical Methods

Microbiological quality. To verify that milk was properly pasteurized and maintained microbial quality over storage period, milk samples were evaluated by standard plate count of aerobic organisms using Petrifilm™ Aerobic Count Plates and Coliform Count Plates (3M, St. Paul, MN). Microbial analysis was based on standard methods (Marshall, 1992). Aerobic count plates were incubated at 32° ±1°C for 48 ± 3h. Coliform Count Plates were incubated at 37°±1°C for 24h. Microbial analysis for aerobic organisms and coliform count to verify quality were taken on day 0 (day of processing) for n = 3 bottles. Microbial testing ensured that fresh milk did not exceed the standard plate count limit for pasteurized milk (20,000 SPC/mL) and the coliform plate count limit (10 CFU/mL) (Jay et al., 2005).

Acceptability of Milk Quality by Sensory Analyses. Sensory analysis was performed using a 9-point hedonic scale for consumer acceptability; scale descriptors were 1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much, 9= like extremely. Just-about-right scale (JARS) was used to give information on flavor and mouthfeel; 1= not nearly enough, 2= not enough, 3= just about right, 4= too much, 5=much too much (Appendix X) (Meilgaard et al., 2007).

Bottles (n= 90) were transported to North Carolina State University (Raleigh, NC) in coolers packed with ice (approximately 1.5°C ± 0.2) from Virginia Tech (Blacksburg, VA; estimated time of travel= 3.5h). Bottles were unloaded from coolers in about 20 min and placed in a dark walk-in cooler in the NC State Food Science Department.

Panelists were prescreened from a pool of about 7,500 participants for milk consumption to ensure panelists were aware of milk quality. Of the participating consumer, 75% drank milk more than 2 times a week; 50% drank 2% milk. Panelists were aged from between 18 to 60 years old (59% were female).

On day of sensory evaluation, fifteen milk bottles (commingled) from each package treatment were tested. Milk was commingled into cleaned and sanitized 5 gal insulated coolers (Igloo Products Corp, Katy, TX). Styrofoam serving cups (6 oz.) were filled with 3 oz. of milk. Sensory testing was conducted in North Carolina State University (Raleigh, NC) sensory evaluation laboratory. Informed consent was obtained from each participant before any sensory testing on each day of testing. A minimum of 150 sensory observations of each treatment was targeted. All treatments (n=6) were presented to panelist in a randomized complete block design with sufficient rest in between samples to avoid fatigue. Panelist were presented with three 3-digit coded samples (10°C ± 2) one at a time with water and crackers on each day of evaluation.

Panelist were in partitioned booths using Compusense® (Guelph, ON, Canada) to record answers. XLSTAT software in Excel was used to analyze data ($P < 0.05$). Liking attributes were analyzed by Fisher's LSD. JARS scale were analyzed using Penalty Analysis.

Degradation of Rb by Fluorometric Analysis. Rb concentration was determined as described in Chapter 3. Three bottles per treatment group (n=15) were tested for each time interval. During frozen storage samples were stored in polypropylene tubes and kept in a dark blast freezer (-20°C). Three samples were tested per package treatment per replication; total of 15 samples tested per package treatment.

Formation of Secondary Oxidation By-Products by Thiobarbituric Acid Reactive Substances Analysis. TBARS was completed as described in Chapter 3. Three bottles per treatment group (n= 15) were tested for each time interval. During frozen storage samples were stored in polypropylene tubes and kept in a dark blast freezer (-20°C). Three samples were tested per package treatment per replication; total of 15 samples tested per package treatment.

Oxygen amount in milk as a result of oxidation. All bottles (n= 90) were tested by a hand held dissolved oxygen meter (LDO101, Hach, Loveland, CO) for dissolved oxygen immediately after each bottle was removed from retail case and opened to reduce atmospheric oxygen from influencing oxygen levels in milk. This analysis provided information on oxygen permeation though packing. The headspace of all packaging were minimized and we do not believe influenced dissolved oxygen readings; they strictly come from the package permeability rate.

Headspace analysis using conducting polymer electronic nose. A conducting polymer electronic nose (Cyranose® 320, Sensigent, Baldwin, CA) was used in this experiment. Settings for the ENose were establish in a preliminary study assessing the draw and purge time of the instrument (Chapter 3,Table 1). Methods and material were the same as Chapter 3.

Volatiles in fresh milk (0h of light exposure) and pkg × light treatments (4h) were tested using the ENose method for every bottle (all replications). For fresh milk, 5 bottles were pulled for duplicate testing. This served as a baseline for what "acceptable" and "good" milk was.

Statistical analyses. Sensory data was analyzed based upon statistical parameters mentioned above and equations outlined in Meilgaard et al. (2007) using XLSTAT on excel.

One way analysis of variance (ANOVA) and student's t-test was used to determine changes in oxidation reported in TBARS, riboflavin concentration, and dissolved oxygen data. Three bottles from each replication were evaluated for TBARS and Rb analysis for all five replications (n=15). Analysis was performed using JMP 10.0.0 Statistical Discovery Software (SAS Institute, Cary, NC). Contrast analysis was performed to test the significance of individual comparisons between treatments using JMP 10.0.0 Statistical Discovery Software (SAS Institute, Cary, NC). Dixon's Q-test was run for extreme observations as mentioned in Snedecor and Cochran (1980). The ENose was analyzed based on multivariate analysis of canonical discrimination analysis from Euclidean distances (CDA) using JMP version 7 software (SAS Institute, Cary, NC).

4.3 Results and Discussions

This project used bottles comparable in size and thickness to half gal HDPE bottles that are commercially used to package fluid milk. The retail case used was a replica of currently used retail cases on market making our study unique to other experiments and representative of milk quality purchased by consumers. The different levels of LPA in this study were 0% (translucent control), 1.3% TiO_2 (loq), 4.9% TiO_2 (high), and yellow (commercially available). Johnson et al. (2015) used packages loaded with up to 4.2% TiO_2. Refrigeration conditions were consistent between replications. Light intensities (lux) were controlled by choosing light intensities between 950 ± 100 lux for fluorescent and LED lights.

Protection of sensory quality. The purpose of this experiment was to determine milk acceptability, as influenced by LPA packages, were most acceptable to consumers based on overall liking and specific attribute liking (appearance, flavor, aroma, mouthfeel, aftertaste, freshness). In addition, the just-about-right (JAR) test was used to give consumer perspective on the milk flavor and mouthfeel/thickness/viscosity. The key objectives of project were 1) to identify if LED lights were any better or worse than fluorescent light comparing light-exposed control and 2) to identify which LPA packages (light-protected control, light-exposed control, yellow, high, and low) protected more effectively under LED light thus having higher acceptability scores.

The minimum number of panelists needed to have sufficient power was 150; 154 responses were attained. The consumer acceptance mean scores are seen in Table 5. It is assumed that light-protected (foil wrapped) control provided the best protection and light-exposed (translucent, 0% TiO_2) control provided no light protection. A score of 7.0 or higher is typically a target for a good product.

Table 5. Summary of statistical significance of sensory testing consumer acceptance scores[1,2] for 2% milk in LPA packages (light-protected (LP), light-exposed (LE), high (4.9% TiO_2), yellow, and low (1.3% TiO_2) at 4h of exposure under fluorescent light (953 ± 54 lux) and light emitting diode (LED) light (976 ± 56 lux).

		LP LED	**High LED**	**Yellow LED**	**Low LED**	**LE LED**	**LE Fluorescent**
Overall Liking		6.5^{a}	6.6^{a}	6.4^{ab}	6.4^{ab}	6.3^{ab}	6.1^{b}
Appearance Liking		7.1^{a}	7.2^{a}	7.1^{a}	7.1^{a}	7.0^{a}	7.0^{a}
Aroma Liking		6.4^{a}	6.4^{a}	6.4^{a}	6.3^{a}	6.3^{a}	6.2^{a}
Flavor Liking		6.5^{ab}	6.6^{a}	6.4^{abc}	6.3^{abc}	6.2^{bc}	6.1^{c}
Mouthfeel/Thickness /Viscosity		6.6^{a}	6.6^{a}	6.6^{a}	6.6^{a}	6.4^{a}	6.4^{a}
Aftertaste Liking		6.1^{a}	5.9^{ab}	5.8^{abc}	5.8^{abc}	5.6^{bc}	5.4^{c}
Aftertaste Intensity		2.3^{b}	2.5^{ab}	2.5^{ab}	2.6^{a}	2.5^{ab}	2.7^{a}
Freshness Perception		6.6^{ab}	6.7^{a}	6.5^{ab}	6.4^{ab}	6.4^{ab}	6.3^{b}
Flavor JAR	Not enough	22.1%^{a}	14.3%^{a}	21.4%^{a}	17.5%^{a}	14.9%^{a}	11.7%^{a}
	JAR	64.3%^{a}	68.2%^{a}	59.1%^{a}	61.0%^{a}	61.0%^{a}	55.8%^{a}

Mouthfeel/Thickness /Viscosity JAR	Too much	13.6%[b]	17.5%[ab]	19.5%[ab]	21.4%[ab]	24.0%[ab]	32.5%[a]
	Not thick enough	16.9%[a]	11.0%[a]	13.0%[a]	11.7%[a]	18.8%[a]	12.3%[a]
	JAR	76.0%[a]	77.3%[a]	77.9%[a]	73.4%[a]	69.5%[a]	75.3%[a]
	Too thick	7.0%[a]	11.7%[a]	9.1%[a]	14.9%[a]	11.7%[a]	12.3%[a]

[1] Liking attributes were scored on a 9-point hedonic scale where dislike extremely =1 and like extremely =9. Liking attributes were analyzed by Fisher's LSD using XLSTAT ($p<0.05$)
Different letters in rows following means signify significant differences ($p<0.05$)
[2] JAR scales were scored on a 5-point scale where too little =1 or 2, just about right =3, and too much=4 or 5. The percentage of consumers that selected these options is presented.
JAR questions were analyzed using the Penalty Analysis function in XLSTAT.

For overall liking, the most highly rated milk was packaged in the LP control and the high package exposed to LED light for 4 h, with acceptability scores of 6.5-6.6 ('like slightly/like moderately'). These packages protected milk more effectively and with a significantly higher acceptability score than the LE milk in translucent HDPE, the commonly used milk package, under fluorescent light ("like slightly"; 6.1). Flavor acceptability for the milk in the high package was significantly higher than for the LE milk in both LED and fluorescent light conditions. The LP control and the high package had higher aftertaste scores than the fluorescent LE milk in the standard translucent package. Milk in the high package had the freshest perception, significantly higher ($P< 0.05$) than LE milk under fluorescent light.

JARS was used to measure the appropriate level of mouthfeel and flavor. The mouthfeel was scored < 70% just-about-right for all packages and seeing no differences ($P > 0.05$) for mean drop for mouthfeel JARS (Table 6). However, flavor showed differences in the percentage of responses (Table 5). The LE package under fluorescent light was scored highest (32.5%) for "too much" flavor. This is also seen in the mean drop for JAR table (Table 6). This shows that the "too strong" flavor impact on the light-exposed packaging under fluorescent light impacted liking by 2.0 points. The LE package under LED light showed a similar evaluation with the mean drop of JARS for flavor being 2.5 (much too high). The LP package was ranked highest for "not

enough" flavor (22.1%). The high package was the highest ranked package for "just about right" and did not show any significant mean drop for the JARS flavor question.

Table 6. Mean drops for JAR questions for 2% milk exposed to fluorescent (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux) light.[1]

		Flavor JAR		Mouthfeel JAR	
		Not enough	Too much	Too thin	Too thick
Light-protected	LED	1.3	2.3	1.4	1.5
High (4.9% TiO_2)	LED	1.4	2.3	0.9	2.2
Yellow	LED	1.0	1.8	0.4	0.4
Low (1.3% TiO_2)	LED	1.7	1.8	0.4	1.0
Light-exposed	LED	1.6	2.5	1.3	1.3
Light-exposed	Fluorescent	1.8	2.0	0.7	1.2

[1]Highlighted cells indicate significant penalties to overall liking ($p<0.05$)

The consumer study shows that LE milk under fluorescent has the lowest overall liking and flavor liking among frequent consumers of milk. Based on Table 5, there is no significant differences among attributes tested between LP control and the high package. The LP control and the high package showed the same consumer liking. Results from the flavor JARS show consumers found the high package flavor as "just about right" indicating the flavor as the perceived ideal. The high package was most accepted by consumers, and would be the package to consider for milk packaging. Milk exposed to fluorescent light was scored lower than milk under LED light even though it was not significantly different. This conclusion concurs with Brothersen et al. (2016) study that fluorescent LE milk is less acceptable than milk under LED light.

Degradation of riboflavin (Rb). It is well known that when riboflavin is exposed to light, degradation will occur (Johnson et al., 2015; Walsh et al., 2015). Rb concentration was analyzed to see if differences occurred between LE controls under fluorescent and LED light. Rb concentration was also observed between all LED packages.

Light-exposed controls were analyzed by a two-sample t-test, comparing light-exposed packages from fluorescent and LED light exposure. Significant differences were seen in Rb amounts ($P < 0.001$) between treatments. Figure 16 shows the boxplot of light-exposed treatments and Table 7 shows average for riboflavin amount. Rb results for the fluorescent LE milk had significantly lower amounts of Rb than the LED LE packages. Riboflavin degradation decreased as oxidation increased from correlation analysis. Consumer liking decreased from "like moderately" (7.1) to "dislike slightly" (3.5) as Rb concentration decreased under fluorescent light for 168h (Walsh et al., 2015). With the significantly lower Rb amounts and low liking for fluorescent LE milk, Rb amount seems to be a good indication of consumer liking.

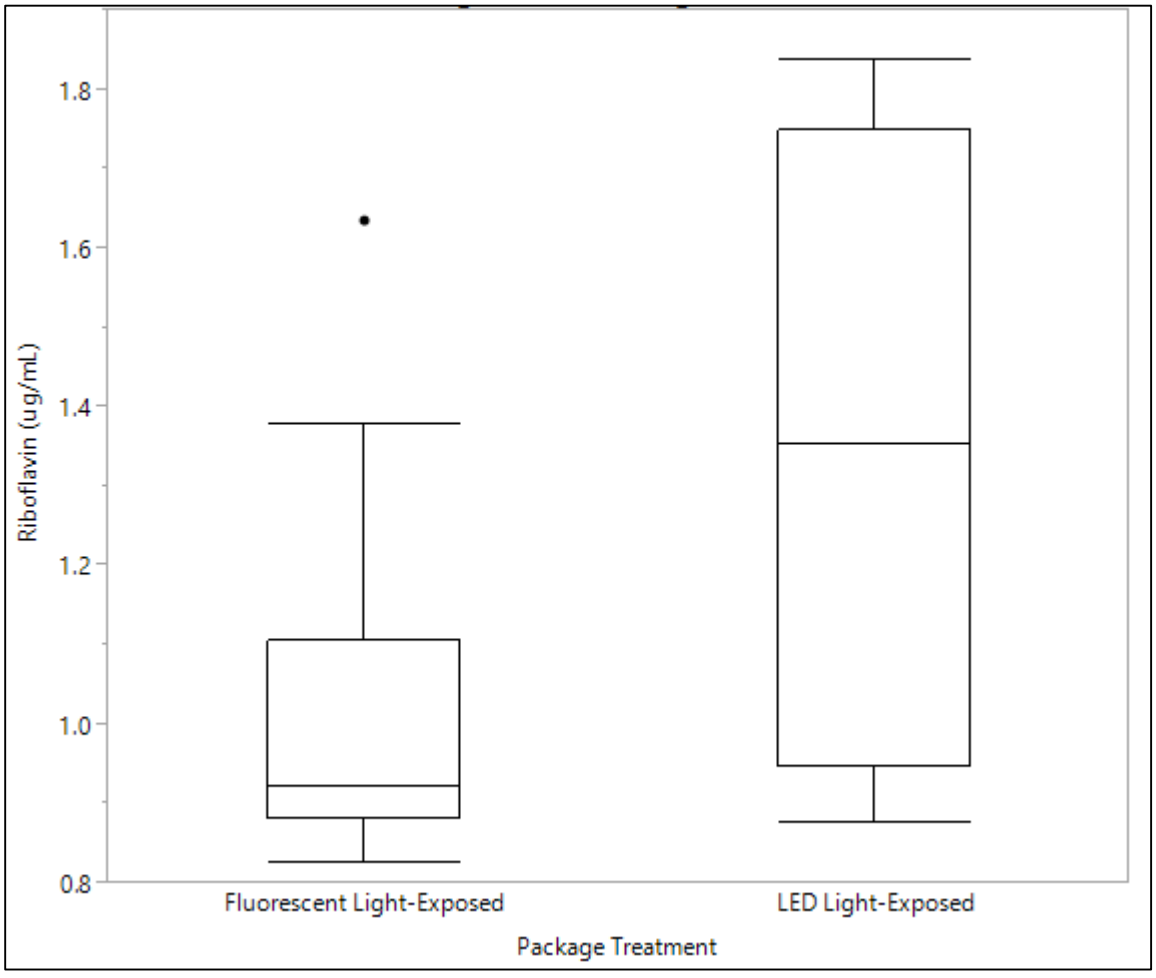

Figure 16. Boxplot of riboflavin amount in 2% milk in light-exposed packages under fluorescent light (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux) for 4h. Two-sample t-test was used to analyze this data; significance indicated by $P < 0.05$.

Table 7. Riboflavin mean and standard deviation for package treatments and light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4h of light exposure.[1]

Package Treatment	Light Treatment	Riboflavin (mg/L)	
		Mean	± sd
Light-protected	LED	1.36[a]	0.39
High (4.9% TiO_2)	LED	1.36 [a]	0.46
Yellow	LED	1.36 [a]	0.57
Low (1.3% TiO_2)	LED	1.31 [a]	0.52
Light-exposed	LED	1.33 [a]	0.47
Light-exposed	Fluorescent	1.04 [a]	0.24

[1] Different letters in rows following means signify significant differences ($p<0.05$).

LED packages were compared with a one-way ANOVA test to detect differences in Rb amount. No significant differences were seen between packages under LED light ($P = 0.799$). At 4h Rb concentration within LED light may not be as affected by the light, but with longer time exposure differences may be seen (as seen in Chapter 3).

Oxidation of milk in packages. Deterioration in food flavor is often times due to lipid oxidation in fat containing foods such as meats and dairy products. Malondialdehyde (MDA), a secondary oxidation product of lipids, and aldehydes are measured by TBARS and has been used as an indicator of off flavors in milk products (Johnson et al., 2015). TBARS value of 1.3 mg/L was reported to be associated with noticeable sensory differences of light induced milk (Johnson et al., 2015), but results from our study are much lower than these reported values. No definite threshold for TBARS has been defined for milk acceptability.

Light-exposed controls were not significant different ($P = 0.114$) in TBARS values for exposure under fluorescent and LED light when analyzed by a two-sample t-test (Figure 17). A one-way ANOVA reported no significant differences (P=0.334) of TBARS between LED package treatments (LP, LE, yellow, low, and high) and the fluorescent LE package. No significant differences (P= 0.089) between TBARS of LE and LP under LED light were found. The values of TBARS are lower than what has been previously reported by Johnson et al. (2015)

(UHT processed milk) and Walsh et al. (2015) (HTST processed milk), but is not surprising based on Chapter 3 results (Table 8).

