

Effects of LED and Fluorescent Light on the Flavor of Milk
and Strategies to Mitigate Milk Oxidation.

A Thesis

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In Partial Fulfillment of the Requirements for the Degree of
Master of Science

by

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ABSTRACT

In this study, skim milk was exposed to LED and fluorescent light at 2000lx to compare their sensory thresholds, flavor profile and consumer acceptance.

Additionally, the effectiveness of PET packaging and antioxidants in milk exposed to LED was determined. The LED sensory threshold was shorter than the fluorescent threshold and when antioxidants (tocopherols and ascorbic acid) were added, the majority of the panelists failed to discriminate milk exposed to LED light. Trained panelists described light exposed milk as significantly higher in cardboard and plastic, with LED exposure resulting in a slightly more plastic aroma, and fluorescent slightly more cardboard. Consumers preferred fluorescent exposed samples over the ones exposed to LED. The antioxidant and the LED engineered light treatments resulted in significantly higher oil, however the former received higher packaging liking scores. The packaging treatment offered protection for 24 hours, with a similar flavor profile as unexposed milk, nevertheless consumers disliked its appearance.

BIOGRAPHICAL SKETCH

Ana Chang was born in Guayaquil, Ecuador in 1990. She studied Food Engineering at ESPOL and was the best graduate of her promotion. She was awarded with the Senescyt Scholarship and is part of the Programa Walter Valdano Raffo, both of which funded her Master in Food Science. She has always liked dairy so she trained on dairy sensory evaluation. On 2017, she won second place on the national dairy evaluation competition in milk, third place on the national dairy evaluation competition in butter and third place on the regional dairy evaluation competition in yogurt. Her research interests include sensory evaluation, product development and nutrition. In her free time, Ana enjoys singing, watching movies and hiking. She also loves to travel, especially to natural areas.

Dedicated

To

My family

For always supporting and encouraging me to succeed academically.

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1. Literature Review

Milk Production and Consumption

Milk production in the United States in April 2017 totaled 18.3 billion pounds, up 2.0 percent from April 2016 (Economic Research Service Staff, Economic Research Service, United States Department of Agriculture, 2017). However, fluid milk per capita consumption has been decreasing since 1975; down 37% in the previous 40 years (Figure 1). Some of this is offset by an increase in cheese and butter production, however people are largely consuming other beverages instead of milk ((Bauer, 2016; Economic Research Service Staff, Economic Research Service, United States Department of Agriculture, 2016; Fisher, Mitchell, Smiciklas-Wright, & Birch, 2001).

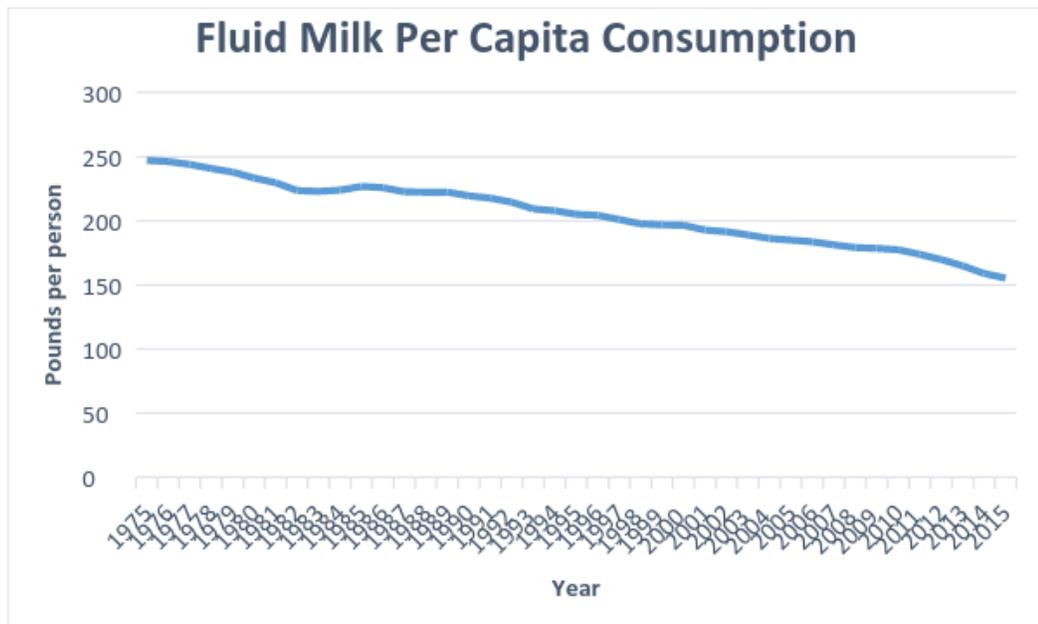


Figure 1: Fluid milk Per capita consumption in the United States. Fluid milk includes the product weight of beverage milks: whole, reduced fat, low fat, skim, flavored, buttermilk and miscellaneous (USDA, 2016).

Milk Composition

Milk is a vital source of energy, protein and fat, contributing on average 134 kcal of energy/capita per day, 8 g of protein/capita per day and 7.3 g of fat/capita per day worldwide in 2009 (FAOSTAT, 2012). The main component is water, followed by lactose. Cow milk accounted for 83% of global milk production in 2010 (FAOSTAT, 2012). Moreover, it contains more calcium, phosphorus, riboflavin and protein than human milk and its protein is of high-quality, with a good balance of all essential amino acids. Also, it is a source of other micronutrients including selenium, magnesium and vitamin B12. Human and cow milk composition is shown in Table 1, with vitamin and mineral composition in Table 2 (FAO, 2013).

Table 1. Proximate composition of human and cow milks (per 100 g of milk).

Proximates	Human	Cow	
	Average	Average	Range
Energy (kJ)	291	262	247–274
Energy (kcal)	70	62	59–66
Water (g)	87.5	87.8	87.3–88.1
Total protein (g)	1.0	3.3	3.2–3.4
Total fat (g)	4.4	3.3	3.1–3.3
Lactose (g)	6.9	4.7	4.5–5.1
Ash	0.2	0.7	0.7–0.7

(FAO, 2013)

Table 2. Vitamin and mineral composition of human and cow milks (per 100 g of milk).