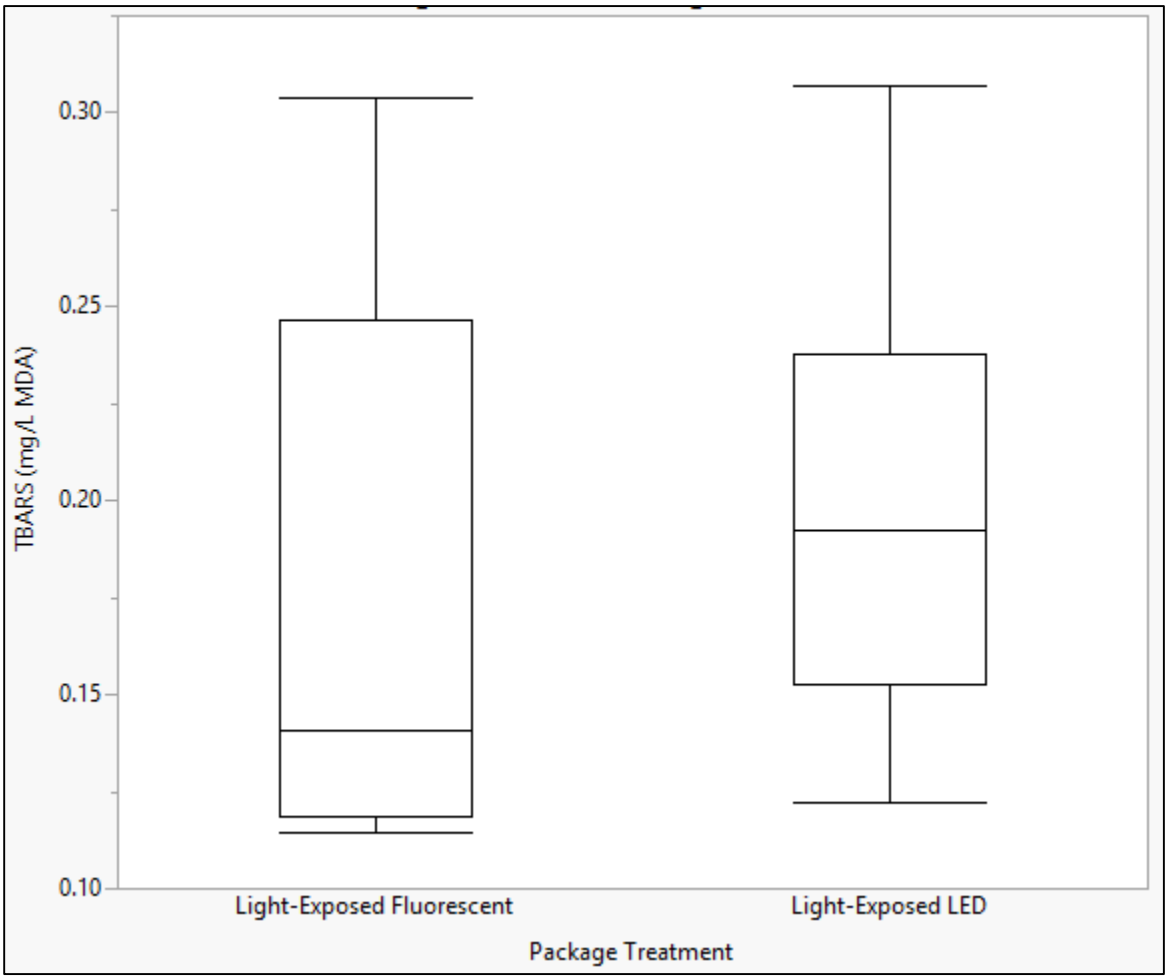

Figure 17. Boxplot of TBARS (mg/ L MDA) in 2% milk in light-exposed packages under fluorescent light (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux) for 4h. Two-sample t-test was used to analyze this data; significance indicated by $P < 0.05$.

Table 8. TBARS (mg/L MDA) mean and standard deviation for package treatments and light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4h of light exposure.[1]

Package Treatment	**Light Treatment**	**TBARS (mg/L MDA)**	
		Mean	± sd
Light-protected	LED	0.16 [a]	0.04
High	LED	0.18 [a]	0.05
Yellow	LED	0.16 [a]	0.04
Low	LED	0.18 [a]	0.03
Light-exposed	LED	0.17 [a]	0.05
Light-exposed	Fluorescent	0.19 [a]	.07

[1] Different letters in rows following means signify significant differences ($p<0.05$).

Dissolved oxidation in packages. Packages were tested for dissolved oxygen immediately after bottles were pulled from the retail case. Dissolved oxygen can serve as an indication of oxidation in milk by measuring the amount of oxygen in the milk. Lipid oxidation involves oxygen through autoxidation and photooxidation as described in Shahidi and Zhong (2009). During autoxidation, light can initiate unsaturated lipids to produce free radicals that react with oxygen to form peroxyl radicals. These radicals later form lipid hydroperoxides that breakdown to oxidation products such as aldehydes and ketones. Photooxidation involves an excited state of oxygen (singlet) that reacts with the double bonds of unsaturated fatty acids. Photosensitizers such as Rb, absorb visible and UV light that cause an excited state that is highly reactive to lipid substrates or triplet oxygen. Oxygen is involved in the two types of lipid oxidation. As oxidation occurs, oxygen will be used to prompt reactions thus changing the amount of oxygen in the milk. Dissolved oxygen can be used as a predictor for oxidation; as dissolved oxygen decreases possible oxidation would have increased.

The fresh (0h exposed) milk had 11.89 ± 0.20 ppm of dissolved oxygen. One-way ANOVA was used to analyze all LPA packages (under fluorescent and LED light) and did not include fresh milk. We observed a difference (P= 0.0001) between packages for dissolved oxygen. The LP control maintained the highest amount of oxygen (11.29 ppm) and LE had the lowest amount of oxygen (10.41 ppm). Table 9 show the mean and standard deviation of dissolved oxygen in packages with the rank analyzed by Tukey's HSD. We observed differences with the LP control under LED light to the rest of the packages (yellow, high, low, LE under LED light, and LE under fluorescent light). The LE packages were different under the different lights representing significant changes in dissolved oxygen amount influenced by different light source.

Table 9. Dissolved oxygen (ppm) mean and standard deviation package treatments and light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4h of light exposure. [1]

Package Treatment	Light	Dissolved Oxygen (ppm)	
		Mean	± s.d.
Light-protected	LED	11.29a	0.15
High (4.9% TiO_2)	LED	10.88b	0.39
Yellow	LED	10.99b	0.26
Low (1.3% TiO_2)	LED	10.86b	0.28
Light-exposed	LED	10.78b	0.21
Light-exposed	Fluorescent	10.41c	0.23

[1] Different letters in rows following means signify significant differences ($p<0.05$).

The LE packages under fluorescent and LED light support the sensory evaluation data; fluorescent had the least amount of dissolved oxygen in the milk indicating highest oxidation reactions that decreased acceptability of the milk product the most. Dissolved oxygen served as a more accurate predictor to sensory evaluation than Rb concentration and TBARS analysis as seen in this study as well as Potts (2016) study.

Electronic nose volatile discrimination of milk in LPA packages. The electronic nose (ENose) is used for a fast, cost efficient way to analyze the quality of milk by discriminating volatiles. Trained panels and gas chromatography-mass spectroscopy (GC-MS) can be used, but require time and money that many companies are not willing to provide. The ENose was used in this study to provide more information to the changes milk undergo in different light sources and different packages.

Figure 18 shows canonical distribution of treatments with fresh milk (0 h of exposure). The circles show the mean of the 32 sensors used to identify milk samples. Unlike Chapter 3, these canonical plots show many data points outside the circles that may cause variation and pull to the canonical circles. According to Figure 20, fresh milk has similar volatile compounds compared to light-exposed packages under fluorescent and LED light. It is unexpected to see the volatile profile of light-protected milk at 4h different than fresh milk (non-intersecting circles)

while the light- exposed are similar.

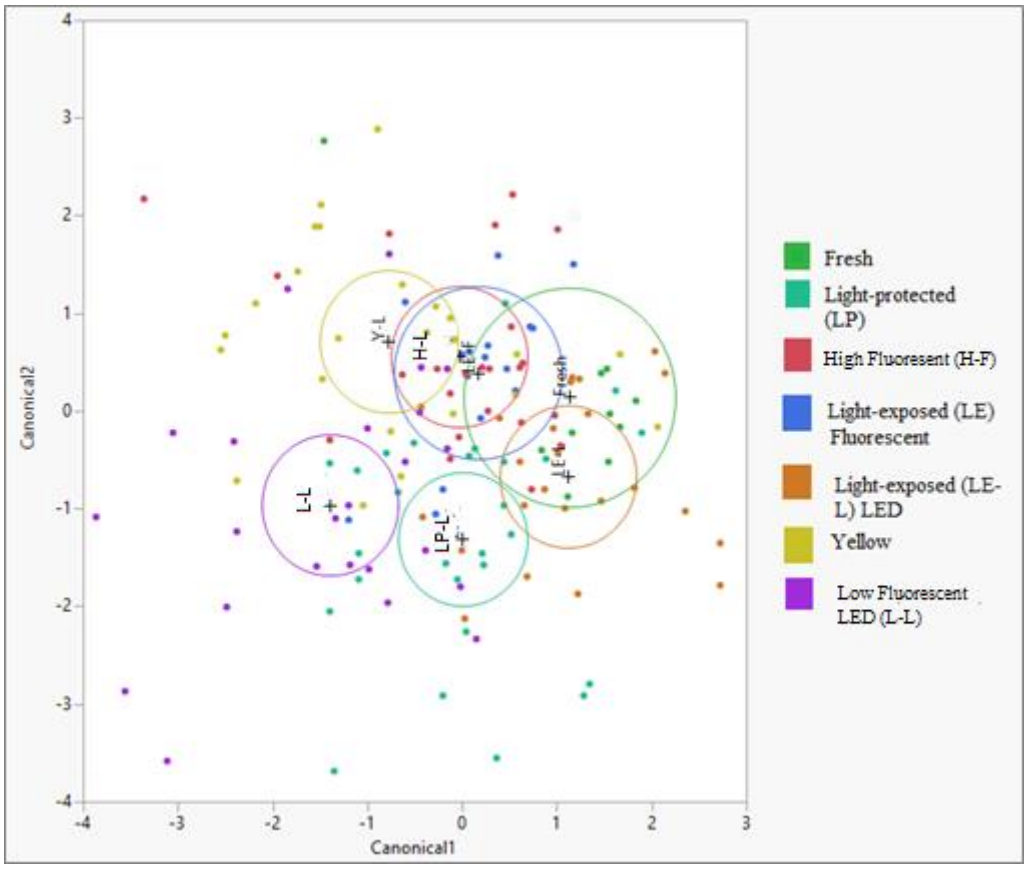

Figure 18. Canonical distribution of differences detected by ENose analysis of LPA HDPE bottles (light-exposed (LE) (fluorescent and LED), light-protected (LP), yellow (Y), high (H), and low (L))) for 4h under fluorescent (F) and light emitting diode (LED) light and of fresh milk (0 light exposure). Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Figure 19 and 20 both show the differences of LPA packages without fresh milk. These canonical plots do not relate to sensory or the dissolved oxygen analysis as previously discussed.

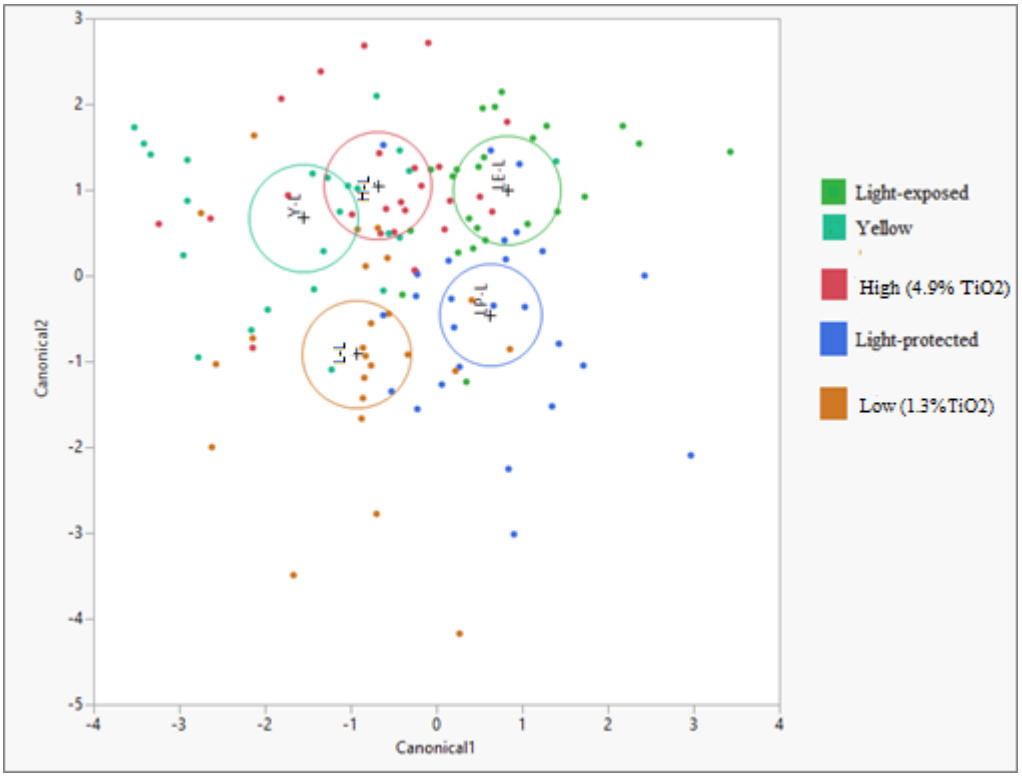

Figure 19. Canonical distribution of differences detected by ENose analysis of LPA HDPE bottles (light-exposed (LE), light-protected (LP), yellow (Y), High (H), and Low (L)) for 4h under light emitting diode (LED) light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

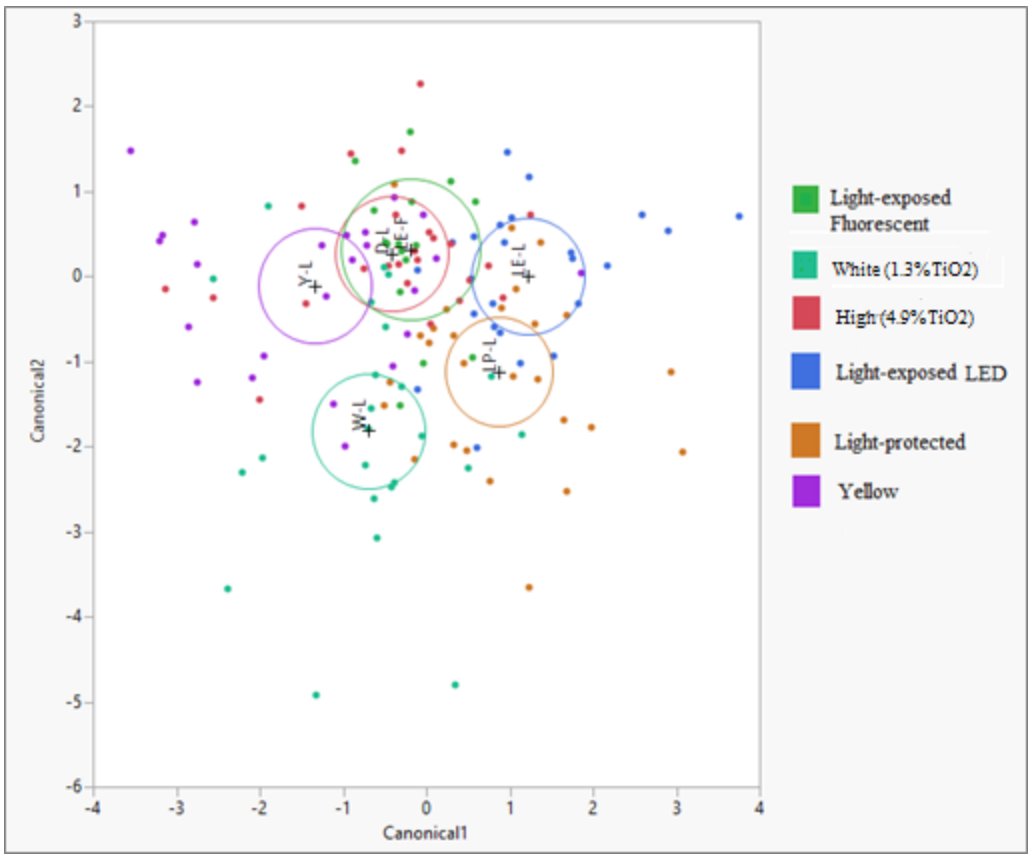

Figure 20. Canonical distribution of differences detected by ENose analysis of LPA HDPE bottles (light-exposed (LE) (fluorescent and LED), light-protected (LP), yellow (Y), high (H), and low (L)) for 4h under fluorescent (F) and light emitting diode (LED) light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

In order to utilize the ENose as a reliable source of discrimination sensitivity and accuracy, intense training needs to be done. The ENose cannot identify a blind sample as a specific kind of milk; as in light-exposed under 4h. The ENose can only deem "good/ acceptable" or bad/unacceptable" once the ENose has been trained properly; trained products can be for the ideal quality or the unacceptable product.

CONCLUSION

This study determined the acceptability of milk exposure to LED and fluorescent light at 4h. Consumers scored the fluorescent light-exposed milk lower in all attributes, but was not seen as significantly different to light- under LED. However, fluorescent light-exposed milk was significantly different than the light-protected control and high package under LED light. The

high and light-protected receive higher scores under LED light for all attributes except for aftertaste liking. The type of light may not have been affecting the milk flavor enough to see significance among the light-exposed packages, but it is apparent that translucent, unpigmented exposed packages were the least acceptable among all package types. The high package (4.9% TiO_2) would be the best package for 4h of LED light exposure out of the LPA packages tested.

Rb and TBARS values followed the same trend and stayed within the same values as Chapter 3. With other photosensitive compounds in milk and low sensitivity of TBARS, other analysis should be considered when predicting milk quality. The ENose analysis would not serve as an accurate analysis for consumer acceptability. Measuring the amount of dissolved oxygen in milk in this study served as a more accurate indicator of flavor of milk. .

This study is important for the dairy industry to understand how consumer liking is impacted by milk exposed to different lights in different packages. The dairy industry may not be able to control the lighting milk is exposed to, but they can provide packaging that will protect milk under different types of lights. This study provides important information on how milk reacts with different lights in different levels of LPA packaging. Natural, unpigmented HDPE bottles are commonly used which allows more light transmission causing adverse reactions with consumers; fluorescent light-exposed milk being less liked than LED light-exposed milk compared to LPA packages. Packages with LPAs are protecting milk better, but may not all be protecting milk equally, indicated by different liking/acceptability scores with different concentrations of LPA. Packages with higher concentrations of titanium dioxide (such as the high package (4.9% TiO_2)) may provide a product more acceptable and ideal to consumers. Providing products consumers like and enjoy can increase consumption and sales of fluid milk. This study was done with only 4h of light exposure. Knowing that significant differences were found in Chapter 3 for LPA packages and light-protected controls beyond 4h of exposure, there needs more research on how milk is liked with longer time exposure under these conditions.

The dairy industry can use this information to understand how light exposure impacts consumer acceptability of milk, and find protective packaging that delivers fresh quality milk for an extended period of time.

REFERENCES

Alvarez, V. B. 2009. Fluid Milk and Cream Products. Pages 73-134 in The Sensory Evaluation of Dairy Products. 2nd ed. Springer Sciences, New York, NY. Print.

Brothersen C., D.J. McMahon, J. Legako, and S. Martini. 2016. Comparison of milk oxidation by exposure to LED and fluorescent light. J. Dairy Sci. 99:1-8.

Chapman, K. 2002. New study validates light blocking efforts: Teens taste light-oxidization in milk and don't like it. Dairy Foods. 103:40-42.

Chapman, K. W., L. J. Whited, and K. J. Boor. 2002. Sensory threshold of light-oxidized flavor defects in milk. J. Food Sci. 67.2770-2773.

Dimick, P. S. 1982. Photochemical effects on flavor and nutrients of fluid milk. Can Institute Food Sci Technol J. 15: 247-256.

Drake, M. A. 2007. Invited review: Sensory analysis of dairy foods. J. Dairy Sci. 90:4925-4937.

Duncan, S. E., and H. H. Chang. 2012. Implications of light energy on food quality and packaging selection. Advances in food and nutrition research. 67:25-73.

Duncan, S. E., and J. B. Webster. 2010. Oxidation and protection of milk and dairy products. Woodhead Publishing, Ltd., Cambridge, UK. Pp. 121-155.

Food Business News, 2013. More ingredients are being used for dairy alternatives. Available from http://www.foodbusinessnews.net/articles/news_home/Consumer_Trends/2013/01/More_ingredients_are_being_use.aspx?ID={9F6B5243-0F3C-48D4-A4A5-694479F96B8C}&cck=1. Accessed 8/4/15

Heer, A. K., S. E., Duncan, and D., Brochetti. 1995. Sensory detection of and consumer response to off-flavors in milk. Dairy, Food and Environmental Sanitation. 15:488–493.

International Dairy Foods Association, 2015. Milk Sales and Consumption. Available from http://www.idfa.org/news-views/media-kits/milk/milk-sales-consumption. Accessed 11/23/14

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Page 511 in Modern Food Microbiology. Springer Sciences. New York, NY.

Johnson, D. S., S.E. Duncan, L. M. Bianchi, H. H. Chang, W. N. Eigel, and S. F. O'Keefe. 2015. Packaging modification for protecting flavor of extended-shelf-life milk from light. J. Dairy Sci. 98: 2205-2214.

Marshall, R. 1992. Standard methods for the examination of dairy products. 16th ed. American Public Health Association. Washington, DC.

Meilgaard, M. C., G. V. Civille, and B. T. Carr. 2007. Sensory Evaluation Techniques. 4th ed. CRC Press, New York.

Potts, H. L. 2016. Oxidation barrier and light protective packaging properties for controlling light induced oxidation in milk. Master's thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA. Print.

Robertson, G. L. 2006. Food packaging: principles and practice. 2nd edition. Boca Ranton: CRC press. Pp. 67-69.

Scheidegger, D., R. P. Pecora, P. M. Radici, and S. C. Kivatinitz. 2010. Protein oxidation changes in whole and skim milk after ultraviolet or fluorescent light exposure. J. Dairy Sci. 93:5101-5109.

Shahidi, F, and Zhong, Y. 2010. Lipid oxidation and improving the oxidative stability. Chem. Soc. Rev. 39: 4067-4079.

Snedecor, G. W. and W. G. Cochran. 1980. Failures in assumptions. Pages 279-280 in Statistical Methods. 7th ed. Iowa State University Press.

van Aardt, M., S. E. Duncan, J. E. Marcy, T. E. Long, and C. R. Hackney. 2001. Effectiveness of poly(ethylene terephthalate) and high-density polyethylene in protection of milk flavor. J. Dairy Sci. 84:1341-1347.

Walsh, A. M., S. E. Duncan, H. L. Potts, and D. L. Gallagher. 2015. Comparing quality and emotional responses as related to acceptability of light-induced oxidation flavor in milk. Food Research Int. 76:293-300.

Zygoura, P, T. Moyssiadi, A. Badeka, E. Kondyli, I. Savvaidis, and M. G. Kontominas. 2004. Shelf-life of whole pasteurized milk in Greece: effect of packaging material. Food Chem. 87:1-9.

APPENDICES

Appendix A: Institutional Review Board Approval Letter

VirginiaTech

Office of Research Compliance
Institutional Review Board
North End Center, Suite 4120, Virginia Tech
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-4606 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE: November 10, 2014

TO: Susan E Duncan, Virginia C Fernandez-Plotka, Laurie M Bianchi, Daryan Stefon Johnson, Kemia Nicole Amin, Hayley Potts

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires April 25, 2018)

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

IRB NUMBER: **11-477**

Effective November 7, 2014, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the Amendment request for the above-mentioned research protocol.

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

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As:	**Exempt, under 45 CFR 46.110 category(ies) 2,6**
Protocol Approval Date:	**July 7, 2011**
Protocol Expiration Date:	**N/A**
Continuing Review Due Date*:	**N/A**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

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

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

Appendix B: Sensory Evaluation Informed Consent

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

Title Project: Light protection of milk flavor and properties by packaging during retail storage

Investigators: Susan E. Duncan, PhD, RD, Kemia Amin

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

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

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

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

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

VI. Compensation
You will be compensated with a small edible treat at the end of every session in which you choose to participate. You will receive a stamp for each panel participated in at upon entering the sensory welcome desk. Your name me will be entered into a drawing for a gift card. The drawing will occur at the end of the study.

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

VIII. Subject's Responsibilities
I voluntarily agree to participate in this study. I have the following responsibilities:

Appendix B: Con't

Evaluate 5 sets (3 samples each) of milk, as presented, and provide answers using a touch screen computer.

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

Date: _____

Subject Signature: _____

Subject Printed Name: _____

Appendix C: Sensory Evaluation Scorecard

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 Evaluation Schedule

Table D: Sensory evaluation schedule for discrimination (triangle) tests for 4, 8, 16, 24, 48, and 72 hours.

Rep 1		Rep 2,3	
Tuesday	Fresh Milk Delivery	Wednesday	Fresh Milk Delivery
Wednesday	16 h	Thursday	16 h
Thursday	24 h	Friday	4 h
Friday	4 h	Monday	72 h
Monday	72 h	Tuesday	X
Tuesday	X	Wednesday	48 h
Wednesday	48 h	Thursday	8 h
Thursday	8 h	Friday	24 h

Appendix E: Microbial Count for 2% Milk

Table E: Microbial growth for [a]fresh (0 hour exposed) 2% milk and [b]18 days from post processing. [a]Samples were tested in triplicates to obtain results. [b]Samples were taken from 120 samples for each test per replication.

	Replication 1		Replication 2		Replication 3	
	Fresh (0 hours)	18 day post process	Fresh (0 hours)	18 day post process	Fresh (0 hours)	18 day post process
Aerobic Plate Count	0%	5%	0%	3.3%	0%	0.8%
Colifom Plate Count	0%	3.3%	0%	0.8%	0%	0%

Appendix F: Package Comparison Definitions

Table F: Definitions for package comparisons and effectiveness against light exposure of fluorescent and LED lights in sensory evaluation (triangle; difference test) of milk.

	Difference	Similarity	Description
Targeted package Condition	$P < 0.05$ Light-exposed Control	$P > 0.05$ Light-protected Control	Best case scenario (ideal condition); treatment package protects milk as well as light-protected (no light exposure) package
Partially Protected Condition	$P > 0.05$ Light-exposed Control	$P > 0.05$ Light-protected Control	Intermediate protection; package treatment protects milk to some extent, but also has similarities to light-exposed milk. There are protective qualities making the package partially protected product.
Partially Ineffective Condition	$P < 0.05$ Light-exposed Control	$P < 0.05$ Light-protected Control	Intermediate protection; package treatment is different than light-protected control, but also is different to light-exposed milk; having differences when compared to light-protected milk moves this package treatment further away from the ideal therefore making it less effective than the partially protected condition.
Ineffective Package Condition	$P > 0.05$ Light-exposed Control	$P < 0.05$ Light-protected Control	Worst case scenario; treatment package does not protect milk any better than standard translucent (0% LPA) HDPE bottle

Appendix G: Package Comparison Significance and Definitions

Table G: Definitions and significance ($P < 0.05$) for package comparisons and effectiveness against light exposure of fluorescent and LED lights in sensory evaluation (triangle; difference test) of milk.

Item		4h		8h		16h	
Fluorescent	LE vs. Yellow	<0.001*	Partially	0.610	Ineffective	0.049*	Partially
Fluorescent	LP vs. Yellow	0.041*	Ineffective	<0.001*		<0.001*	Ineffective
Fluorescent	LE vs. High	0.026*	Targeted	0.945	Ineffective	0.197	Ineffective
Fluorescent	LP vs. High	0.118		0.045*		<0.001*	
Fluorescent	LE vs. Low	0.288	Partially	0.687	Ineffective	0.973	Ineffective
Fluorescent	LP vs. Low	0.064	Protected	0.007*		<0.001*	
LED	LE vs. Yellow	0.019*	Targeted	0.006*	Partially	0.013*	Targeted
LED	LP vs. Yellow	0.064		<0.001*	Ineffective	0.237	
LED	LE vs. High	0.064	Partially	0.289	Ineffective	0.022*	Partially
LED	LP vs. High	0.391	Protected	0.010*		<0.001*	Ineffective
LED	LE vs. Low	0.555	Ineffective	0.472	Ineffective	0.258	Ineffective
LED	LP vs. Low	<0.001*		<0.001*		<0.001*	

Item		24h		48h		72h	
Fluorescent	LE vs. Yellow	0.033*	Partially	<0.001*	Partially	0.110	Ineffective
Fluorescent	LP vs. Yellow	<0.001*	Ineffective	<0.001*	Ineffective	<0.001*	
Fluorescent	LE vs. High	0.003*	Partially	0.016*	Partially	0.057	Ineffective
Fluorescent	LP vs. High	<0.001*	Ineffective	<0.001*	Ineffective	<0.001*	
Fluorescent	LE vs. Low	0.335	Ineffective	0.184	Ineffective	0.010*	Partially
Fluorescent	LP vs. Low	<0.001*		<0.001*		<0.001*	Ineffective
LED	LE vs. Yellow	0.308	Ineffective	0.071	Ineffective	0.708	Ineffective
LED	LP vs. Yellow	0.012*		0.005*		<0.001*	
LED	LE vs. High	0.557	Ineffective	0.094	Ineffective	0.608	Ineffective
LED	LP vs. High	<0.001*		0.002*		<0.001*	
LED	LE vs. Low	0.087	Ineffective	0.635	Ineffective	0.708	Ineffective
LED	LP vs. Low	<0.001*		<0.001*		<0.001*	

Appendix H: 1-2 Wavelength Spectrum of Light Protective Additive Packages

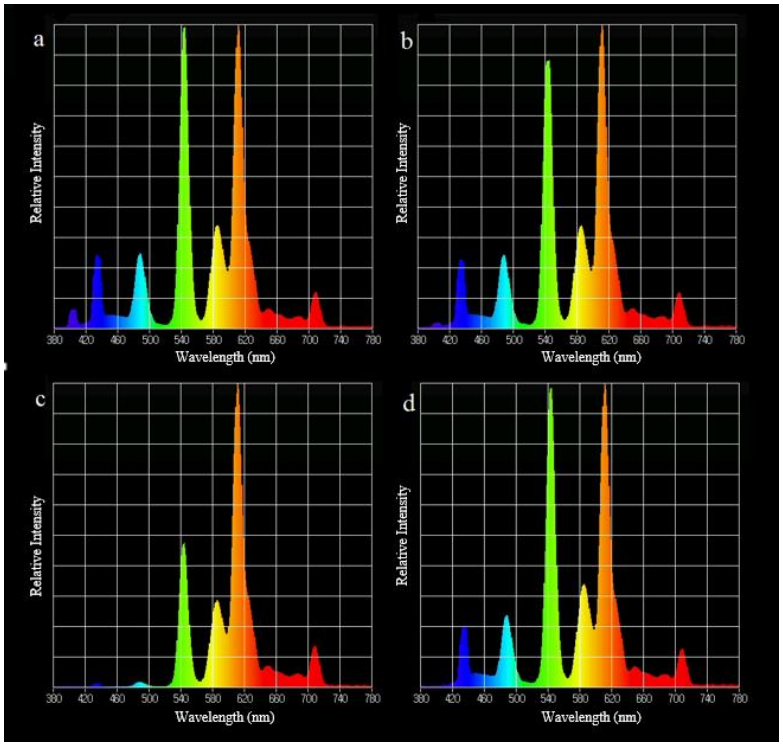

Figure H-1: Wavelength intensities for fluorescent light with package barrier for [a]unpigmented HDPE, [b]High, [c]Yellow, [d]Low.

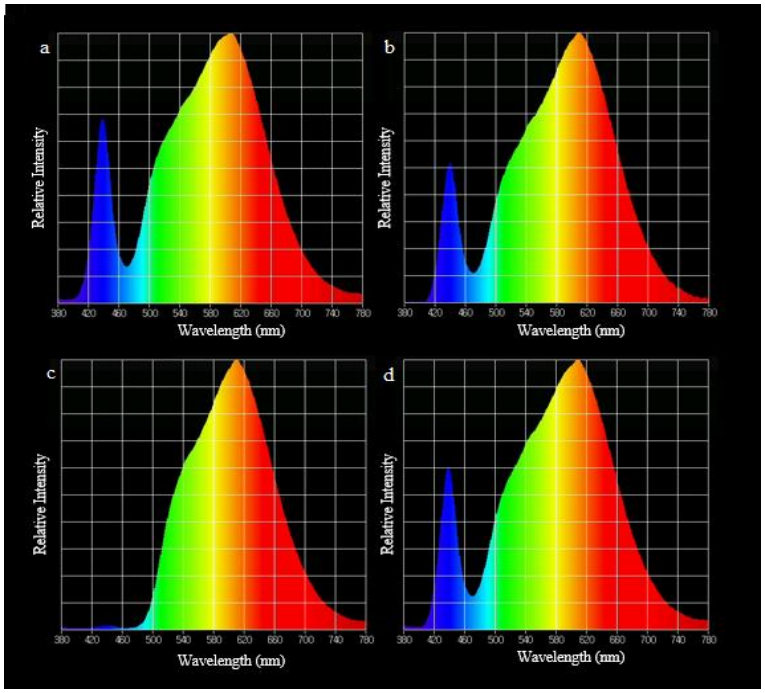

Figure H-2: Wavelength intensities for LED light package barrier for [a]unpigmented HDPE, [b]High, [c]Yellow, [d]Low.

Appendix I 1-2: TBARS Mean and Standard Deviation for Individual Bottles

Table I: Mean and standard deviation of malondialdehyde concentration (mg/L) of individual bottles of 2% milk (Rep.1 & 2), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent)

Individual Bottles (Rep. 1 & 2) Under Fluorescent Light (1,617 ± 505 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light Protected (foil covered)	0.08	±	0.00	0.10	±	0.01	0.11	±	0.04	0.12	±	0.02	0.11	±	0.04	0.15	±	0.01	0.17	±	0.03
High	0.08	±	0.00	0.11	±	0.00	0.12	±	0.06	0.14	±	0.02	0.14	±	0.05	0.16	±	0.02	0.17	±	0.01
Yellow	0.08	±	0.00	0.11	±	0.01	0.11	±	0.05	0.13	±	0.03	0.13	±	0.06	0.16	±	0.06	0.20	±	0.02
Low	0.08	±	0.00	0.17	±	0.06	0.13	±	0.06	0.13	±	0.02	0.14	±	0.05	0.17	±	0.05	0.18	±	0.02
Light Exposed (translucent)	0.08	±	0.00	0.11	±	0.01	0.14	±	0.09	0.14	±	0.03	0.14	±	0.05	0.17	±	0.05	0.19	±	0.05
Individual Bottles (Rep. 1 & 2) Under LED Light (929 ± 97 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	0.08	±	0.00	0.11	±	0.01	0.10	±	0.03	0.15	±	0.04	0.13	±	0.04	0.16	±	0.03	0.14	±	0.03
High	0.08	±	0.00	0.13	±	0.02	0.11	±	0.05	0.13	±	0.02	0.14	±	0.04	0.15	±	0.04	0.16	±	0.02
Yellow	0.08	±	0.00	0.12	±	0.03	0.12	±	0.05	0.17	±	0.05	0.18	±	0.04	0.19	±	0.07	0.16	±	0.01
Low	0.08	±	0.00	0.17	±	0.10	0.12	±	0.05	0.12	±	0.01	0.15	±	0.04	0.16	±	0.03	0.18	±	0.03
Light Exposed (translucent)	0.08	±	0.00	0.11	±	0.01	0.10	±	0.04	0.19	±	0.05	0.15	±	0.05	0.17	±	0.04	0.17	±	0.03

Appendix J 1-2: TBARS Results for Commingled Bottles

Table J: Mean and standard deviation of malondialdehyde concentration (mg/L) of commingled bottles of 2% milk (Rep.1, 2, & 3), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent).

Commingled 2% Milk (Rep. 1, 2, & 3) Under Fluorescent Light (1,882 ± 993 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.
Light Protected (foil covered)	0.09	±	0.02	0.14	±	0.05	0.13	±	0.03	0.15	±	0.04	0.13	±	0.03	0.16	±	0.03	0.14	±	0.02
High	0.09	±	0.02	0.18	±	0.02	0.17	±	0.03	0.16	±	0.06	0.17	±	0.03	0.17	±	0.04	0.18	±	0.05
Yellow	0.09	±	0.02	0.16	±	0.01	0.16	±	0.03	0.17	±	0.08	0.15	±	0.06	0.18	±	0.04	0.20	±	0.04
Low	0.09	±	0.02	0.17	±	0.03	0.17	±	0.03	0.17	±	0.08	0.14	±	0.05	0.18	±	0.04	0.18	±	0.06
Light Exposed (translucent)	0.09	±	0.02	0.16	±	0.03	0.17	±	0.02	0.18	±	0.06	0.17	±	0.04	0.19	±	0.08	0.19	±	0.01
Commingled Bottles (Rep. 1, 2, & 3) Under LED Light (915 ± 150 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	0.09	±	0.02	0.14	±	0.05	0.13	±	0.02	0.13	±	0.02	0.14	±	0.06	0.14	±	0.03	0.13	±	0.03
High	0.09	±	0.02	0.22	±	0.07	0.20	±	0.08	0.17	±	0.05	0.17	±	0.02	0.21	±	0.01	0.18	±	0.06
Yellow	0.09	±	0.02	0.19	±	0.00	0.18	±	0.06	0.15	±	0.05	0.17	±	0.05	0.17	±	0.06	0.17	±	0.05
Low	0.09	±	0.02	0.20	±	0.01	0.17	±	0.01	0.20	±	0.07	0.17	±	0.03	0.18	±	0.01	0.19	±	0.07
Light Exposed (translucent)	0.09	±	0.02	0.17	±	0.02	0.21	±	0.11	0.18	±	0.06	0.19	±	0.03	0.21	±	0.07	0.19	±	0.08

Appendix K 1-2: Riboflavin Mean and Standard Deviation for Individual Bottles

Table K: Mean and standard deviation of riboflavin concentration (mg/L) of individual bottles of 2% milk (Rep.1 & 2), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent).