	Human	Cow	
	Average	Average	Range
<i>Minerals</i>			
Calcium (mg)	32	112	91–120
Iron (mg)	Tr	0.1	Tr–0.2
Magnesium (mg)	3	11	10–11
Phosphorus (mg)	14	91	84–95
Potassium (mg)	51	145	132–155
Sodium (mg)	17	42	38–45
Zinc (mg)	0.2	0.4	0.3–0.4
Copper (mg)	0.1	Tr	Tr–Tr
Selenium (µg)	1.8	1.8	1.0–3.7
Manganese (µg)		8	4–10
<i>Vitamins</i>			
Retinol (µg)	60	35	29–45
Carotene (µg)	7	16	7–23
Vitamin A (µg RE)	61	37	30–46
Vitamin E (mg)	0.08	0.08	0.07–0.08
Thiamin (mg)	0.01	0.04	0.02–0.04
Riboflavin (mg) (vit B ₂)	0.04	0.20	0.17–0.20
Niacin (mg)	0.18	0.13	0.09–0.20
Niacin equivalent (mg)		0.79	0.70–0.80
Pantothenic acid (mg)	0.22	0.43	0.34–0.58
Vitamin B ₆ (mg)		0.04	0.03–0.06
Folate (µg)	5.0	8.5	5.0–8.0
Biotin (µg)		2.0	1.4–2.5
Vitamin B ₁₂ (µg)	0.05	0.51	0.25–0.90
Vitamin C (mg)	5.0	1.0	0.0–2.0
Vitamin D (µg)	0.1	0.2	0.1–0.3

(FAO, 2013)

Milk Quality

Milk quality is a complex term, depending on several factors, including chemical composition, microbial content, somatic cell count and flavor. To assess the quality of milk, important methods include: beta lactam test, somatic cell count, standard plate count and sensory evaluation. High somatic cell counts are an indicator of mastitis, which alter milk composition, yield and shelf-life. The current regulatory limit for raw milk in the United States defined in the Grade “A” Pasteurized Milk Ordinance is 750,000 cells per milliliter. On the other hand, Standard Plate Count (SPC) is used as an estimate of the total number of viable aerobic bacteria present and should not exceed 100,000 cfu/ml. Microbial contamination causes milk spoilage by degrading milk components. This may result in milk with off-flavors which can also be unsafe for consumption.

Milk flavor defects are generally categorized in 4 groups by the causes of the different off-flavors: absorbed, bacterial, chemical and delinquency. Delinquency examples are flat or foreign, absorbed flavors are related to poor milk storage conditions and inadequate farm management practices (i.e., barny or cowy), while chemical off-flavors are caused by metals or oxidizing factors such as light (Alvarez, 2009).

Off-Flavors Development in Light Exposed Milk

Milk oxidation occurs due to its photosensitive components, which include: protoporphyrin, hematoporphyrin, uroporphyrin, chlorophyll and riboflavin (Wold et al., 2005). From these, riboflavin is the most studied with the mechanism by which off-flavors are developed well known. Photosensitizers in milk absorb energy from light, become excited and then undergo an intersystem crossing to an excited triplet-state. At this point, the excited triplet sensitizer can follow the two pathways shown in Figure 2. In Type I, the triplet sensitizer reacts with

milk components by transferring and accepting hydrogen or electron to produce free radicals or free-radical ions, causing a free-radical chain reaction. This pathway is favored in milk due to the reduced availability of oxygen (Lledias & Hansberg, 2000; Min & Boff, 2002). Type II occurs when it reacts with triplet oxygen to form singlet oxygen, while the sensitizer returns to ground state.

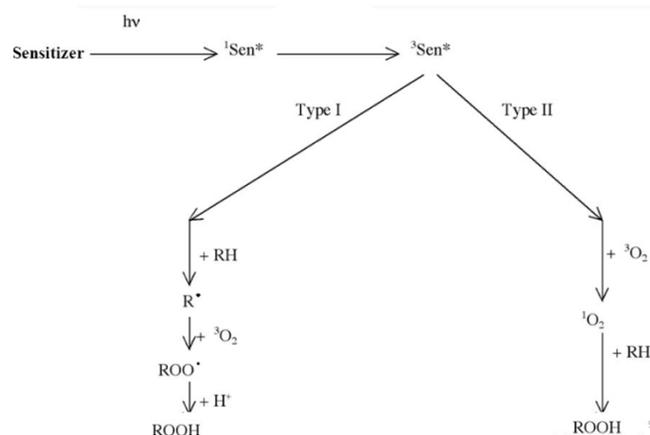


Figure 2: Formation of excited triplet sensitizer ($^3\text{Sen}^*$) and its reaction with substrate via type I and Type II reactions (Sharman et al., 2000).

Light oxidized characteristic off-flavors have been reported to be produced by the breakdown of proteins and lipids in milk. Chemical changes in amino acids result in an off-flavor commonly described as light activated. Singlet oxygen reacts with tryptophan, histidine, tyrosine, methionine, and cysteine (Michaeli & Feitelson, 1995, 1997). It has been reported that light activated flavor is caused by methanethiol, dimethyl sulfide, and dimethyl disulfide, which are products of the reaction between singlet oxygen and methionine (Foote, 1976; Samuelson, 1962). Dimethyl disulfide has been highly correlated with the sunlight flavor and has been described as boiled cabbage and burnt feather odor (Forss, 1978; Friedrich & Acree, 1998; Jung, Yoon, Lee, & Min, 1998). Light activated milk has been described as burnt, burnt feathers, cabbage, plastic or mushroom (Dimick, 1982). Jung et al. (1998) found that the

presence of riboflavin and light are necessary for the breakdown of methionine to occur. Milk fat degradation contributes to light-oxidized off-flavors. Unsaturated fatty acids and esters are oxidized by singlet oxygen. The number of double bonds is positively related to the reaction rates of these molecules (Min & Boff, 2002). UFA oxidation products are: aldehydes, ketones and alcohols such as 2-methylpropanal, n-pentanal, n-hexanal and 1-octen-3-one (J. H. Lee & Min, 2009; van Aardt et al., 2005). The respective odor descriptors for these compounds are dark chocolate, cut-grass, green and mushroom (Cadwallader & Howard, 1998). On the other hand, fat content has a protective effect because the light-degradation rate of riboflavin is slower as lipid content increases (Allen & Parks, 1979; Gaylord, Warthesen, & Smith, 1986).

Sensory Evaluation of Light Oxidized Milk

Sensory thresholds of light oxidized flavor defects in 2% milk have been calculated by Chapman et al. (2002) and Heer et al. (1995). Untrained panelists were able to detect off-flavors between 54 min and 2 h. However, trained panelists needed only 15 to 30 min of light exposure to detect off-flavors in fluorescent light exposed milk (Chapman, Whited, & Boor, 2002). No reports on thresholds for LED exposed milk have been published. Nevertheless, consumer analysis of light exposed milk has been performed by several authors. Martin et al. (2016) reported a decrease in liking for LED exposed milks, even more than microbial contaminated milk. Brothersen et al. (2016) exposed 1% milk to LED at 4000 lx and fluorescent light at 2200 lx for 12 and 24 hours, these samples were then assayed via consumer and descriptive sensory. Flavor liking for fluorescent light exposed milk dropped at 12 h of exposure, whereas milk exposed to LED showed a significant drop at 24 h of exposure. Similarly, Potts et al. (2017) discovered that overall liking was significantly

penalized only for fluorescent light exposed milk.