Individual Bottles (Rep. 1 & 2) Under Fluorescent Light (1,617 ± 505 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	X	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	1.45	±	0.07	1.49	±	0.14	1.27	±	0.09	1.27	±	0.37	1.47	±	0.66	1.15	±	0.18	0.95	±	0.37
High	1.45	±	0.07	1.35	±	0.17	1.27	±	0.08	1.22	±	0.04	1.08	±	0.18	1.16	±	0.19	0.92	±	0.10
Yellow	1.45	±	0.07	1.33	±	0.11	1.33	±	0.03	1.30	±	0.26	0.99	±	0.11	1.15	±	0.09	0.89	±	0.15
Low	1.45	±	0.07	1.39	±	0.20	1.31	±	0.10	1.05	±	0.26	1.23	±	0.15	1.34	±	0.21	0.66	±	0.16
Light Exposed (translucent)	1.45	±	0.07	1.25	±	0.21	1.17	±	0.12	1.15	±	0.07	1.07	±	0.34	1.07	±	0.01	0.75	±	0.20
Individual Bottles (Rep. 1 & 2) Under LED Light (929 ± 97 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light Protected (foil covered)	1.45	±	0.07	1.49	±	0.89	1.35	±	0.16	1.17	±	0.47	1.24	±	0.05	1.12	±	0.17	0.76	±	0.21
High	1.45	±	0.07	1.34	±	0.26	1.31	±	0.21	1.20	±	0.41	1.07	±	0.33	1.15	±	0.09	0.89	±	0.10
Yellow	1.45	±	0.07	1.34	±	0.12	1.13	±	0.16	1.13	±	0.22	1.64	±	0.30	1.25	±	0.15	0.80	±	0.11
Low	1.45	±	0.07	1.37	±	0.08	1.25	±	0.05	1.35	±	0.32	1.12	±	0.07	1.33	±	0.13	0.80	±	0.12
Light Exposed (translucent)	1.45	±	0.07	1.22	±	0.36	1.08	±	0.08	1.12	±	0.18	1.05	±	0.02	1.06	±	0.24	0.76	±	0.14

Appendix L 1-2: Riboflavin Mean and Standard Deviation for Commingled Bottles

Table L: Mean and standard deviation of riboflavin concentration (mg/L) of commingled bottles of 2% milk (Rep.1, 2, & 3), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent).

Commingled 2% Milk (Rep. 1, 2, & 3) Under Fluorescent Light (1,882 ± 993 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.
Light Protected (foil covered)	1.46	±	0.01	1.45	±	0.22	1.45	±	0.06	1.40	±	0.20	1.31	±	0.41	1.22	±	0.16	0.99	±	0.25
High	1.46	±	0.01	1.25	±	0.07	1.26	±	0.14	0.87	±	0.22	1.08	±	0.07	1.19	±	0.15	0.75	±	0.16
Yellow	1.46	±	0.01	1.33	±	0.04	1.27	±	0.10	1.16	±	0.28	1.15	±	0.04	1.03	±	0.12	0.86	±	0.09
Low	1.46	±	0.01	1.46	±	0.06	1.30	±	0.16	1.14	±	0.12	1.03	±	0.32	0.95	±	0.44	0.78	±	0.11
Light Exposed (translucent)	1.46	±	0.01	1.19	±	0.07	1.14	±	0.06	1.04	±	0.16	0.91	±	0.16	0.95	±	0.19	0.73	±	0.20
Commingled Bottles (Rep. 1, 2, & 3) Under LED Light (915 ± 150 lux) Analytical Testing																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	1.46	±	0.01	1.79	±	0.14	1.41	±	0.09	1.31	±	0.24	1.17	±	0.33	1.01	±	0.15	0.90	±	0.13
High	1.46	±	0.01	1.38	±	0.07	1.29	±	0.30	0.94	±	0.24	1.10	±	0.35	0.99	±	0.22	0.79	±	0.28
Yellow	1.46	±	0.01	1.18	±	0.05	1.21	±	0.31	1.08	±	0.16	1.25	±	0.53	1.19	±	0.27	0.72	±	0.14
Low	1.46	±	0.01	1.33	±	0.10	1.24	±	0.35	1.16	±	0.34	1.45	±	0.08	1.08	±	0.11	0.72	±	0.15
Light Exposed (translucent)	1.46	±	0.01	1.47	±	0.09	1.09	±	0.03	0.93	±	0.13	1.00	±	0.08	1.08	±	0.10	0.78	±	0.13

Appendix M 1-2: TBARS Mean and Standard Deviation for 18 Day Shelf Life Study

Table M: Mean and standard deviation of malondialdehyde concentration (mg/L) of 18 day shelf life study bottles of 2% milk (Rep.1, 2, & 3), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent).

2% Milk (Rep. 1, 2, & 3) Under Fluorescent Light (1,984 ± 932 lux) Analytical Testing for 18 Shelf Life Study																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	0.08	±	0.02	0.11	±	0.01	0.12	±	0.02	0.11	±	0.01	0.14	±	0.03	0.13	±	0.02	0.16	±	0.03
High	0.08	±	0.02	0.11	±	0.02	0.12	±	0.03	0.11	±	0.03	0.16	±	0.02	0.15	±	0.03	0.14	±	0.04
Yellow	0.08	±	0.02	0.12	±	0.12	0.11	±	0.03	0.13	±	0.02	0.15	±	0.01	0.15	±	0.04	0.16	±	0.03
Low	0.08	±	0.02	0.12	±	0.02	0.12	±	0.03	0.13	±	0.03	0.15	±	0.02	0.16	±	0.01	0.14	±	0.02
Light Exposed (translucent)	0.08	±	0.02	0.12	±	0.02	0.12	±	0.03	0.14	±	0.03	0.17	±	0.03	0.16	±	0.03	0.15	±	0.04
2 % Milk (Rep. 1, 2, & 3) Under LED Light (917 ± 122 lux) Analytical Testing for 18 Shelf Life Study																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.
Light Protected (foil covered)	0.08	±	0.02	0.11	±	0.01	0.13	±	0.03	0.11	±	0.02	0.13	±	0.02	0.16	±	0.03	0.14	±	0.04
High	0.08	±	0.02	0.12	±	0.02	0.12	±	0.01	0.11	±	0.03	0.14	±	0.03	0.16	±	0.03	0.14	±	0.02
Yellow	0.08	±	0.02	0.11	±	0.02	0.12	±	0.01	0.12	±	0.03	0.13	±	0.03	0.16	±	0.01	0.16	±	0.06
Low	0.08	±	0.02	0.11	±	0.02	0.13	±	0.02	0.12	±	0.03	0.15	±	0.03	0.16	±	0.02	0.14	±	0.03
Light Exposed (translucent)	0.08	±	0.02	0.11	±	0.01	0.12	±	0.03	0.13	±	0.03	0.16	±	0.03	0.17	±	0.02	0.16	±	0.04

Appendix N 1-2:

Table N: Mean and standard deviation of riboflavin concentration (μg/ml) of 18 day shelf life study bottles of 2% milk (Rep.1, 2, & 3), exposed to fluorescent and LED light over 72 hours in 5 different treatment bottles; light-protected (foil wrapped), high (4.9% TiO_2), yellow, low (1.3% TiO_2), and light-exposed (translucent).

2% Milk (Rep. 1, 2, & 3) Under Fluorescent Light (1,984 ± 932 lux) Analytical Testing for 18 Shelf Life Study																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	x	±	s.d.	x	±	s.d.	x	±	s.d.	x	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	1.46	±	0.01	1.23	±	0.14	1.17	±	0.16	1.27	±	0.37	1.24	±	0.46	1.24	±	0.12	1.05	±	0.12
High	1.46	±	0.01	1.19	±	0.11	1.13	±	0.12	1.03	±	0.06	1.03	±	0.07	1.10	±	0.17	0.96	±	0.04
Yellow	1.46	±	0.01	1.27	±	0.20	1.13	±	0.15	1.09	±	0.08	1.18	±	0.27	1.08	±	0.06	1.02	±	0.10
Low	1.46	±	0.01	1.39	±	0.19	1.12	±	0.08	1.12	±	0.06	1.12	±	0.25	1.03	±	0.14	0.98	±	0.13
Light Exposed (translucent)	1.46	±	0.01	1.34	±	0.39	1.42	±	0.67	1.09	±	0.15	1.06	±	0.22	0.95	±	0.10	0.95	±	0.05
2 % Milk (Rep. 1, 2, & 3) Under LED Light (917 ± 122 lux) Analytical Testing for 18 Shelf Life Study																				
PACKAGE TREATMENT	HOURS																			
	0			4			8			16			24			48			72		
	X	±	s.d.	x	±	s.d.	X	±	s.d.	X	±	s.d.	X	±	s.d.	x	±	s.d.	X	±	s.d.
Light Protected (foil covered)	1.46	±	0.01	1.35	±	0.25	1.24	±	0.20	1.24	±	0.38	1.22	±	0.33	1.08	±	0.12	1.02	±	0.14
High	1.46	±	0.01	1.18	±	0.18	1.21	±	0.26	1.14	±	0.19	1.01	±	0.08	1.02	±	0.11	0.97	±	0.04
Yellow	1.46	±	0.01	1.29	±	0.27	1.36	±	0.21	1.20	±	0.23	1.00	±	0.07	1.03	±	0.08	0.96	±	0.07
Low	1.46	±	0.01	1.15	±	0.15	1.29	±	0.48	1.15	±	0.17	1.06	±	0.10	0.99	±	0.07	0.97	±	0.05
Light Exposed (translucent)	1.46	±	0.01	1.24	±	0.19	1.19	±	0.15	1.03	±	0.14	1.03	±	0.10	0.96	±	0.07	0.94	±	0.07

Appendix O: Light Intensity Readings for Retail Case

Table O: Light intensity readings (lux) at each position within retails case for all treatments under fluorescent and LED lights.[1]

Fluorescent Light (lux)							
	AA1	**AA2**	**AA3**	**AA4**	**AA5**	**AA6**	**AA7**
Mean	1399.33	2603.83	977.67	1095.83	1120.83	1489.50	2299.33
± s.d	464.30	255.97	72.36	124.88	138.85	59.16	62.19
Median	1138.00	1643.50	1069.00	985.50	1131.50	1354.50	2421.50
	AB1	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**
Mean	2151.67	2751.33	1873.00	1409.00	1671.00	2765.33	5067.83
± s.d	189.56	474.16	293.32	361.30	353.89	376.26	419.74
Median	2195.00	2799.50	1782.50	1553.50	1553.50	2691.50	5055.00
	AC1	**AC2**	**AC3**	**AC4**	**AC5**	**CA6**	**AC7**
Mean	2280.83	2833.33	1914.33	1357.17	1629.50	2240.00	4458.50
± s.d	216.39	209.41	296.71	203.71	262.57	811.20	949.15
Median	2275.00	2830.50	1878.50	1351.50	1683.00	2655.00	4770.00
	AD1	**AD2**	**AD3**	**AD4**	**AD5**	**AD6**	**AD7**
Mean	2076.83	2711.00	1755.50	1361.17	1573.17	2270.67	4176.50
± s.d	266.06	883.45	156.69	228.21	439.89	871.72	1377.36
Median	2070.00	3055.00	1755.00	1341.50	1817.50	2730.00	4910.00
	BA1	**BA2**	**BA3**	**BA4**	**BA5**	**BA6**	**BA7**
Mean	2498.17	1472.83	1395.67	1094.00	1330.33	1747.50	1600.33
± s.d	440.87	372.63	691.34	334.15	245.65	82.93	489.06
Median	2545.50	1309.00	993.00	958.00	1260.50	1729.50	1382.00
	BB1	**BB2**	**BB3**	**BB4**	**BB5**	**BB6**	**BB7**
Mean	4911.50	2571.67	1813.83	1367.00	1753.33	2937.00	2776.50
± s.d	372.10	673.57	632.22	334.49	89.33	484.53	399.50
Median	4320.00	2255.00	1459.00	1267.00	1742.00	3105.00	2740.00
	BC1	**BC2**	**BC3**	**BC4**	**BC5**	**BC6**	**BC7**
Mean	4214.00	2076.17	1386.83	1312.67	1730.67	-2993.83	2661.00
± s.d	850.75	430.45	217.97	265.99	341.22	738.23	242.68
Median	4430.00	2175.00	1428.00	1307.50	1831.00	3340.00	2623.00
	BD1	**BD2**	**BD3**	**BD4**	**BD5**	**BD6**	**BD7**
Mean	4194.00	2103.33	1335.17	1280.50	1805.50	2751.00	2598.67
± s.d	887.13	480.87	273.40	180.16	217.38	645.33	399.98
Median	4660.00	2355.00	1411.00	1297.50	1857.00	2805.00	2540.00
LED Light (lux)							
	AA1	**AA2**	**AA3**	**AA4**	**AA5**	**AA6**	**AA7**
Mean	1020.00	892.67	832.83	831.83	935.83	953.67	1069.67
± s.d	353.15	64.46	76.41	91.84	118.61	59.16	62.91
Median	884.00	879.00	76.41	91.84	118.61	59.16	62.91

	AB1	AB2	AB3	AB4	AB5	AB6	AB7
Mean	1224.67	1253.67	982.50	1019.33	988.50	1005.83	1121.17
± s.d	605.73	716.03	41.66	38.75	39.18	45.41	92.06
Median	605.73	966.00	978.00	1004.50	973.50	990.50	1150.50
	AC1	**AC2**	**AC3**	**AC4**	**AC5**	**AA6**	**AC7**
Mean	1065.17	2833.33	984.83	943.17	982.00	1090.83	1229.33
± s.d	571.36	35.70	36.78	34.48	57.85	88.27	157.44
Median	1069.00	1008.00	976.00	932.50	1008.00	1115.50	1303.50
	AD1	**AD2**	**AD3**	**AD4**	**AD5**	**AD6**	**AD7**
Mean	886.17	903.80	889.17	914.83	896.67	949.67	1112.33
± s.d	49.26	33.27	31.93	64.86	105.84	51.83	94.61
Median	887.00	903.50	880.50	922.50	906.00	959.50	1147.50
	BA1	**BA2**	**BA3**	**BA4**	**BA5**	**BA6**	**BA7**
Mean	894.50	844.83	772.67	812.83	784.83	794.33	747.50
± s.d	59.13	80.40	101.72	127.23	90.57	91.52	206.66
Median	877.50	836.50	739.00	863.00	751.50	760.00	631.50
	BB1	**BB2**	**BB3**	**BB4**	**BB5**	**BB6**	**BB7**
Mean	1040.50	901.67	895.17	864.67	845.33	896.00	766.17
± s.d	62.39	56.60	65.72	41.98	46.10	17.12	127.29
Median	1017.50	895.50	881.00	863.00	836.50	899.50	732.00
	BC1	**BC2**	**BC3**	**BC4**	**BC5**	**BC6**	**BC7**
Mean	1077.17	990.83	941.50	877.50	861.50	888.83	850.00
± s.d	107.26	87.74	31.99	41.46	46.48	58.71	138.56
Median	1093.50	1004.00	946.50	875.00	874.00	890.00	839.00
	BD1	**BD2**	**BD3**	**BD4**	**BD5**	**BD6**	**BD7**
Mean	862.00	862.00	906.83	876.67	841.17	851.50	777.67
± s.d	54.05	54.05	31.60	48.88	77.38	71.22	66.52
Median	871.00	871.00	900.50	870.00	829.50	830.50	787.50

1 Position AA4, AB4, AC4, AD4, BA4, BB4, BC4, BD4 were light readings of water half-gal bottles used as place holders for study; no LPA packages were in this position.

Appendix P: 1-2: Absorption and Transmission of Packages

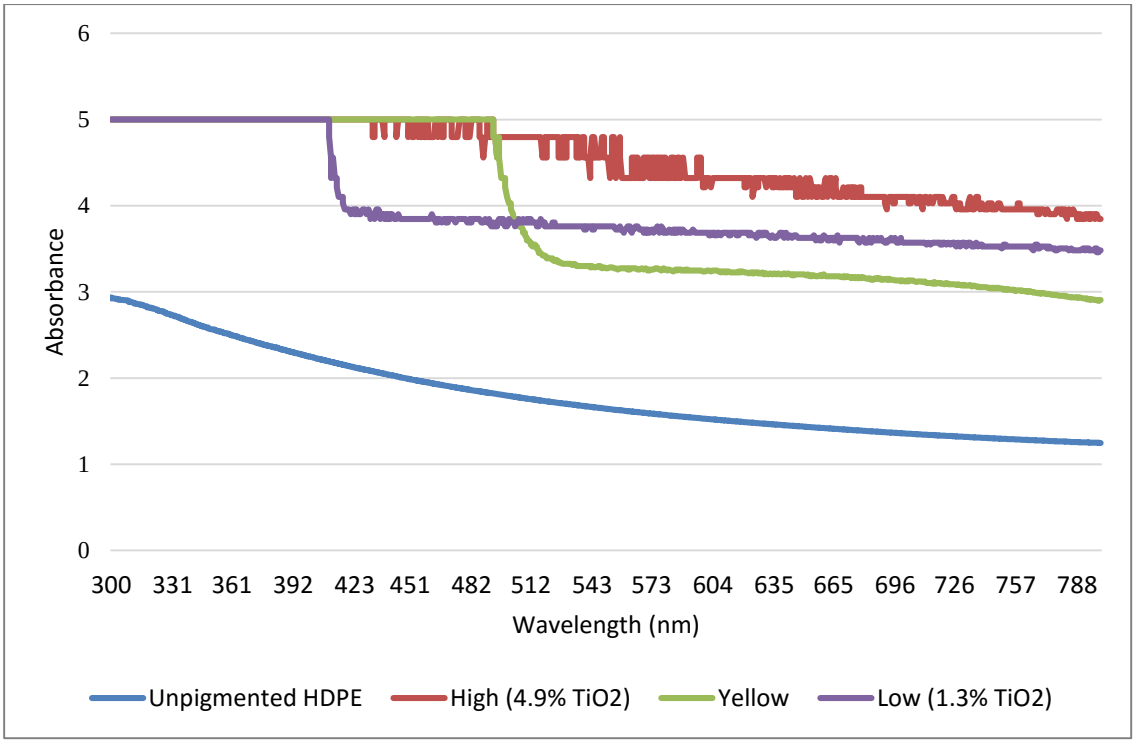

Figure P-1: Absorbance of LPA packages at 300 to 800 nm.