The negative effect of light in milk has been documented via descriptive analysis as well. Martin et al. (2016) performed descriptive analysis with 12 panelists. They found that the following sensory attributes were significantly different between non light exposed and light exposed samples: cream, plastic, putrid, white, visual thickness, sweet taste, sweet aftertaste and mouth coating. Other terms that have been reported to be predominant attributes in light exposed milks are: mushroom, cooked, milkfat, butterscotch, cardboard, and, astringency (Brothersen, McMahon, Legako, & Martini, 2016; Cadwallader & Howard, 1998).

Color changes in milk are evident after light exposure. The milk turned slightly brown after Toba et al. (1980) exposed milk for 1-2 h to direct sunlight. Additionally, Lee et al. (1998) determined the Hunter L, a, b values in milk exposed to fluorescent light at 3300 lux for 1, 2, 3, 5, and 10 h. As a result, the absolute values of lightness (L), greenness (a) and yellowness (b) decreased after 10 h of illumination (K. Lee, Jung, & Kim, 1998). Likewise, Mestdagh et al. (2005) found a decrease in the b value and an increase in the a value (increase in red color). In this study, milk was exposed to fluorescent light at 2500 lux for several days. This color change has been described to be caused by the degradation of yellow/green colored compounds like riboflavin, β -carotene and vitamin A. An increase in red color could be due to the browning during tryptophan and tyrosine degradation (Mestdagh, De Meulenaer, De Clippeleer, Devlieghere, & Huyghebaert, 2005; Toba, Adachi, & Arai, 1980).

Nutritional Losses

Milk oxidation causes off-flavors and nutritional losses at the same time. Vitamins A, C, D, and E can be destroyed due to riboflavin oxidation (Choe, Huang, & Min, 2005). Riboflavin losses have been reported to be as high as 80% and ascorbic acid can be totally lost, depending

on the light intensity, wavelength, exposure time and package (Herreid, Ruskin, Clark, & Parks, 1952). Riboflavin maximum absorbance range is from 400 to 550 nm with a wavelength of 450 nm found to be the most destructive (Maniere & Dimick, 1976; Min & Boff, 2002; Webster, Duncan, Marcy, & O'Keefe, 2009). Wold et al. (2005) reported that porphyrins and chlorins become excited in the 600-750 nm region, which they found to have the highest correlation with oxidized flavors. However, these photosensitive molecules are more effectively excited at wavelengths between 400-420 nm (Wold et al., 2005).

Strategies to Mitigate Milk Oxidation

The most studied strategies to protect milk from light oxidation are antioxidant enrichment and the use of protective packaging materials. The effectiveness of antioxidants on milk flavor has been investigated by Hall et al. (2009), van Aardt et al. (2004), Jung et al. (1998) and Lee et al. (1998). It has been reported that ascorbic acid can lower the formation of dimethyl disulfide by acting as a singlet oxygen quencher (Jung et al., 1998). Moreover, Hall et al. (2009) reported that ascorbic acid is a more effective quencher than trolox and that both antioxidants protected milk from riboflavin destruction in a concentration dependent manner. In a similar way, α -Tocopherol with ascorbic acid protected milk for 10 h of fluorescent light exposure (van Aardt et al., 2005). Tocopherols have demonstrated the ability to prevent lipid oxidation (Ho Lee, Soon An, Cheol Lee, Jin Park, & Sun Lee, 2004; Min & Boff, 2002).

Packaging materials can prevent or reduce off-flavor development in milk. Translucent materials such as clear glass and transparent HDPE readily transmit light, allowing food oxidation to occur. HDPE and paperboard are commonly used in milk packaging, despite the fact that these materials will still transmit 57% and 4% of fluorescent light respectively

(Brothersen et al., 2016). Milk producers are now selecting protective materials and/or adding pigmentation to their packages as a way to mitigate milk oxidation (Shropshire, 2017). It has been reported that three-layered (white-black-white) polyethylene terephthalate (PET) can successfully protect milk from oxidation for 60 days at 2500 lux (Mestdagh et al., 2005). Furthermore, the addition of titanium dioxide has been studied. 1.3% TiO₂ treated HDPE packages protected milk for 4 h of LED light at 1460 lux (Potts et al. 2017). However, Johnson et al. (2015) reported that 1.3% TiO₂ addition is not as effective as a high TiO₂ package (4.3%), which protected milk for 22 days of exposure to fluorescent light at 2,186 lx (Johnson et al., 2015). Tinted packages provide additional protection, besides consumers prefer white or cream colored containers for milk (White, 1985).

2. Introduction

Cow's milk is consumed around the world due to its nutritional composition; it is a major source of dietary energy, protein and fat. Moreover, it contains more calcium, phosphorus, riboflavin and protein, than human milk and its protein is of high-quality. Also, it is a source of other micronutrients like selenium, magnesium and vitamin B12. Per capita milk consumption has increased worldwide from 1987 to 2007 (FAO, 2013). However, in the United States it decreased by 30% since the 1970s (Stewart, Dong, & Carlson, 2013) and in 2015 low fat and skim milk sales dropped 10%. Part of the reason behind this is increasing consumption of other beverages and flavored milk (Bauer, 2016; Fisher et al., 2001). More importantly, this trend may be influenced by milk quality at the moment of purchase, which is known to decrease rapidly due to a number of factors, one of which being light oxidation. As a result, Americans are not fulfilling their daily recommended intake of dairy products (Stewart et al., 2013). Milk flavor and nutritional content are affected by light. This process begins with porphyrins, chlorins, riboflavin and other photosensitive components (Wold et al., 2005), once activated, produce singlet oxygen that can react with proteins, vitamins and lipids (Choe et al., 2005). The off-flavors generated are mainly attributed to amino acid (AA) oxidation which form methionine sulfoxide and dimethyl disulfide and unsaturated fatty acid (UFA) oxidation that produce aldehydes and ketones (Jung et al., 1998; Min & Boff, 2002; van Aardt et al., 2005). Some combination of aromatic compounds can be perceived after between 54 and 120 minutes of fluorescent light exposure at 2000 lx (Chapman et al., 2002).

LED light usage in retail stores is increasing, but little is known about the effects of this type of illumination in milk. Previous studies have shown that consumers respond negatively to milk exposed to light. Brothersen et al. (2016) found that fluorescent when compared to LED light had faster and higher production of off-flavor generating compounds. Besides this, only fluorescent light reduced riboflavin and vitamin A content. On the other hand, Martin et al. (2016) discovered that consumers prefer milk with microbial defects over LED-exposed milk. Consequently, there is a necessity to characterize and compare the sensory and nutritional impact of both types of light on fluid milk, and moreover to determine possible protective strategies to ensure milk quality.

Packaging materials and antioxidants have been studied as possible interventions to prevent milk off-flavors from developing during milk shelf-life. Potts et al. (2017) exposed milk for 4 h to LED and fluorescent light (1460 lx) in HDPE and PET packages with and without TiO₂. Consumers overall acceptability only decreased for the fluorescent light condition in both PET and HDPE (Potts, Amin, & Duncan, 2017), indicating pigmentation effectively protected the product. In a similar manner, a mixture of tocopherol and ascorbic acid was able to avoid off-flavor development after 10 h of fluorescent light exposure (1100 to 1300 lx) (van Aardt et al., 2005). Oxidation is mitigated because antioxidants react with singlet oxygen protecting the photosensitive compounds (Hall, Chapman, Kim, & Min, 2010).