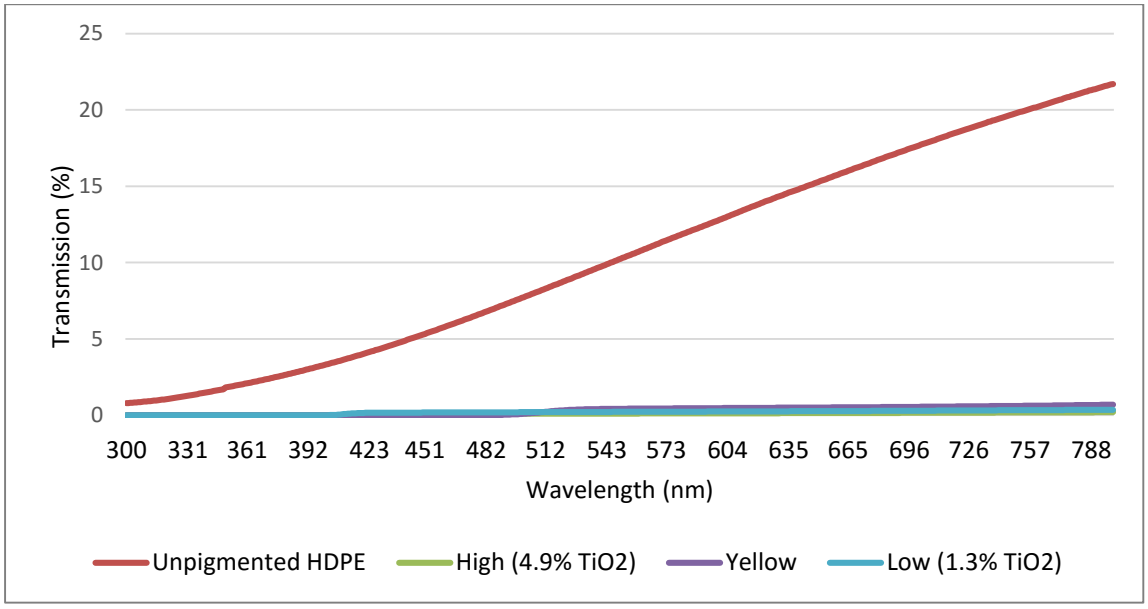

Figure P-2: Transmission of LPA packages at 300 to 800 nm.

Appendix Q: **Retail Milk Survey Light Intensities**

Table Q: Retail milk survey of light intensities (lux) for fluorescent (1,185 ± 1,114) and LED (907 ± 644) light at various locations within retail case in multiple retail markets.

Store		Shelf	Position Within Shelf			Light
			Left	Middle	Right	
1	Grocery	1	1,580	1,661	1,758	Fluorescent
	Grocery	2	1,409	1,398	1,407	Fluorescent
2	Supermarket	1	3,060	687	620	Fluorescent
	Supermarket	2	1,630	1,378	1,826	Fluorescent
	Supermarket	3	636	899	7,950	Fluorescent
	Supermarket	4	952	1,132	1,230	Fluorescent
3	Grocery	1	1,372	1,021	1,237	Fluorescent
	Grocery	2	1,241	1,181	1,107	Fluorescent
4	Convenient	1	119	164	112	Fluorescent
	Convenient	2	50	101	47	Fluorescent
5	Convenient	1	164	183	425	Fluorescent
	Convenient	2	119	153	116	Fluorescent
6	Convenient	1	1,437	1,366	1,361	Fluorescent
	Convenient	2	2,320	1,831	2,350	Fluorescent
7	Gas Station	1	2,470	1,098	2,460	Fluorescent
	Gas Station	2	1,989	1,044	2,190	Fluorescent
8	Gas Station	1	4,580	819	2,040	Fluorescent
	Gas Station	2	4,700	994	2,430	Fluorescent
9	Convenient	1	806	433	414	Fluorescent
	Convenient	2	438	555	500	Fluorescent
10	Convenient	1	1,006	1,424	1,688	Fluorescent
	Convenient	2	816	723	813	Fluorescent
11	Supermarket	1	643	376	309	Fluorescent
	Supermarket	2	577	418	361	Fluorescent
	Supermarket	3	270	510	2,050	Fluorescent
	Supermarket	4	292	388	495	Fluorescent
12	Grocery	1	2,070	500	683	Fluorescent
	Grocery	2	2,690	258	752	Fluorescent
	Grocery	3	1,404	411	495	Fluorescent
	Grocery	4	856	376	634	Fluorescent
13	Grocery	1	1,558	1,633	1,664	Fluorescent
	Grocery	2	1,260	1,317	1,283	Fluorescent
14	Supermarket	1	1,456	689	1017	LED
	Supermarket	2	987	420	371	LED
	Supermarket	3	1,264	441	214	LED
	Supermarket	4	371	340	221	LED
15	Grocery	1	914	687	1,290	LED
	Grocery	2	1,728	489	2,760	LED
	Grocery	3	591	1,142	1,011	LED
	Grocery	4	949	1,010	1,830	LED

16	Convenient	1	1,973	620	2,970	LED
	Convenient	2	979	324	2,140	LED
17	Grocery	1	1,075	205	858	LED
	Grocery	2	254	189	212	LED
	Grocery	3	199	208	577	LED
	Grocery	4	237	233	214	LED
18	Grocery	1	1,241	959	1,524	LED
	Grocery	2	1,229	916	1,348	LED
	Grocery	3	1,329	444	276	LED
	Grocery	4	918	466	1,189	LED
19	Convenient	1	381	524	2,200	LED
	Convenient	2	486	533	613	LED
20	Convenient	1	2,020	509	1,030	LED
	Convenient	2	1180	869	1,638	LED
	Convenient	3	485	393	831	LED
	Convenient	4	2,470	655	984	LED
Total	Mean		1,236	708	1,252	
	± s.d		971	438	1,186	

Appendix R: Riboflavin Concentration for 18 Day Shelf Life Study

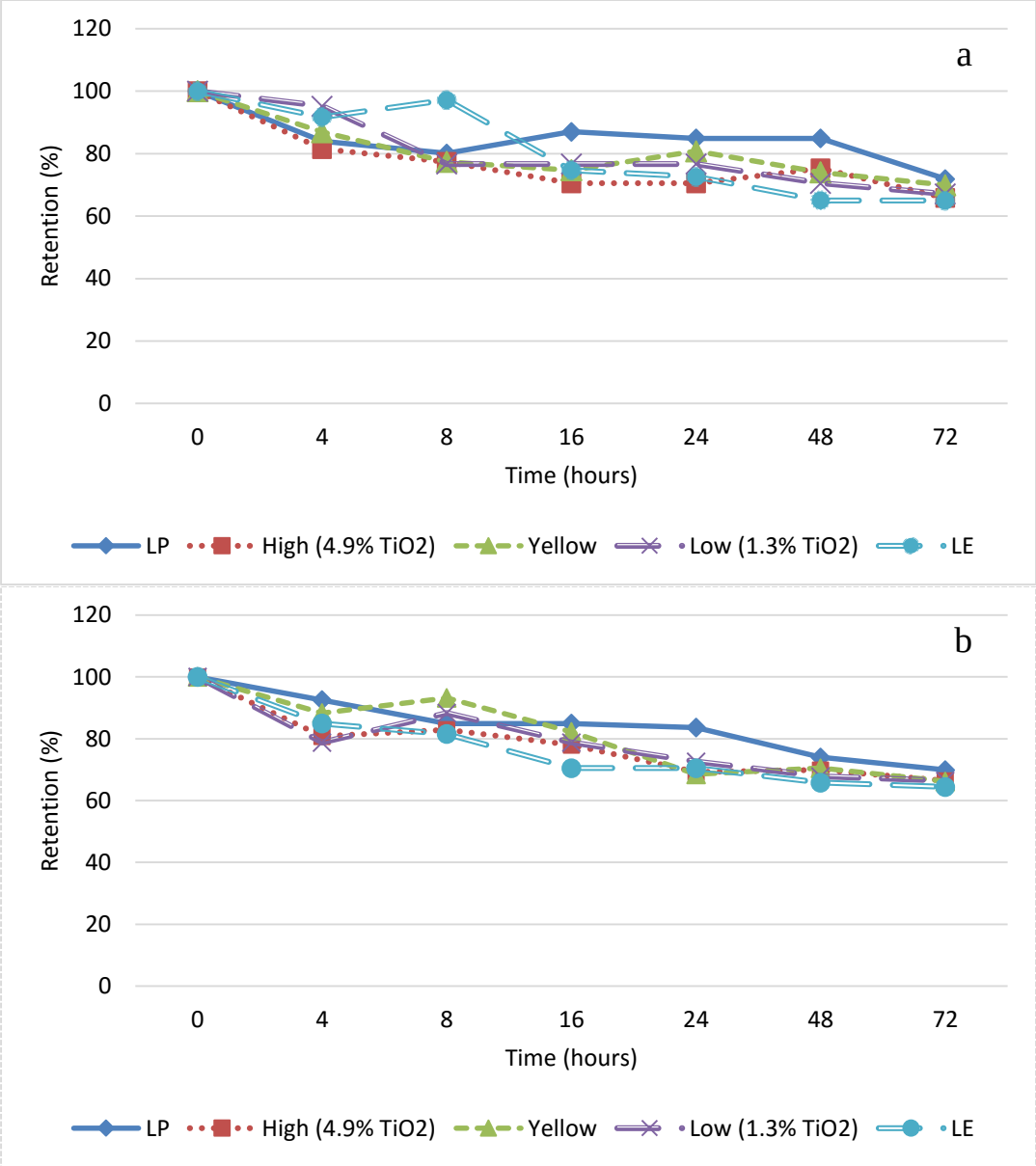

Figure R: Riboflavin retention (mg/L) in 18 day shelf life study of 2% milk packaged in high-density polyethylene (HDPE) with different light protective additive levels (yellow, low, high) compared with controls (light exposed, LE); light protected (LP) over 72 h of [a]fluorescent light (1,984 ± 932 lux) and [b]light emitting diode (LED) (917 ± 122 lux) exposure.

Appendix S: TBARS Results for 18 Day Shelf Life Study

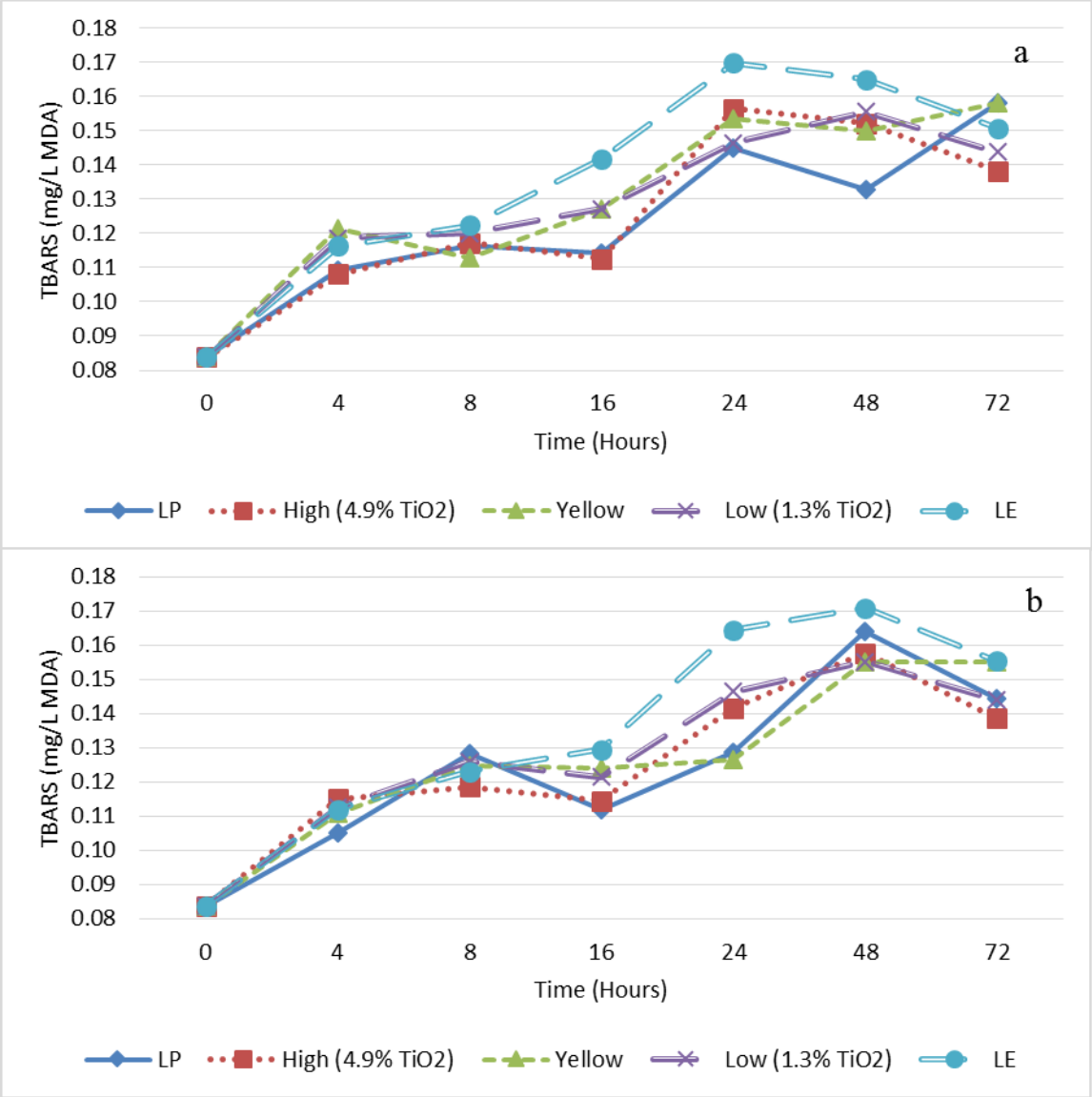

Figure S: Malondialdehyde concentration in 18 day shelf life study of 2% milk packaged in high-density polyethylene (HDPE) with different light protective additive levels (yellow, low, high) compared with controls (light exposed, LE); light protected (LP) over 72 h of [a]fluorescent light (1,984 ± 932 lux) and [b]light emitting diode (LED) (917 ± 122 lux) exposure.

Appendix T: **Table T: Power analysis and sensory evaluation data of 2% Milk**

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	4	0.0414	43	104	0.30	0.025	20%	Similarity	0.4135	12%	0.0483	0.0408	0.1996
LP vs Low	4	0.0635	42	104	0.30	0.025	20%	Similarity	0.4039	11%	0.0481	0.0266	0.1849
LP vs High	4	0.1180	40	103	0.30	0.025	20%	Similarity	0.3884	8%	0.0480	0.0035	0.1615
LP vs LE	4	0.0021	48	103	0.05	0.150	20%	Difference	0.4660	20%	0.0492	0.1182	0.2799
LE vs Y	4	0.0000	46	104	0.05	0.150	20%	Difference	0.4423	16%	0.0487	0.0834	0.2436
LE vs Low	4	0.2884	37	103	0.05	0.150	20%	Difference	0.3592	3%	0.0473	-0.0389	0.1166
LE vs High	4	0.0261	44	104	0.05	0.150	20%	Difference	0.4231	13%	0.0485	0.0549	0.2143
LED													
LP vs Y		0.0635	42	104	0.30	0.025	20%	Similarity	0.4039	11%	0.0481	0.0266	0.1849
LP vs Low	4	0.0000	68	104	0.30	0.025	20%	Similarity	0.6539	48%	0.0467	0.5575	0.4040
LP vs High	4	0.3905	36	104	0.30	0.025	20%	Similarity	0.3462	2%	0.0467	-0.0575	0.0960
LP vs LE	4	0.0009	50	105	0.05	0.150	20%	Difference	0.4762	21%	0.0487	0.1341	0.2945
LE vs Y	4	0.0192	45	105	0.05	0.150	20%	Difference	0.4286	14%	0.0483	0.2223	0.0634
LE vs Low	4	0.5549	34	104	0.05	0.150	20%	Difference	0.3269	-0.962%	0.0460	-0.0853	0.0661
LE vs High	4	0.0635	42	104	0.05	0.150	20%	Difference	0.4039	11%	0.0481	0.0266	0.1849
LP (F) vs LP (L)	4	0.5055	34	104	0.30	0.025	20%	Similarity	0.3269	-0.96%	0.0460	0.0661	-0.0853
LE (F) vs LE (L)	4	0.0007	50	104	0.30	0.025	20%	Similarity	0.4762	21%	0.0487	0.1341	0.2945

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	8	0.0002	52	104	0.30	0.025	20%	Similarity	0.5000	25%	0.0490	0.3307	0.1693
LP vs Low	8	0.0074	46	103	0.30	0.025	20%	Similarity	0.4466	17%	0.0490	0.0893	0.2505
LP vs High	8	0.0464	42	102	0.30	0.025	20%	Similarity	0.4118	12%	0.0487	0.0375	0.1978
LP vs LE	8	0.0004	50	102	0.05	0.150	20%	Difference	0.4902	24%	0.0495	0.1539	0.3167
LE vs Y	8	0.6095	33	103	0.05	0.150	20%	Difference	0.3204	-1.90%	0.0460	-0.0950	0.0562
LE vs Low	8	0.6869	32	103	0.05	0.150	20%	Difference	0.3107	-3.40%	0.0456	-0.1090	0.0410
LE vs High	8	0.9445	27	104	0.05	0.150	20%	Difference	0.2596	-11%	0.0430	-0.1813	-0.0399
LED													
LP vs Y	8	0.0005	50	103	0.30	0.025	20%	Similarity	0.4854	23%	0.0492	0.1471	0.3092
LP vs Low	8	0.0004	50	102	0.30	0.025	20%	Similarity	0.4902	24%	0.0495	0.1539	0.3167
LP vs High	8	0.0104	45	102	0.30	0.025	20%	Similarity	0.4412	16%	0.0492	0.0809	0.2426
LP vs LE	8	0.2884	37	103	0.05	0.150	20%	Difference	0.3592	4%	0.0473	-0.0389	0.1166
LE vs Y	8	0.0063	46	103	0.05	0.150	20%	Difference	0.4466	17%	0.0490	0.0893	0.2505
LE vs Low	8	0.4717	34	101	0.05	0.150	20%	Difference	0.3366	0.50%	0.047	-0.0724	0.0823
LE vs High	8	0.2884	37	103	0.05	0.150	20%	Difference	0.3592	4%	0.0473	-0.0389	0.1166
LP (F) vs LP (L)	8	0.4717	31	102	0.30	0.025	20%	Similarity	0.3039	-4%	0.0455	-0.1190	0.0308
LE (F) vs LE (L)	8	0.0464	42	102	0.30	0.025	20%	Similarity	0.4118	12%	0.0487	0.0375	0.1978

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	16	0.0000	52	98	0.30	0.025	20%	Similarity	0.5306	30%	0.0504	0.2130	0.3788
LP vs Low	16	0.0000	54	95	0.30	0.025	20%	Similarity	0.5684	35%	0.0508	0.2690	0.4362
LP vs High	16	0.0000	52	98	0.30	0.025	20%	Similarity	0.5306	30%	0.0404	0.2130	0.3788
LP vs LE	16	0.0000	64	99	0.05	0.150	20%	Difference	0.6465	47%	0.0480	0.3907	0.5487
LE vs Y	16	0.0493	40	97	0.05	0.150	20%	Difference	0.4124	12%	0.0500	0.0363	0.2008
LE vs Low	16	0.9725	24	99	0.05	0.150	20%	Difference	0.2424	-14%	0.0431	-0.2070	-0.0660
LE vs High	16	0.1967	37	99	0.05	0.150	20%	Difference	0.3737	6%	0.0486	-0.0190	0.1406
LED													
LP vs Y	16	0.2373	36	98	0.30	0.025		Similarity	0.3673	5%	0.0487	-0.0290	0.1311
LP vs Low	16	0.0000	54	98	0.30	0.025	20%	Similarity	0.5510	33%	0.0502	0.2439	0.4092
LP vs High	16	0.0007	48	99	0.30	0.025	20%	Similarity	0.4848	23%	0.0502	0.1446	0.3099
LP vs LE	16	0.0000	54	97	0.05	0.150	20%	Difference	0.5567	34%	0.0504	0.2521	0.4180
LE vs Y	16	0.0134	43	98	0.05	0.150	20%	Difference	0.4388	16%	0.0501	0.0757	0.2406
LE vs Low	16	0.2578	35	96	0.05	0.150	20%	Difference	0.3750	6%	0.0494	-0.0190	0.1438
LE vs High	16	0.0224	42	98	0.05	0.150	20%	Difference	0.4286	14%	0.0500	0.0606	0.2251
LP (F) vs LP (L)	16	0.6145	30	94	0.30	0.025	20%	Similarity	0.3191	-2%	0.0481	-0.1000	0.0578
LE (F) vs LE (L)	16	0.6145	30	94	0.30	0.025	20%	Similarity	0.3191	-2%	0.0481	-0.1000	0.0578