The purpose of this study is (1) to determine sensory thresholds for exposure to fluorescent and LED light in skim milk, plus that treated and untreated with

antioxidants. (2) compare the flavor profile via descriptive sensory of milk exposed to three retail lighting conditions (LED, LED2 and fluorescent), as well as determine the effectiveness of PET packaging and antioxidants in milk exposed to LED. Finally, (3) characterize consumer acceptance for all conditions.

3. Methods

3.1 Sensory Thresholds

The objective was to determine the minimum exposure time required to generate light-oxidized flavors that can be detected by the consumer.

Three sessions were scheduled to find the sensory threshold for each of the following: Fluorescent, LED and LED with antioxidants.

For each session, 33 half-gallon bottles of skim milk were purchased from the Cornell dairy plant. The milk came from the same production run and was processed a week before sample testing. Control milk bottles were wrapped in aluminium foil and were not exposed to light. Milk was stored at 4°C in HDPE containers.

The bottles for the fluorescent light treatment group were stored at 3 to 6°C in front of fluorescent lights (Laboratory Supplies Co.) at 2000 lux \pm 100 lux. The LED bottles were placed in a glass-front refrigerator illuminated with LED (Zhejiang Yankon

Group Co.,Ltd) at 2000 lux \pm 100 lux. Finally, for the LED with antioxidants treatment mixed tocopherols and L-ascorbic acid were purchased from SIGMA-ALDRICH. Skim milk was spiked with 0.025% of mixed tocopherols and 0.025% of L-ascorbic acid. Milk was warmed to 50°C in stainless steel pots before antioxidants were added and blended with a hand blender for 30 s. Spiked milk was returned to HDPE containers and then exposed to LED light.

30 ml of control and light-exposed milk samples were poured into 5oz plastic cups with plastic lids and stored at 4°C in carton boxes until sensory testing.

An ascending exposure forced choice method was used. Participants were presented with sets of three samples in which two samples were the same (control) and one different (light-exposed). They had to choose the different sample before moving to the next set. Skim milk was exposed to LED and fluorescent light for 0.5, 1, 2, 4, 8, 12, 24 and 48 hours. Participants evaluated the sets starting with the least exposed (0.5 hour) sample and two controls, and each successive set contained the sample with the next level of exposure, ending with the most exposed (48 hour) sample. Samples were presented at 4 to 6°C.

Each session was conducted on separate days, panelists were recruited from Cornell University. Participants received a \$5 compensation for each session, and a \$10 bonus if they attended all three sessions. 56 panelists attended the fluorescent session, 67

panelists the LED session and 66 panelists the LED with antioxidants session. All panelists agreed to participate by reading and signing a consent form approved by the Cornell Institutional Review Board for testing with human subjects.

Testing was done at the Cornell Sensory Center, participants performed the test on separate sensory booths on computers using RedJade sensory software. Instructions were presented before the test and with each set of samples. Participants were asked to smell and taste the samples on the order presented, from left to right and to pick the different sample, based on smell and taste. They were asked to expectorate the samples and clean their palate with water after tasting each sample. The presentation order was randomized for every panelists and sample set. After selecting the odd sample, they were asked to consume crackers and water to cleanse their palates during a mandatory 10 s break.

The sensory threshold for each treatment was calculated as the geometric mean of the individual thresholds. The individual thresholds were calculated as the geometric mean of the last incorrect judgement and the value at which they first answered correctly and all higher concentrations were also correct. For the panelists that answered incorrectly all the questions, the geometric mean of the highest level (48 hours) and the next level that would have been used (96 hours) was calculated (Lawless & Heymann, 2010).

3.2 Colorimetry

Milk exposed to fluorescent and LED light for 0.5, 1, 2, 4, 8, 12, 24 and 48 hours was examined using a Macbeth Color-eye with Hunter Lab scaling. Milk was poured to an Erlenmeyer flask and heated to 25°C in a hot plate before testing. Hunter L, a, and b values were determined in duplicate and an average of these values was calculated. Pearson's correlation was calculated between the exposure time and colorimeter readings.

3.3 Descriptive Analysis

Descriptive sensory evaluations were performed, by a trained panel, on 9 skim milk samples. Each sample was presented in duplicate and panelists rated a total of 6 samples per session. There were three testing sessions, which occurred on different days. This analysis was conducted in accordance with Cornell University's Institutional Review Board's guidelines.

A total of 12 panelists participated, 6 of them were members of the Cornell Voluntary Shelf Life Milk Panel and 2 had previous milk sensory training. The other 4 panelists were screened (odor and taste) and selected from Cornell's staff and students.

Panelists were trained for a total of 24 hours with sessions that included ballot training, followed by identification and rating of 16 attributes in physical references

and spiked samples. The attributes were divided into five categories: aroma, appearance, taste, aftertaste and texture. Old oil, plastic aroma and cardboard taste are related to light exposure. Training was performed using Compusense At-Hand feedback calibration on iPad minis (Findlay, Castura, & Lesschaeve, 2007). Panelists were also asked to comment on and rate additional attributes in all the categories to prevent dumping into incorrect categories. Unstructured 10 point-line scales were used to rate all attributes during both the training and testing periods.

For testing, panelists used Compusense At-Hand sensory evaluation software. During testing sessions, panelists were situated on individual sensory booths. 4.5 gallons of skim milk were obtained from the Cornell dairy plant and were stored for a week at 4°C before evaluation. Milk was exposed to fluorescent and two LED lights: 3500 K, (Zhejiang Yankon Group Co.,Ltd) and an engineered LED light (LED2), 4669 K with a dominant wavelength of 580nm, centroid wavelength of 550nm and a peak wavelength at 479nm (Envirolux Energy Systems). Milk was exposed for 24 hours at an intensity of 2000 lux \pm 100 lux. Milk with antioxidants and in a PET bottle (PolyOne) was also exposed to LED. Samples were poured under low lighting on 5oz plastic cups with plastic lids. Before testing, all samples were heated to 15-21°C and swirled. Then, panelists were instructed to smell and evaluate visual attributes. After this, they tasted the sample and rated taste attributes. Finally, they resampled, and rated aftertaste and mouthfeel. Breaks were included during the test to minimize fatigue. Also, panelists were instructed to cleanse their palates with crackers and

water. Sample order was randomized for each panelist and repetition. Participants received a \$10 compensation for hour of training and testing session.

3.4 Consumer Analysis

10.5 gallons of skim milk were obtained from the Cornell dairy plant and stored for eight days at 4°C before testing. Milk was exposed to fluorescent and two LED lights for 24 hours at an intensity of 2000 lux \pm 100 lux. Milk with antioxidants and in a PET package (PolyOne) was also exposed to LED. Samples were poured under low lighting on 5oz plastic cups and covered with plastic lids. Samples were served at the range of 4 to 6°C.

A total of 130 participants from Cornell University completed the test. Consumers were selected with the following restrictions: no milk allergy or intolerance, no taste or smell disorders and drinkers of milk. This analysis was conducted in accordance with Cornell University's Institutional Review Board's guidelines and each panelist received a \$5 compensation.

Panelists first observed 7 milk bottle pictures, each showing a treatment condition: fluorescent light exposed, LED exposed (4669 K, Envirolux Energy Systems), protective packaging, LED exposed (3500 K, Zhejiang Yankon Group Co.,Ltd) and milk with antioxidants LED exposed (3500 K, Zhejiang Yankon Group Co.,Ltd). The last two conditions were duplicated to serve as controls. The pictures were presented

in random order. After each picture the respondent was asked about the overall liking of the appearance of the product and purchase intent. Then, they tasted 7 milk samples in a randomized complete block design and evaluated each sample for overall liking (appearance, overall acceptability, aroma and flavor) and freshness perception on a 5 point scale (1=not fresh; 5=fresh). They were also asked Just About Right (JAR) questions to rate the level of whiteness, visual consistency and oral thickness (5 point JAR), if they detected any off-flavor or off-aroma and purchase intent. At the end of the test, respondents answered demographic and usage questions. Each session lasted approximately 30 minutes.

3.5 Statistical Analysis

Statistical analysis was conducted using XLSTAT and Microsoft Excel. One-way ANOVA and T-tests were calculated to evaluate if the treatments were significantly different ($P < 0.05$). Principal components analysis, cluster analysis and preference mapping were completed in XLSTAT statistical analysis software to understand the relationships among the sensory attributes and consumer's preferences. Tukey's HSD and Fisher's LSD in XLSTAT were employed to analyze the difference between the treatments for each attribute.

4. Results

4.1 Sensory Thresholds

The sensory threshold for detecting fluorescent light-exposed milk was 12 hours. This was not in agreement with previous findings of around 2 hours (Chapman et al., 2002; Heer, Duncan, & Brochetti, 1995). However, it is important to mention that the method and the milk fat content used in this study were different. Chapman et al. (2002) used the semi-ascending paired difference method; in this method panelists are given a reference and 3 samples, then they are asked to compare each sample to the reference and rate the intensity of the difference (Chapman et al., 2002). It has been shown that panelists have better sensitivity in detecting differences when they have been provided a reference. Besides, when the threshold is based on statistical significance, it tends to be lower than the mean of individual thresholds, due to the large number of observations (Lawless & Heymann, 2010).

The threshold for LED light was 9 hours, meaning people can detect off-flavors of LED-exposed milk sooner than when exposed to fluorescent light. Despite this, the addition of antioxidants resulted in significantly fewer panelists discriminating the LED exposed sample from controls vs either LED or fluorescent (both $p < 0.001$), and a 49-hour threshold. It should be noted however that the control samples for this test also contained antioxidants, and thus panelists may find this formulation inherently less pleasant.

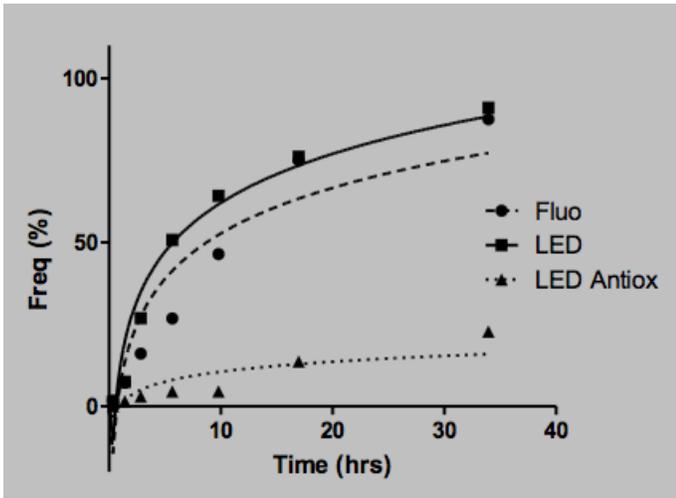


Figure 3: Results from the threshold tests.

4.2 Colorimetry

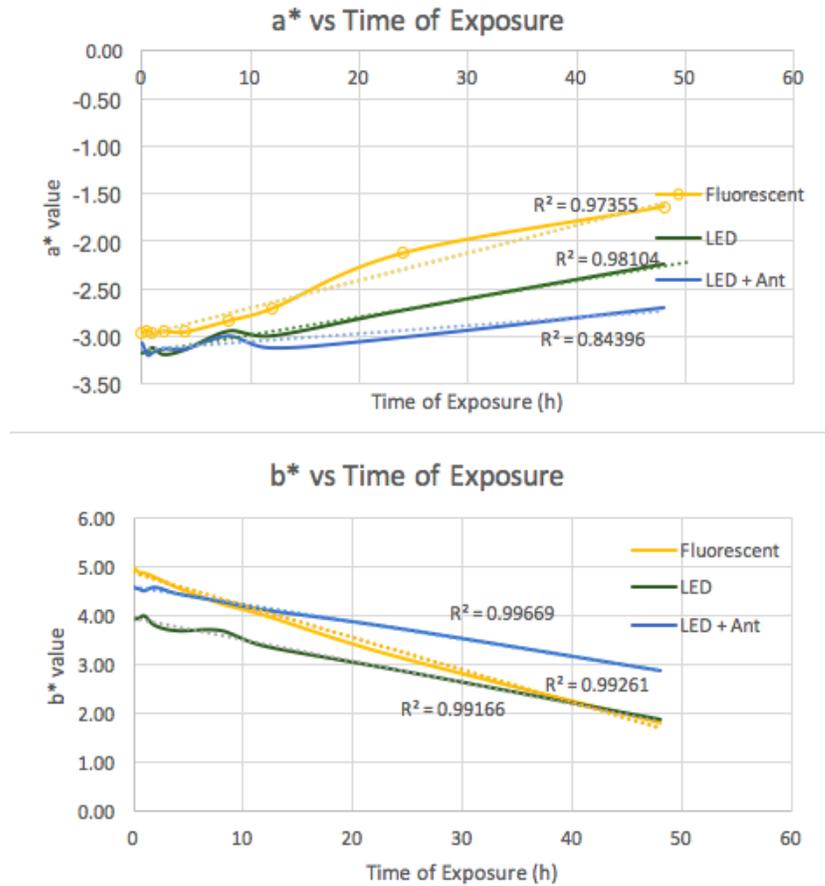


Figure 4a: a* values of skim milk with (LED only) and without antioxidant supplementation after exposure to LED and fluorescent light (2000 lux). Figure 4b: b* values of skim milk with (LED only) and without antioxidant supplementation after exposure to LED and fluorescent light (2000 lux).