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	24	0.0000	55	99	0.30	0.025	20%	Similarity	0.5556	33%	0.0499	0.2512	0.4155
LP vs Low	24	0.0000	54	98	0.30	0.025	20%	Similarity	0.5510	33%	0.0502	0.2439	0.4092
LP vs High	24	0.0000	59	100	0.30	0.025	20%	Similarity	0.5900	39%	0.0492	0.3041	0.4659
LP vs LE	24	0.0000	53	98	0.05	0.150	20%	Difference	0.5408	31%	0.0503	0.2284	0.3940
LE vs Y	24	0.0329	42	100	0.05	0.150	20%	Difference	0.4200	13%	0.0494	0.0488	0.2112
LE vs Low	24	0.3347	35	99	0.05	0.150	20%	Difference	0.3535	3%	0.0480	-0.0490	0.1093
LE vs High	24	0.0028	46	99	0.05	0.150	20%	Difference	0.4646	20%	0.0501	0.1145	0.2794
LED													
LP vs Y		0.0118	44	100	0.30	0.025		Similarity	0.4400	16%	0.0496	0.0783	0.2417
LP vs Low	24	0.0000	57	100	0.30	0.025	20%	Similarity	0.0570	36%	0.0495	0.2736	0.4364
LP vs High	24	0.0000	53	99	0.30	0.025	20%	Similarity	0.5354	30%	0.0501	0.2206	0.3855
LP vs LE	24	0.0000	57	100	0.05	0.150	20%	Difference	0.5700	36%	0.0495	0.2736	0.4364
LE vs Y	24	0.3083	35	98	0.05	0.150	20%	Difference	0.3571	4%	0.0484	-0.0440	0.1153
LE vs Low	24	0.0873	39	98	0.05	0.150	20%	Difference	0.3980	10%	0.0494	0.0156	0.1783
LE vs High	24	0.5565	32	98	0.05	0.150	20%	Difference	0.3265	-1%	0.0474	-0.0880	0.0677
LP (F) vs LP (L)	24	0.5565	32	98	0.30	0.025	20%	Similarity	0.3265	-1%	0.0474	-0.0880	0.0677
LE (F) vs LE (L)	24	0.0076	44	98	0.30	0.025	20%	Similarity	0.4490	17%	0.0502	0.0908	0.2561

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	48	0.0000	69	104	0.30	0.025	20%	Similarity	0.6635	50%	0.0463	0.4190	0.5714
LP vs Low	48	0.0000	65	104	0.30	0.025	20%	Similarity	0.6250	44%	0.0475	0.3594	0.5156
LP vs High	48	0.0014	49	104	0.30	0.025	20%	Similarity	0.4712	21%	0.0489	0.1262	0.2872
LP vs LE	48	0.0000	56	104	0.05	0.150	20%	Difference	0.5285	31%	0.0489	0.2273	0.3881
LE vs Y	48	0.0002	52	105	0.05	0.150	20%	Difference	0.4952	24%	0.0488	0.1626	0.3231
LE vs Low	48	0.1835	39	104	0.05	0.150	20%	Difference	0.3750	6%	0.0475	-0.0160	0.1406
LE vs High	48	0.0158	45	104	0.05	0.150	20%	Difference	0.4327	15%	0.0486	0.0691	0.2290
LED													
LP vs Y	48	0.0051	47	104	0.30	0.025	20%	Similarity	0.4519	18%	0.0488	0.0976	0.2582
LP vs Low	48	0.0000	54	101	0.30	0.025	20%	Similarity	0.5347	30%	0.0496	0.2203	0.3836
LP vs High	48	0.0019	47	100	0.30	0.025	20%	Similarity	0.4700	21%	0.0499	0.1229	0.2871
LP vs LE	48	0.0000	55	101	0.05	0.150	20%	Difference	0.5446	32%	0.0496	0.2353	0.3983
LE vs Y	48	0.0706	41	102	0.05	0.150	20%	Difference	0.4020	10%	0.0485	0.0231	0.1828
LE vs Low	48	0.6353	33	104	0.05	0.150	20%	Difference	0.3173	-2%	0.0456	-0.0990	0.0510
LE vs High	48	0.0937	41	104	0.05	0.150	20%	Difference	0.3942	9%	0.0479	0.0125	0.1702
LP (F) vs LP (L)	48	0.4997	35	105	0.30	0.025	20%	Similarity	0.3330	0%	0.0460	-0.0760	0.0757
LE (F) vs LE (L)	48	0.0293	43	102	0.30	0.025	20%	Similarity	0.4216	13%	0.0489	0.0519	0.2128

Comparison	Time of exposure (hrs)	P-Value	Total number of correct appraisals (number)	Total number of appraisals (number)	alpha	beta	Proportion of discriminators - Preset	Testing	Pc prop correct	Proportion of discriminators	Standard Deviation	Lower Confidence Level 95%	Upper Confidence Level 95%
Fluorescent													
LP vs Y	72	0.0000	68	108	0.30	0.025	20%	Similarity	0.6296	44%	0.0465	0.3680	0.5209
LP vs Low	72	0.0000	56	108	0.30	0.025	20%	Similarity	0.5185	28%	0.0481	0.1987	0.3569
LP vs High	72	0.0001	54	107	0.30	0.025	20%	Similarity	0.5047	26%	0.0483	0.1775	0.3365
LP vs LE	72	0.0000	64	104	0.05	0.150	20%	Difference	0.6154	42%	0.0477	0.3446	0.5016
LE vs Y	72	0.1102	42	108	0.05	0.150	20%	Difference	0.3889	8%	0.0469	0.0062	0.1605
LE vs Low	72	0.0100	47	107	0.05	0.150	20%	Difference	0.4393	16%	0.0480	0.0800	0.2378
LE vs High	72	0.0570	43	106	0.05	0.150	20%	Difference	0.4057	11%	0.0477	0.0300	0.1869
LED													
LP vs Y	72	0.0000	64	107	0.30	0.025	20%	Similarity	0.5981	40%	0.0474	0.3192	0.4752
LP vs Low	72	0.0003	52	106	0.30	0.025	20%	Similarity	0.4906	24%	0.0486	0.1560	0.3157
LP vs High	72	0.0000	58	108	0.30	0.025	20%	Similarity	0.5370	31%	0.0480	0.2266	0.3845
LP vs LE	72	0.0000	62	108	0.05	0.150	20%	Difference	0.5741	36%	0.0476	0.2828	0.4394
LE vs Y	72	0.7075	33	107	0.05	0.150	20%	Difference	0.3084	-4%	0.0446	-0.1110	0.0361
LE vs Low	72	0.7075	33	107	0.05	0.150	20%	Difference	0.3084	-4%	0.0446	-0.1110	0.0361
LE vs High	72	0.6080	34	106	0.05	0.150	20%	Difference	0.3208	-2%	0.0453	-0.0930	0.0557
LP (F) vs LP (L)	72	0.5541	35	107	0.30	0.025	20%	Similarity	0.3271	-1%	0.0454	-0.0840	0.0653
LE (F) vs LE (L)	72	0.2670	38	105	0.30	0.025	20%	Similarity	0.3619	4%	0.0469	-0.0340	0.1200

Appendix U: 1-6: Electric Nose Canonical Distribution for Commingled Milk

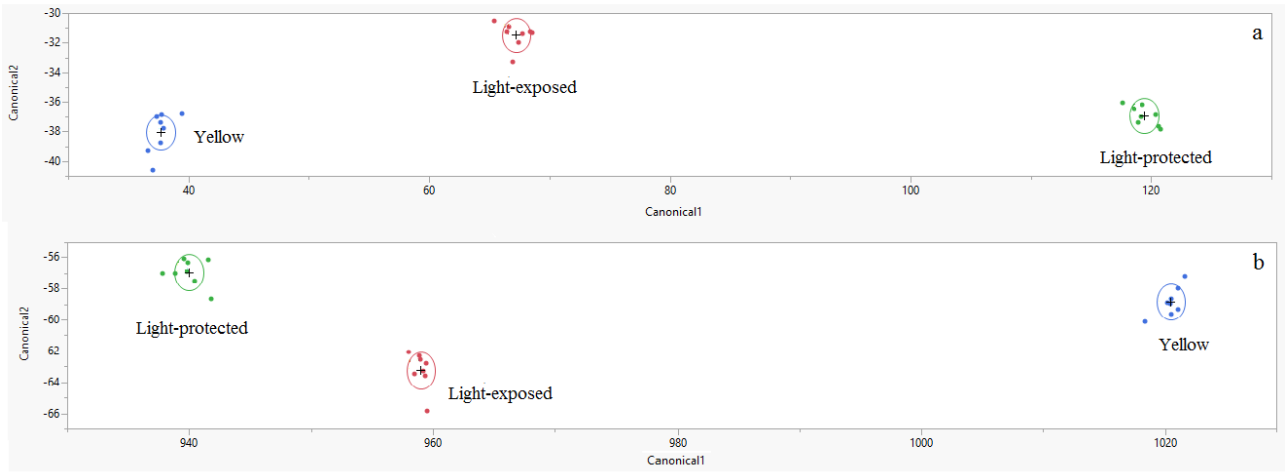

Figure U-1: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and yellow HDPE packages for 8 hours under fluorescent[a] and LED[b] light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

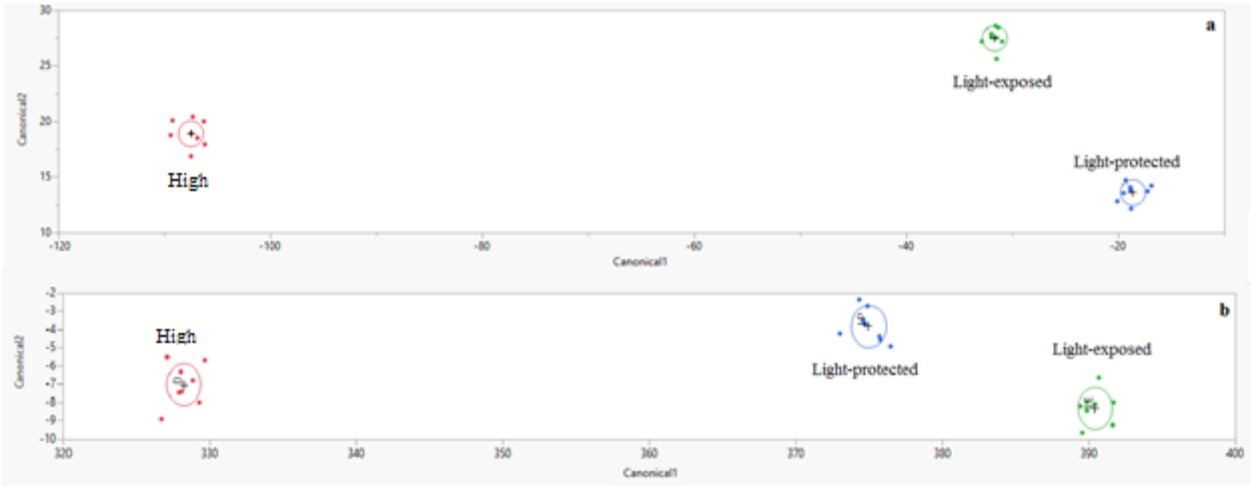

Figure U-2: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and high HDPE packages for 8 hours under fluorescent[a] and LED[b] light. Significance differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Cont. Appendix U: 1-6

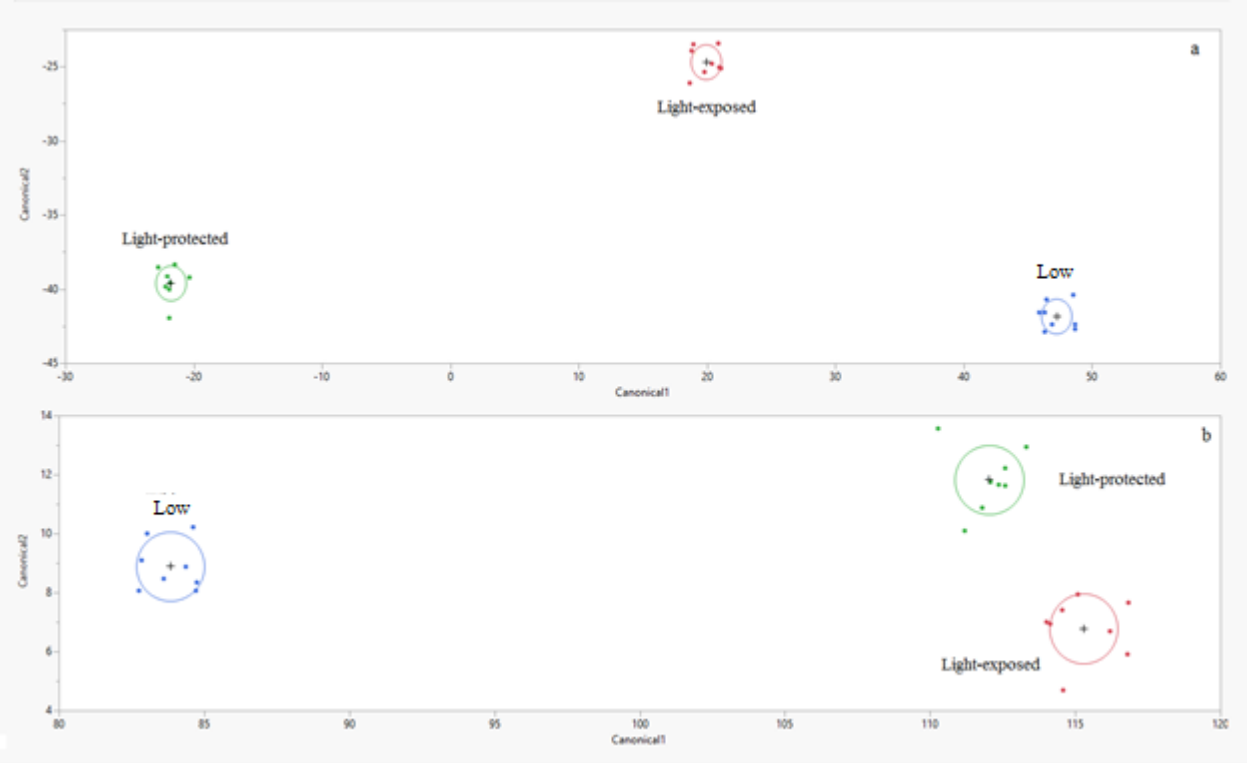

Figure U-3: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and low HDPE packages for 8 hours under fluorescent[a] and LED[b] light. Significance differences at α = 0.05 level indicated by nonintersecting circles.

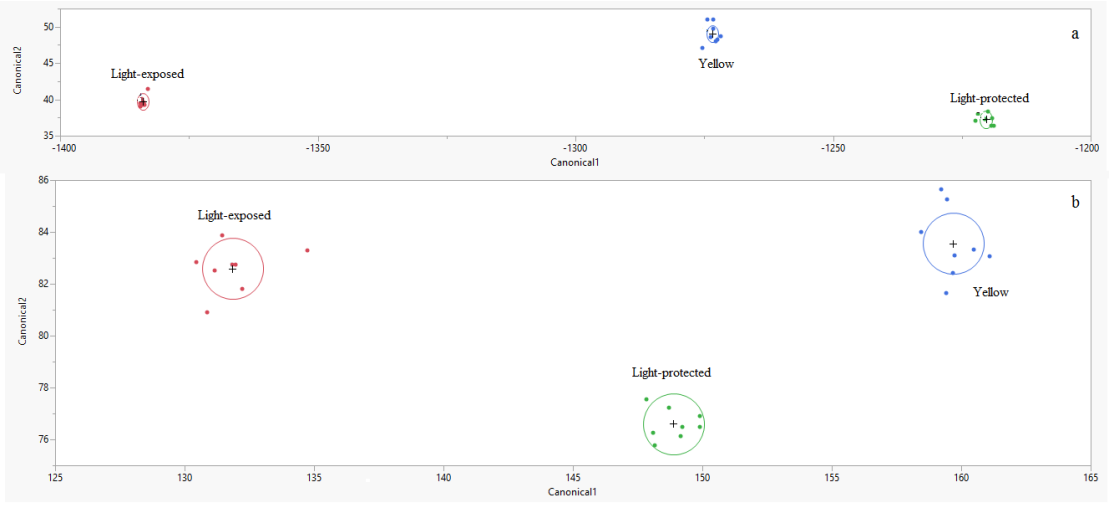

Figure U-4: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and yellow HDPE packages for 24 hours under fluorescent[a] and LED[b] light. Significance differences at α = 0.05 level indicated by nonintersecting circles.

Cont. Appendix T: 1-6

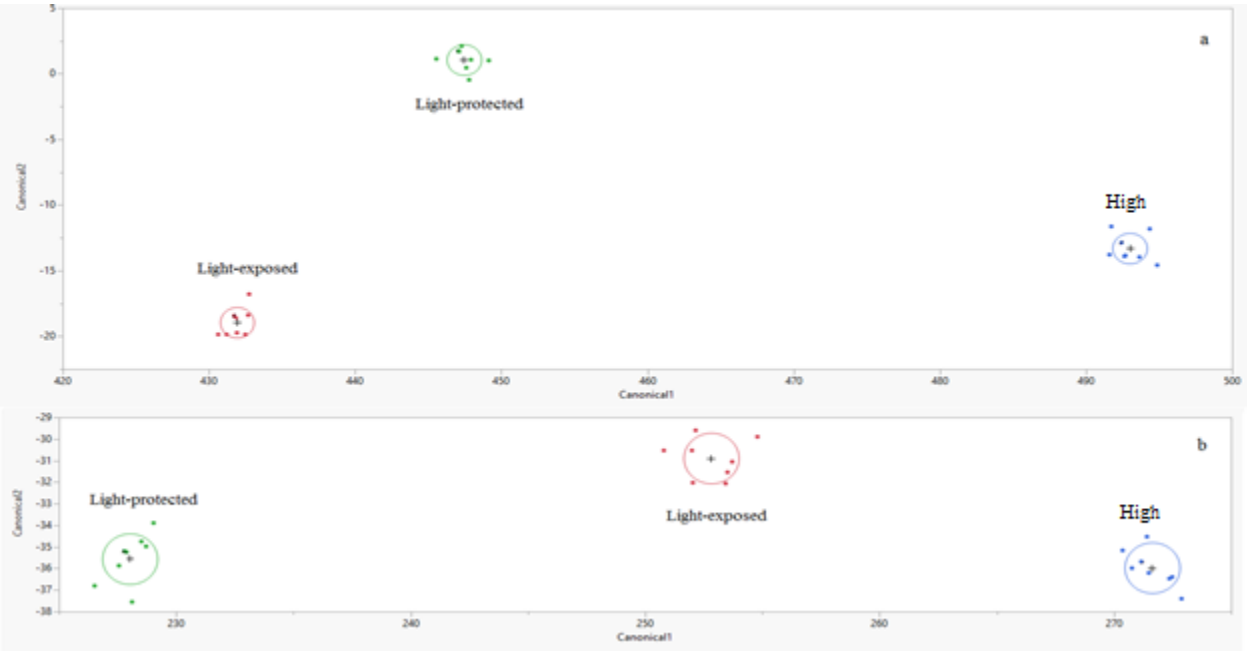

Figure U-5: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and high HDPE packages for 24 hours under fluorescent[a] and LED[b] light. Significance differences at α = 0.05 level indicated by nonintersecting circles.