The same trend was observed for all the treatments: The a* value increased with exposure time (becoming more red and less green) and the b* value decreased when time of exposure increased, milk samples were becoming more blue (Figure 4). The a* value of fluorescent light-exposed milk changed faster and more, followed by LED and finally LED + ant.

4.3 Descriptive Analysis

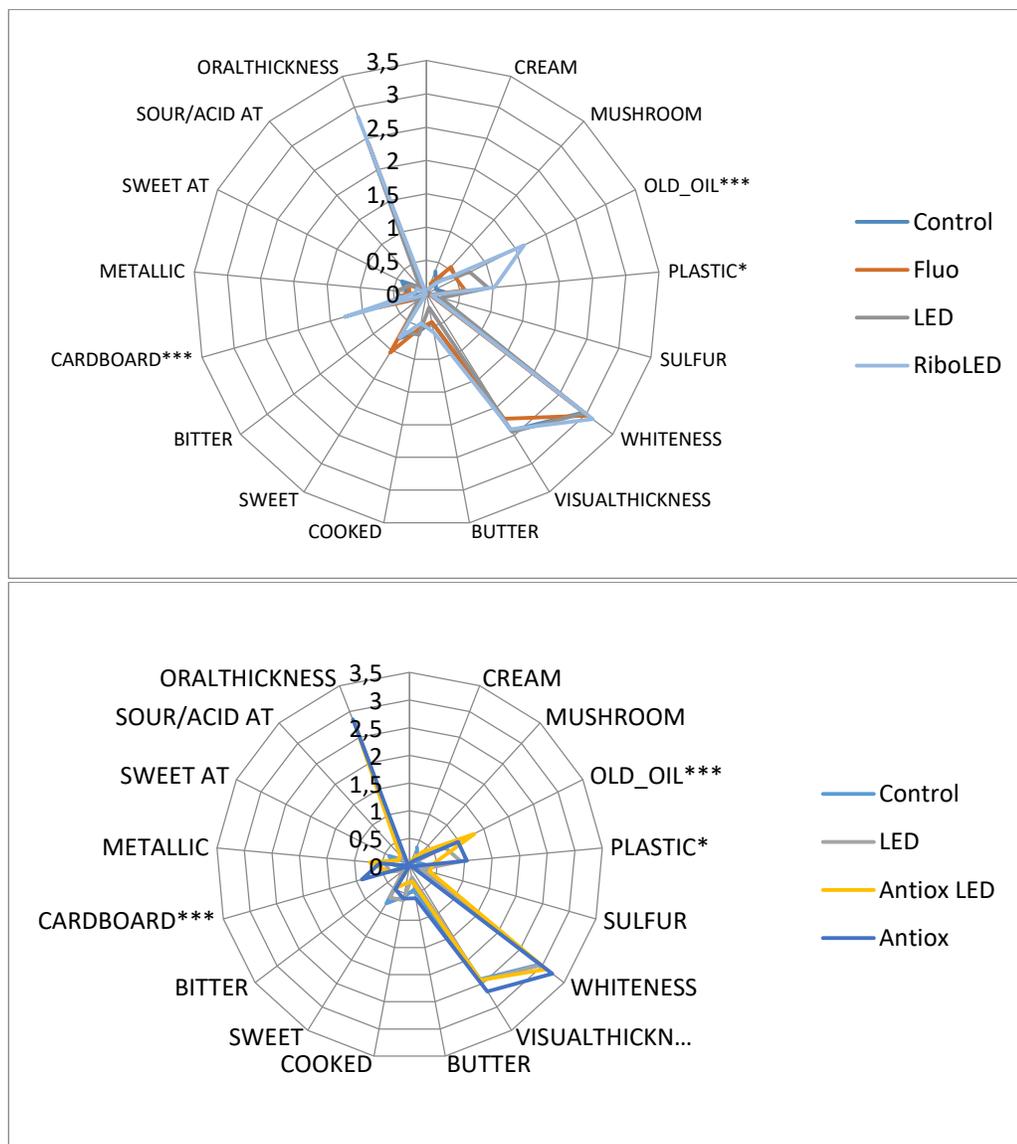


Figure 5a: Descriptive attributes of light-exposed skim milk. Figure 5b: Descriptive attributes of LED light-exposed skim milk with antioxidants. The *, **, *** indicate significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

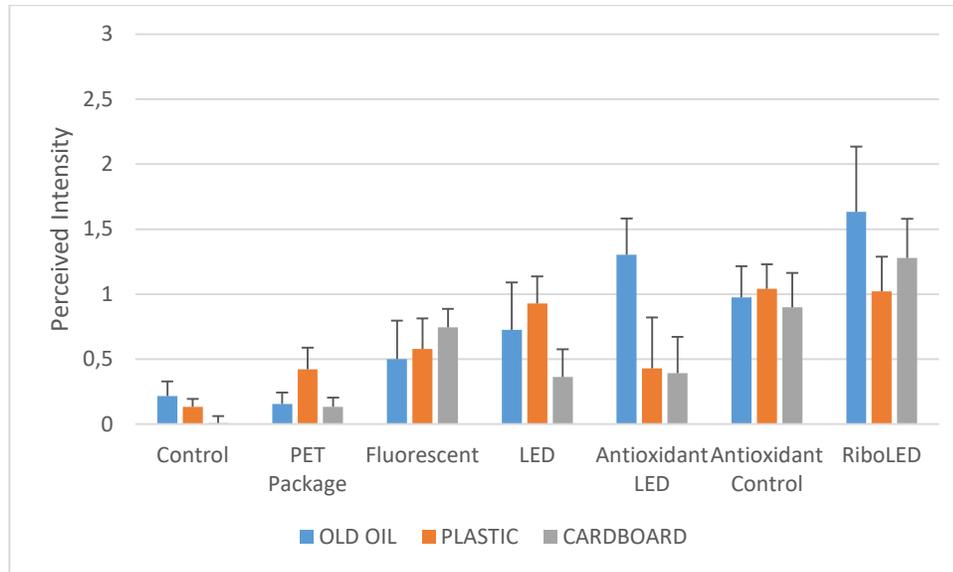


Figure 6: The old oil, plastic and cardboard attributes of light-exposed milk.

The old oil aroma was significantly different between treatments (ANOVA, $p < 0.0001$). This aroma was significantly higher in RiboLED exposed milk than the control, as well as the packaging treatment (Tukey Test, $p < 0.01$). Moreover, there was a significant difference for this attribute when comparing antioxidant supplemented milk with control milk (Fisher's LSD Test, $p < 0.05$). The plastic aroma was significantly higher in LED and RiboLED exposed milk than in control milk (Fisher's LSD Test, $p < 0.05$). Finally, in RiboLED exposed milk cardboard aftertaste was significantly higher when compared to the control and packaging (Tukey Test, $p < 0.0001$). Fluorescent samples also shown higher cardboard, but this difference was not significant.