Figure U-6: Canonical distribution of differences detected by ENose analysis of commingled light-exposed, light-protected, and low HDPE packages for 24 hours under fluorescent[a] and LED[b] light. Significance differences at α = 0.05 level indicated by nonintersecting circles.

Appendix V: 1-6: Boxplot for Electronic Nose Analysis for Commingled

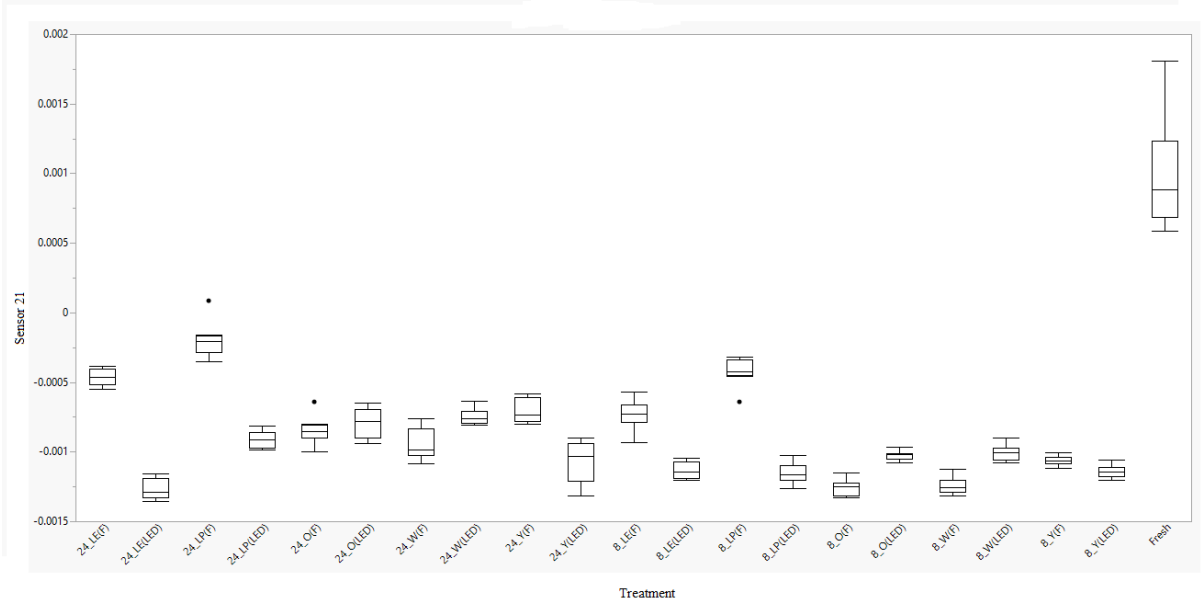

Figure V-1: Boxplot of ENose sensor 21with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; Low (1.3% TiO_2), D; High (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

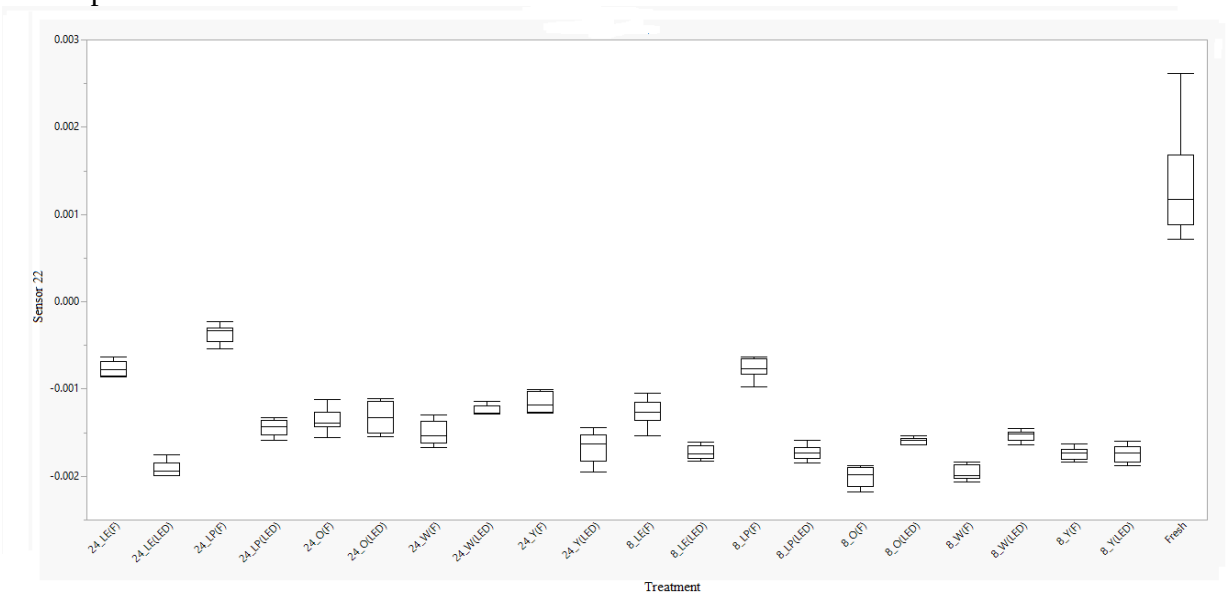

Figure V-2: Boxplot of ENose sensor 22 with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; low (1.3% TiO_2), D; high (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

Cont. Appendix V: 1-6

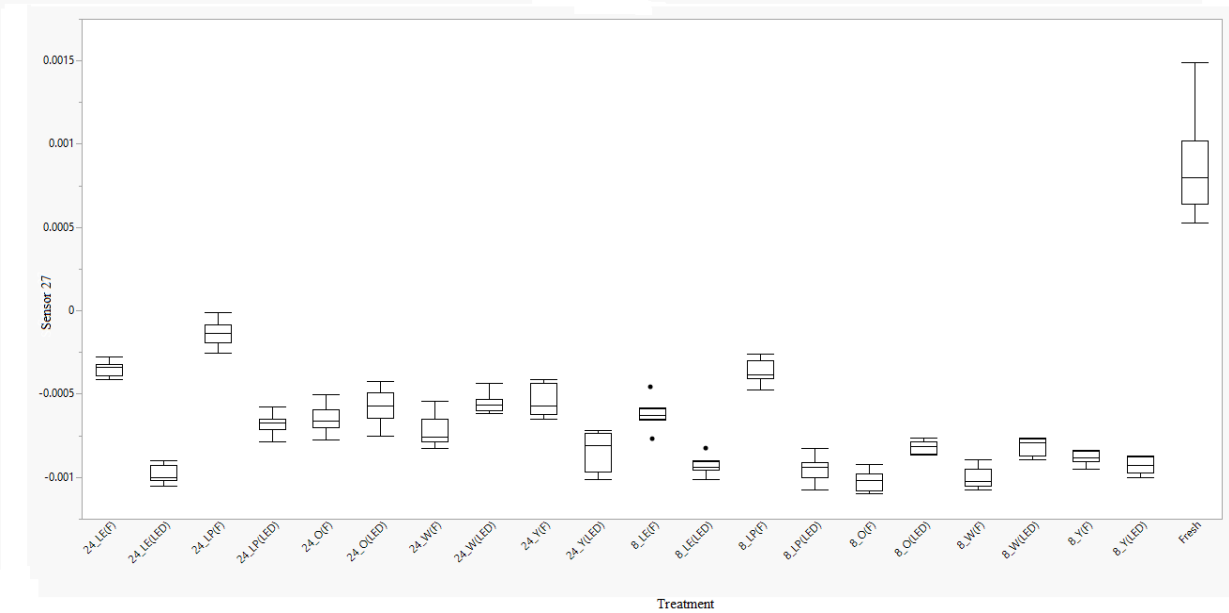

Figure V-3: Boxplot of ENose sensor 27 with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; low (1.3% TiO_2), D; high (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

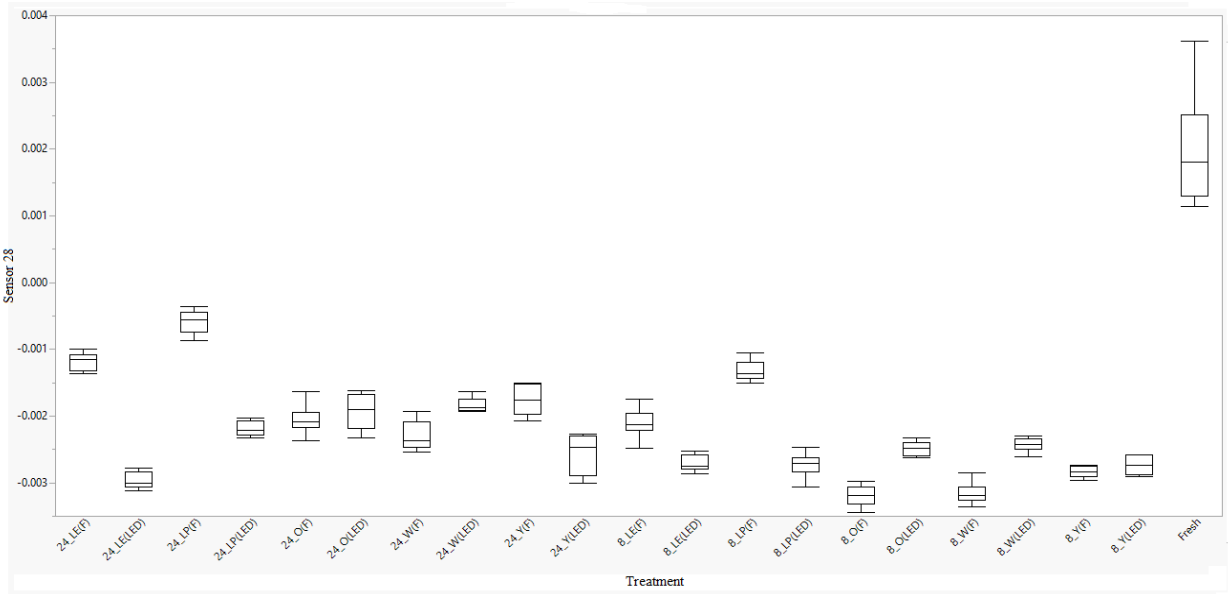

Figure V-4: Boxplot of ENose sensor 28 with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; low (1.3% TiO_2), D; high (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

Cont. Appendix V: 1-6

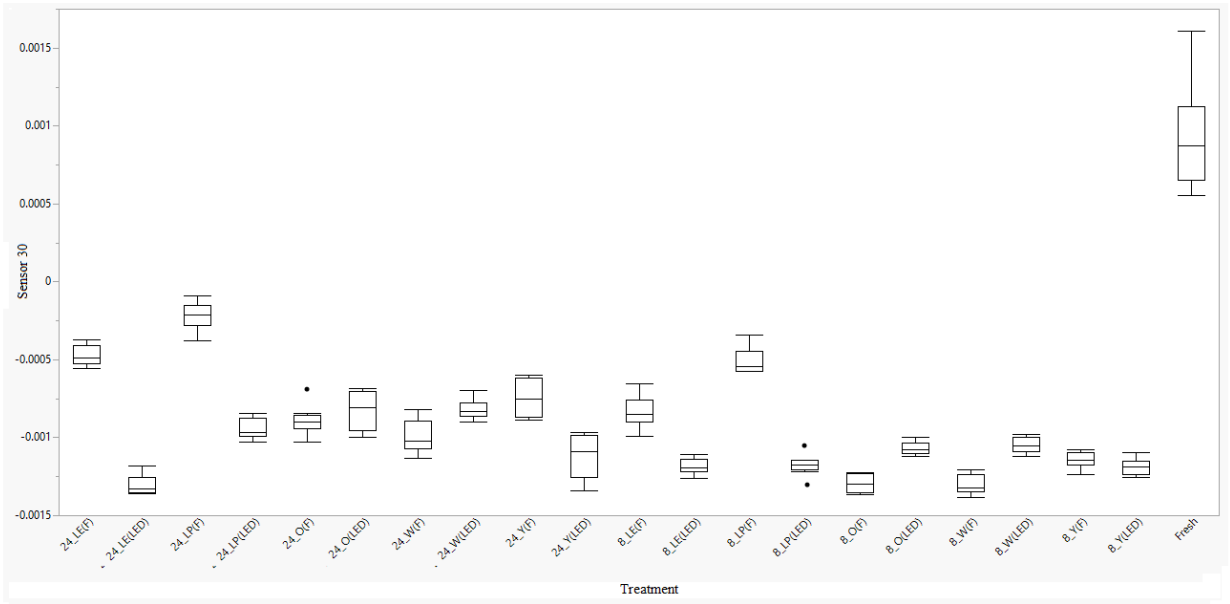

Figure V-5: Boxplot of ENose sensor 30 with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; low (1.3% TiO_2), D; high (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

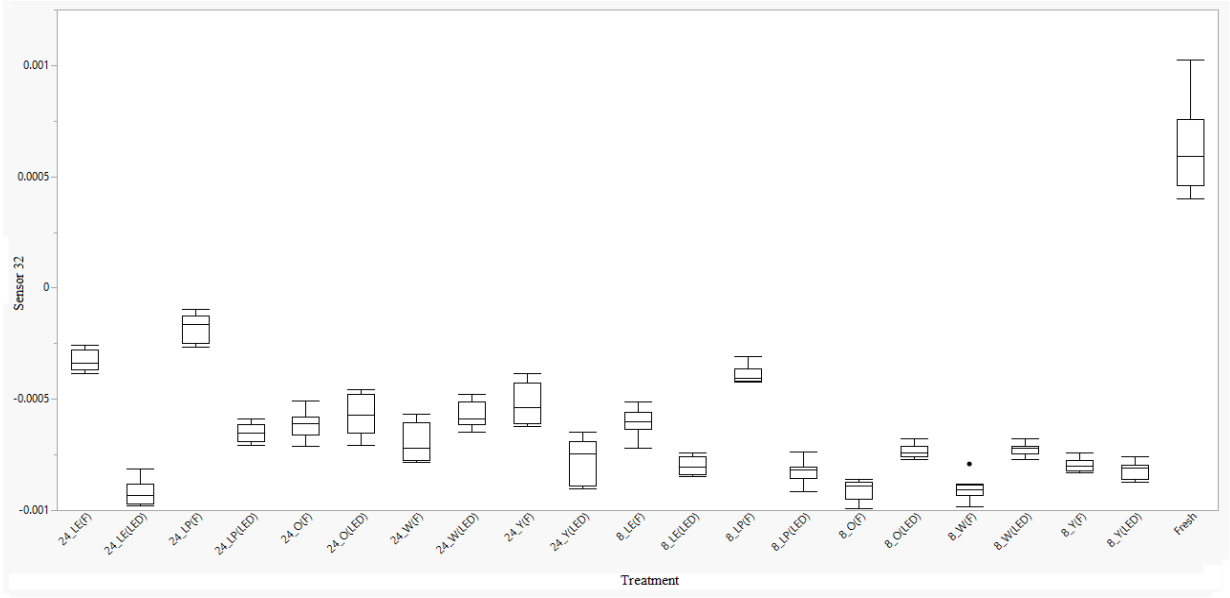

Figure V-6: Boxplot of ENose sensor 32 with treated ((labeled with time (8, 24h) package (LP; light-protected, LE; light-exposed, Y; yellow, W; low (1.3% TiO_2), D; high (4.9% TiO_2) and light (F; fluorescent, LED)) and fresh milk. Treatments with positive values are considered "good" milk in relation to fresh milk; treatments with negative values were deemed as unacceptable milk.

Appendix W: Electronic Nose Means Plot for Sensors 21, 22, 27, 28, 30, and 32

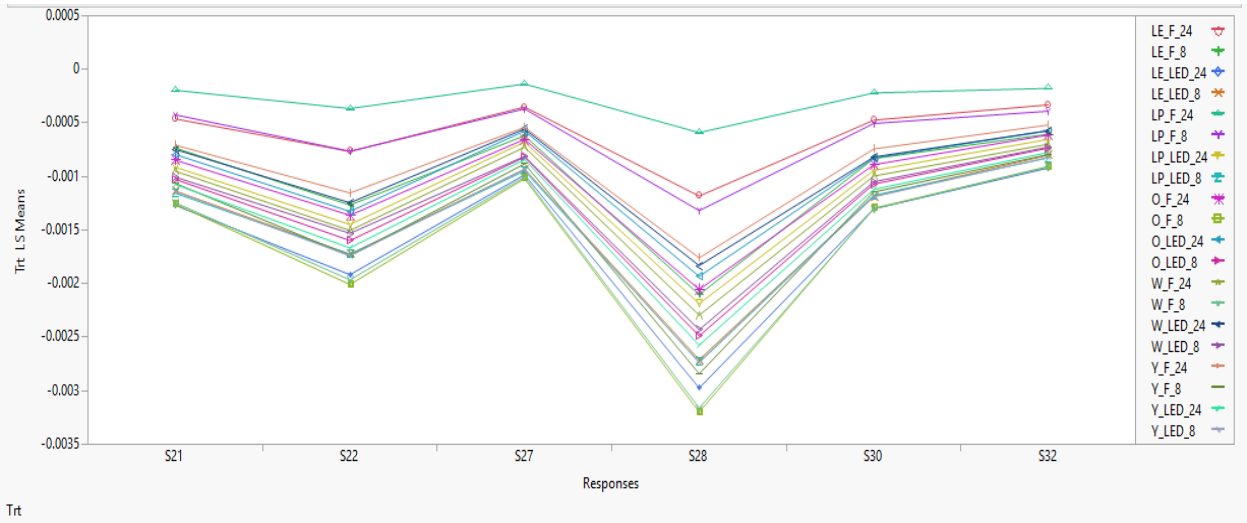

Figure W: MANOVA means plot for treatments at important sensors for milk quality (21, 22, 27, 28, 30, 32) in HDPE bottles. LPA packages with different light protective additive levels (yellow (Y), low (W), high (D)) and controls (light-exposed, LE); light-protected (LP), time (8 and 24h), and light (fluorescent (F) and LED) were combined in combinations to form treatment names.