PET packaging resulted in very similar ratings to controls, it protected milk from light damage for 24 hours from both LED and fluorescent lighting exposures.

Multidimensional Analysis

Principal Component Analysis was performed to visualize and understand the interrelationships of the attributes. The first two factors accounted for 80% of the variation, representing (1) cardboard, whiteness, plastic, old oil, sweet aftertaste, cream, and oral thickness; (2) sulfur. When localizing the samples in the multidimensional space, control milk and packaging were low in both factors, while LED2 and antioxidant control were high in factor 1 (Figure 7). Antioxidant LED and LED exposed milk were high in “sulfur”, while the fluorescent exposed sample is low on this factor.

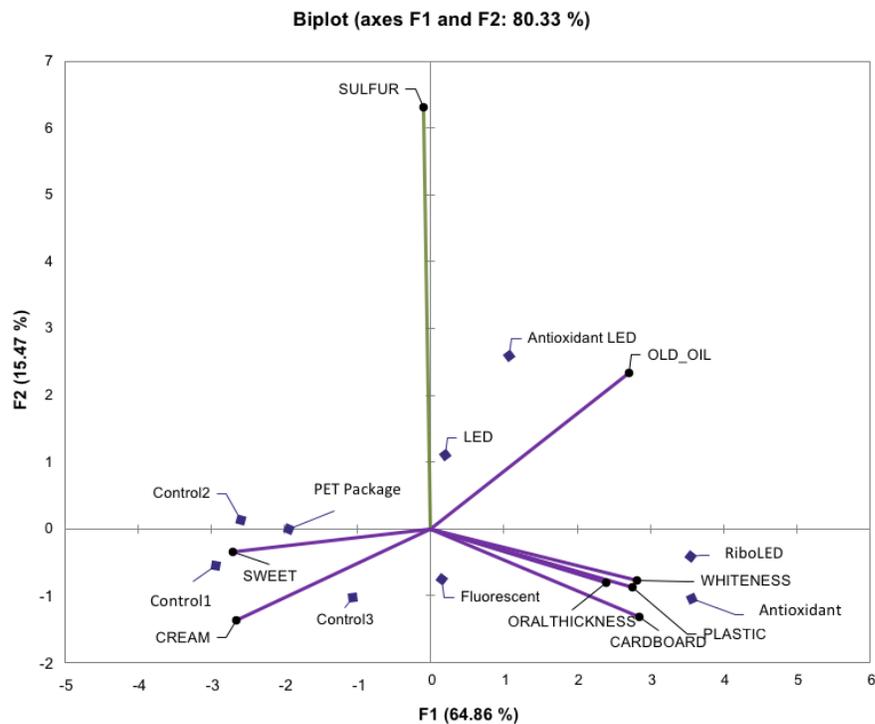


Figure 7: Principal Components Analysis Biplot

4.4 Consumer Analysis

Flavor, aroma and overall liking were significantly different between treatments (ANOVA, $p < 0.0001$). Consumers reported a higher preference for control and milk in protective packaging, followed by antioxidant enriched milk, fluorescent light-exposed milk, and LED light-exposed milk (Figure 8). In accordance with the descriptive panel, consumers didn't show a preference between control and packaging.

Table 3. Overall Liking for all Treatments.

	Antiox Control	Antiox LED	Control	Fluorescent	LED	LED 2	Packaging
Mean	5.14	4.51	6.02	5.13	4.73	4.00	6.32
Post hoc	B	C	A	B	C	D	A

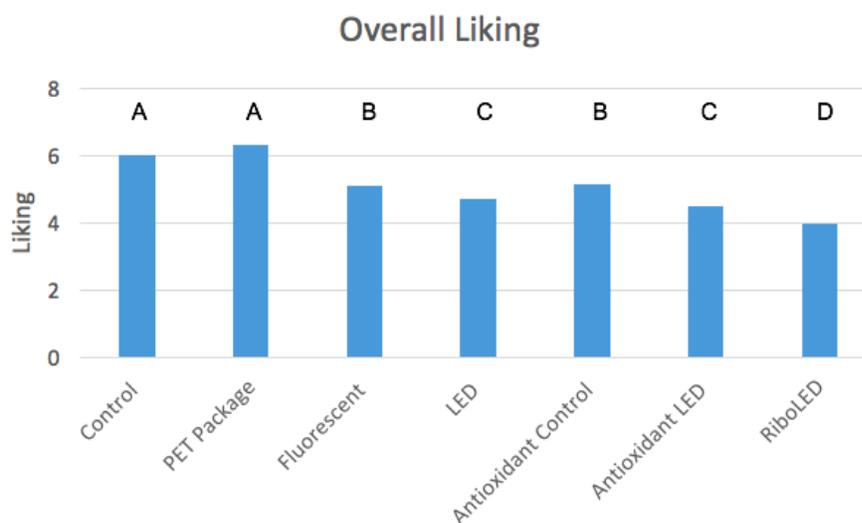


Figure 8: Overall liking for all treatments.

Preference Mapping

Cluster analysis identified 2 main groups of consumers (Figures 9, 10). The first cluster comprised by 27 consumers, preferring LED over fluorescent exposed milk, while the remaining consumers (n=103) preferred fluorescent light-exposed milk. Both segments preferred control and packaging samples over all other samples. However, group 1 preferred higher “sulfur” and a lower level of factor 1, whereas group 2 preferred lower “sulfur.”

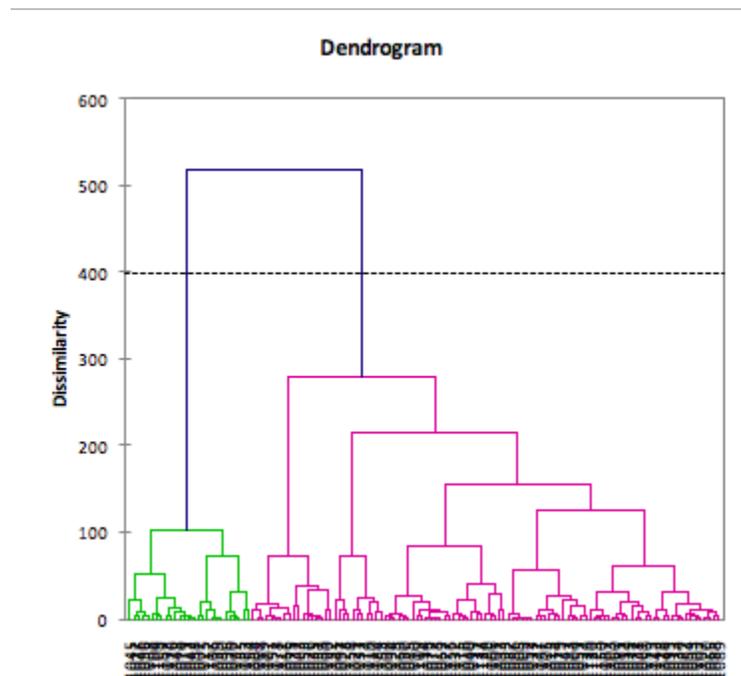


Figure 9: Consumer preferences dendrogram.