Appendix X: 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

January 27, 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:_____

Appendix X: Sensory Evaluation Informed Consent and Scorecard for Consumer Study

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
☐	☐	☐	☐	☐	☐	☐	☐	☐

\

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
☐	☐	☐	☐	☐	☐	☐	☐	☐

Appendix X: Con't Sensory Evaluation Informed Consent and Scorecard for Consumer Study

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

Appendix X: Con't Sensory Evaluation Informed Consent and Scorecard for Consumer Study

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

Appendix Y: Consumer Test Demographics

		%
What is your gender?	Male	41
	Female	59
To which age group do you belong?	18 to 25 years old	20
	26 to 35 years old	21
	36 to 45 years old	21
	46 to 55 years old	21
	56 to 65 years old	17
What is your ethnicity?	Asian	19
	Black/African American	17
	Hispanic/Latino	1
	White, Non-Hispanic	60
	Other	2
How often do you drink milk?	Never	0
	A few times per year	0
	At least once in the last 3 months	0
	At least 2-3 times per month	10
	At least once per week	15
	Two or more times per week	75
How often do you purchase milk from the grocery store?	Never	0
	A few times per year	0
	At least once in the last 3 months	3
	At least 2-3 times per month	25
	At least once per week	49
	Two or more times per week	23
Which fat content milk do you consume? (Choose all that apply)	Skim or Nonfat	41
	Low fat or 1%	64
	Reduced fat or 2%	81
	Whole or Regular	64
Which fat content milk do you consume most often?	Skim or Nonfat	0
	Low fat or 1%	26
	Reduced fat or 2%	50
	Whole or Regular	25

Appendix Z: Boxplot for Riboflavin Concentration for Consumer Study

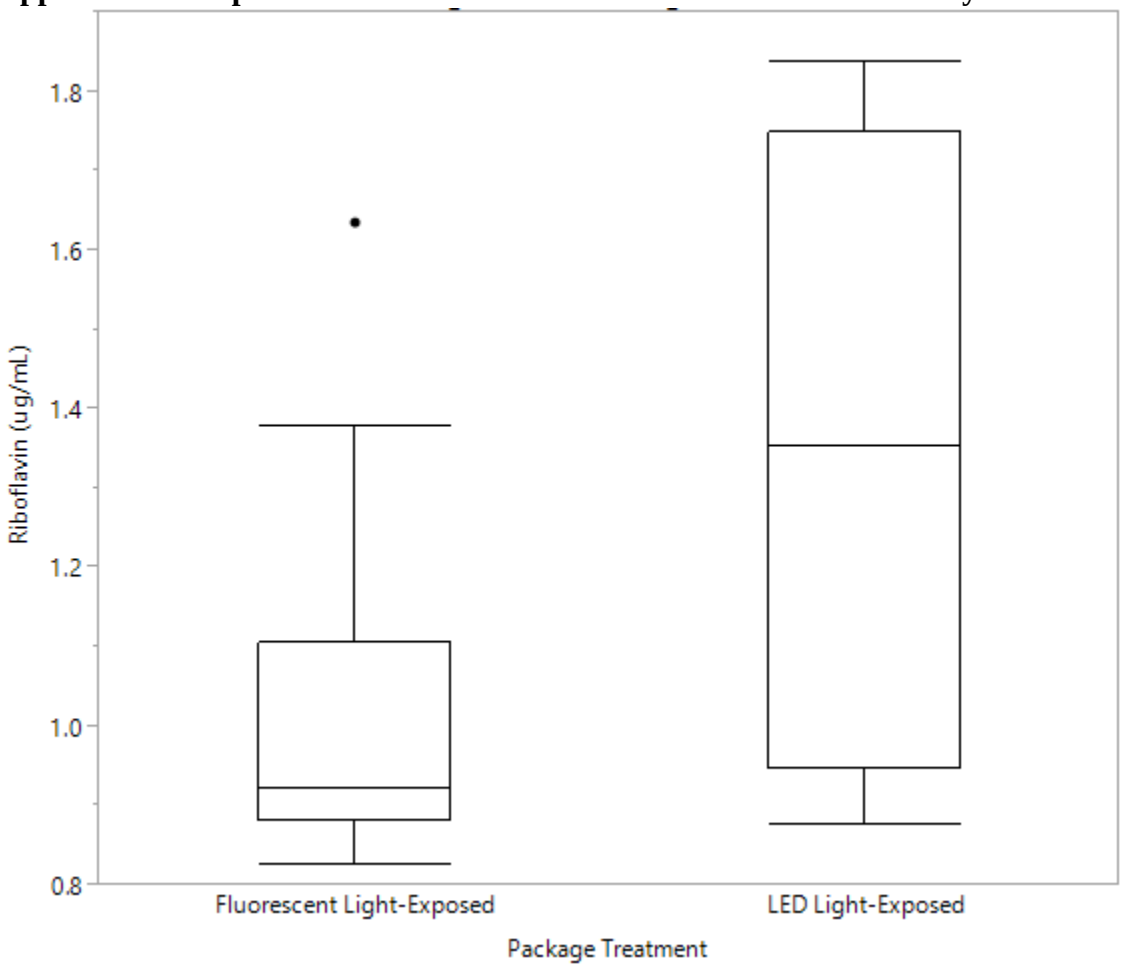

Figure Z: Boxplot of riboflavin amount in 2% milk in light-exposed packages under fluorescent light (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux). Two-sample t-test was used to analyze this data; significance indicated by $P < 0.05$.

Appendix AA: Consumer Acceptability of Light Protective Additive Packages

Table AA: Consumer acceptability of LPA packages (LP; light-protected, LE; light-exposed, Y; yellow, Low (1.3% TiO_2), High (4.9% TiO_2) under light (F; fluorescent, L; LED)

			95% Confidence		
	Mean	**SD**	**Lower**	**Upper**	**Median**
	Overall Liking				
LP-L	6.5[a]	1.62	6.22	6.77	7
High-L	6.6[a]	1.74	6.31	6.86	7
Yellow-L	6.4[ab]	1.77	6.14	6.69	7
Low-L	6.4[ab]	1.62	6.09	6.63	7
LE-L	6.3[ab]	1.76	6.01	6.56	7
LE-F	6.1[b]	1.84	5.79	6.34	6
	Appearance Liking				
LP-L	7.1[a]	1.32	6.89	7.31	7
High-L	7.2[a]	1.72	6.99	7.40	8
Yellow-L	7.1[a]	1.82	6.87	7.27	7
Low-L	7.1[a]	1.78	6.91	7.32	7
LE-L	7.0[a]	1.33	6.81	7.23	7
LE-F	7.0[a]	1.38	7.19	7.75	7
	Aroma Liking				
LP-L	6.4[a]	1.56	6.20	6.69	6
High-L	6.4[a]	1.56	6.20	6.69	6.5
Yellow-L	6.4[a]	1.46	6.20	6.93	7
Low-L	6.3[a]	1.57	6.01	6.50	6
LE-L	6.3[a]	1.59	6.07	6.56	6.5
LE-F	6.2[a]	1.57	5.96	6.45	6
	Flavor Liking				
LP-L	6.5[ab]	1.73	6.21	6.78	7
High-L	6.6[a]	1.73	6.36	6.93	7
Yellow-L	6.4[abc]	1.78	6.16	6.73	7
Low-L	6.3[abc]	1.82	5.99	6.57	7
LE-L	6.2[bc]	1.87	5.92	6.49	6.5
LE-F	6.1[c]	1.92	5.79	6.36	6
	Mouthfeel Liking				
LP-L	6.6[a]	1.62	6.38	6.88	7
High-L	6.6[a]	1.64	6.31	6.82	7
Yellow-L	6.6[a]	1.54	6.36	6.86	7
Low-L	6.6[a]	1.45	6.33	6.84	7
LE-L	6.4[a]	1.58	6.16	6.66	7
LE-F	6.4[a]	1.71	6.17	6.74	7
	Aftertaste Liking				
LP-L	6.1[a]	1.66	5.77	6.35	6

High-L	5.9[ab]	1.66	5.66	6.24	6
Yellow-L	5.8[abc]	1.89	5.52	6.09	6
Low-L	5.8[abc]	1.82	5.48	6.05	6
LE-L	5.6[bc]	1.91	5.32	5.90	5
LE-F	5.4[c]	1.86	5.15	5.72	5
	Aftertaste Intensity				
LP-L	2.3[b]	0.91	2.18	2.46	2
High-L	2.5[ab]	0.88	2.38	2.67	2
Yellow-L	2.6[a]	0.95	2.38	2.67	2
Low-L	2.6[a]	0.92	2.47	2.76	3
LE-L	2.5[ab]	0.88	2.36	2.64	2
LE-F	2.7[a]	0.85	2.52	2.80	3
	Freshness Perception				
LP-L	6.6[ab]	1.97	6.30	6.88	7
High-L	6.4[ab]	1.75	6.42	7.01	7
Yellow-L	6.3[b]	1.85	6.20	6.78	7
Low-L	6.7[a]	1.79	6.14	6.72	7
LE-L	6.4[ab]	1.84	6.09	6.67	7
LE-F	6.5[ab]	1.85	5.97	6.55	6

Appendix BB: Riboflavin Concentration for Consumer Study

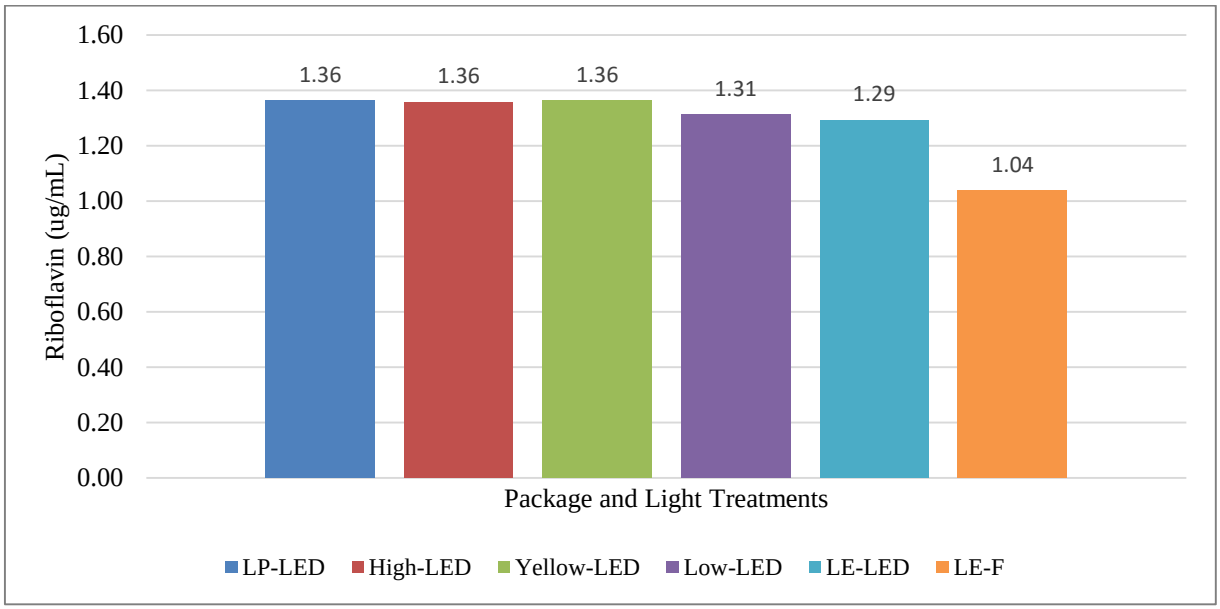

Figure BB: Riboflavin mean amount for package treatments and light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4 hr of light exposure

Appendix CC: TBARS Values for Consumer Study

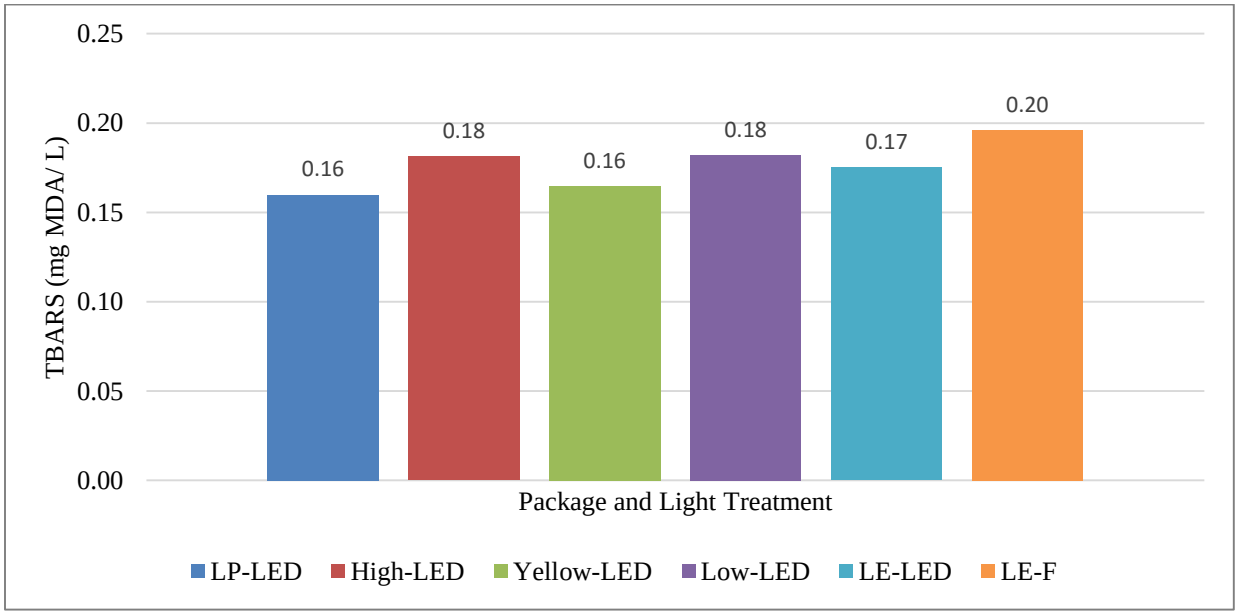

Figure CC: TBARS mean amount for package treatments and light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4 hr of light exposure.

Appendix DD: Dissolved Oxygen for Consumer Study

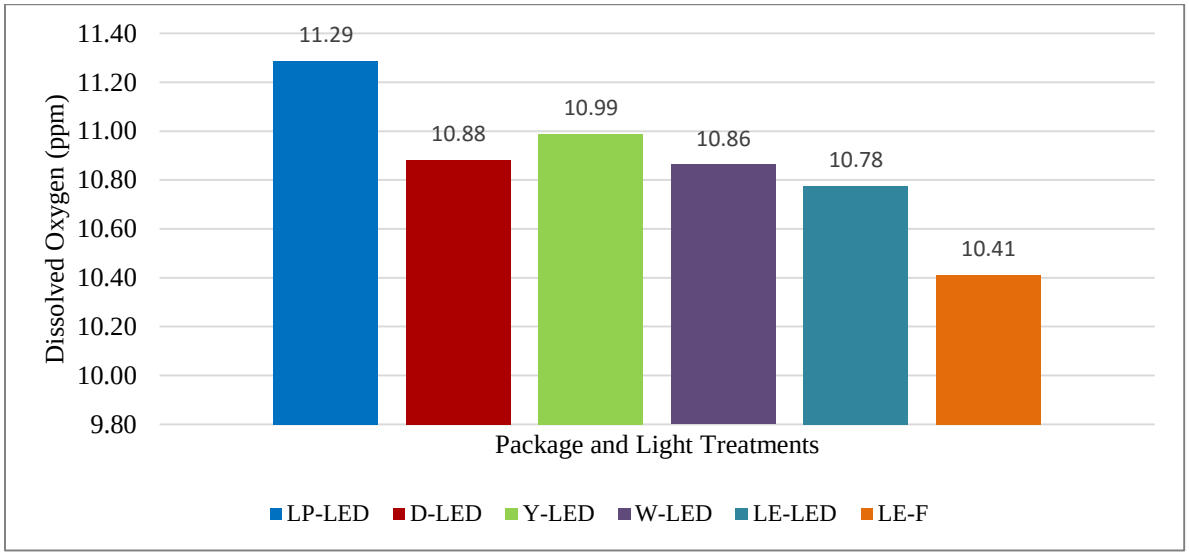

Figure DD: Dissolved oxygen (ppm) mean for light treatments (953 ± 54 lux) and light emitting diode (LED) (976 ± 56 lux)) at 4 hr of light exposure.

Appendix EE: Written copyright permission for optical wavelength spectrum.

VirginiaTech
Invent the Future

Kemia Amin <kamin2@vt.edu>

Fwd: Copyright permission for figure

2 messages

David Ingalls <dingalls@intl-lighttech.com> Tue, Feb 23, 2016 at 1:58 PM
To: kamin2@vt.edu

Kemia:

Thank you for requesting permission to use Figure 1.1 in the "The Light Measurement Handbook". With proper citation, permission is granted.

You have an interesting thesis - what are your findings regarding the effects of different light sources on milk in retail cases?

regards,

Dave Ingalls

Marketing Mgr.
ILT

---------- Forwarded message ----------
From: ilsales <ilsales@intl-lighttech.com>
To: David Ingalls <dingalls@intl-lighttech.com>
Cc:
Date: Tue, 23 Feb 2016 07:57:17 -0500
Subject: Fwd: Copyright permission for figure

>>> Kemia Amin <kamin2@vt.edu> 2/23/2016 6:39 AM >>>
Good morning,

I am a masters student at Virginia Tech University in the Food Science Department. I am studying the effects of retail case light (fluorescent and LED) on milk flavor and nutritional quality in various HDPE packages, I will be defending in March.

I read "the Light Measurement Handbook" by Alex Ryder and found Figure 1.1 The optical portion of the electromagnetic. I would like to ask for permission/copyright permission to use this figure as part of my thesis to help explain the different wavelengths of light. The figure will be cited and used upon approval.

This figure would aid in my explanation of lighting differences within retail cases. I am happy to answer any questions you may have.

Thank you
–

Kemia Amin

Master Candidate

Appendix FF: Written copyright permission for use of cool and warm LED wavelength spectrum.

VirginiaTech
Invent the Future

Kemia Amin <kamin2@vt.edu>

ABC's of LED figures

3 messages

Kemia Amin <kamin2@vt.edu> Tue, Feb 23, 2016 at 6:30 AM
To: hq@ledrise.com

Good morning,

I am a masters student at Virginia Tech University in the Food Science Department. I am studying the effects of retail case light (fluorescent and LED) on milk flavor and nutritional quality in various HDPE packages, I will be defending in March.

I read the article in LinkedIn by Victor Adrian Floroiu, "The ABC's of truly energy efficient LED lighting" and would like to ask permission/ copyright permission to use and reprint the Cool LED and Warm LED figure in my masters thesis. The figure will be cited and used upon approval.

This figure would aid in my explanation of lighting differences within retail cases. I am happy to answer any questions.

Thank you,
–
Kemia Amin
Master Candidate
Department of Food Science & Technology
Virginia Tech
865-696-9318

LedRise, Victor Floroiu <hq@ledrise.com> Tue, Feb 23, 2016 at 7:29 AM
Reply-To: hq@ledrise.com
To: Kemia Amin <kamin2@vt.edu>

Dear Kemia,

You have my permission to use.

I wish you great success with your masters thesis. After you have submitted it i would appreciate if i could quote it in a future article, provided you share your findings with me.
[Quoted text hidden]
–

Thank you,

Victor Floroiu,
Sales Manager

LedRise

Check out our Portfolio