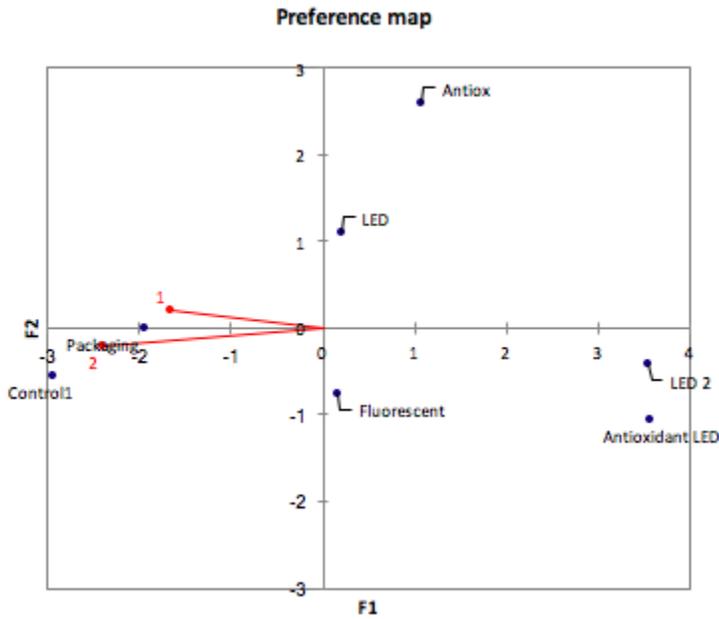


Figure 10: Preference map of consumer clusters. Factor ranges: (F1) cardboard, whiteness, plastic, old oil, sweet aftertaste, cream, and oral thickness; (F2) sulfur.

Most of the consumers preferred to drink 2% and whole milk (Figure 11). However, a statistically greater number of consumers that drank skim milk preferred the fluorescent-exposed milk than the non-skim milk consumers.

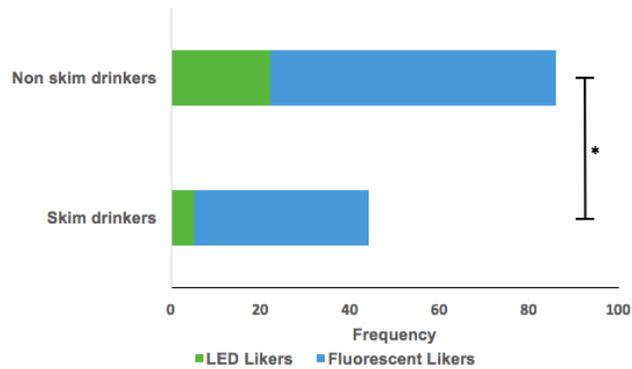


Figure 11: LED and fluorescent light likers by type of milk consumption.

Package Appearance Testing

Package appearance liking was significantly different between treatments (ANOVA, $p < 0.0001$). Milk in protective packaging obtained the highest score for the liking of the sample itself, but consumers expressed discomfort about this container (Figure 13). The preferred category was LED lacking riboflavin ban followed by fluorescent (Figure 12).

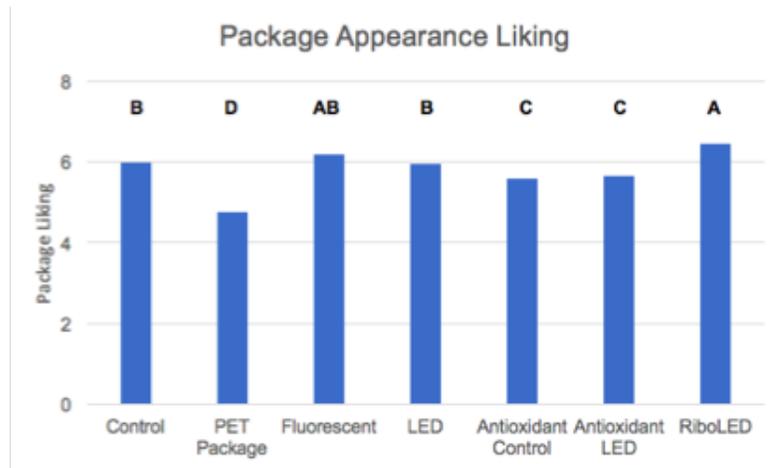


Figure 12: Package appearance liking.

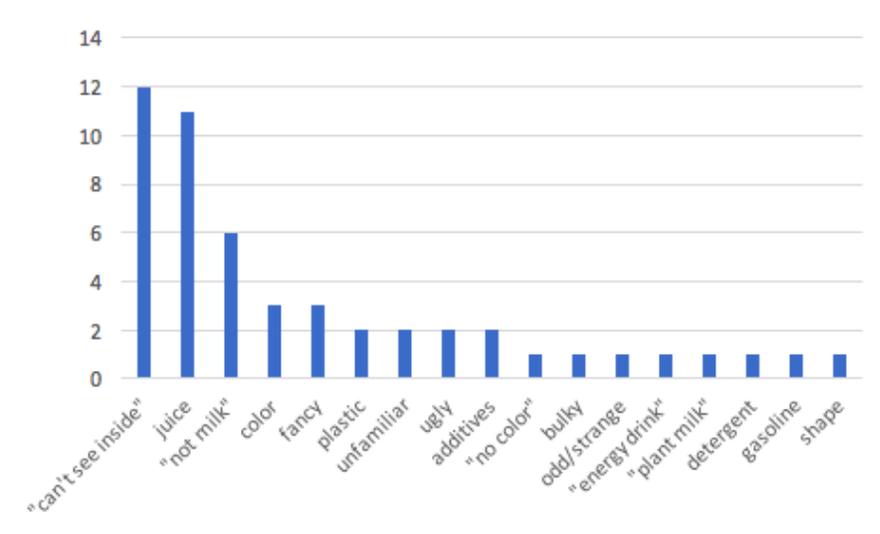


Figure 13: Comments on the protective packaging.

5. Discussion

LED and fluorescent light seemed to have different effects on milk flavor. LED lighting off-flavor detection threshold was shorter (thus detected sooner, although not statistically). Additionally, in the colorimetry analysis the variation on the a^* value of fluorescent light-exposed samples was greater. The increase in red color could be due to degradation of amino acids and the loss of yellow due to the degradation of riboflavin or vitamin A (Mestdagh et al., 2005). Moreover, the flavor profile between these light treatments differed. LED samples were characterized by a significantly higher plastic aroma, whereas fluorescent samples presented a higher cardboard aftertaste. Consumer overall, flavor and aroma liking ratings were higher when milk was exposed to fluorescent light when compared to exposure from LED lighting. Generally, LED lighting appears to be more damaging and caused more off-flavors than fluorescent light.

An engineered LED light to lack riboflavin absorption bands did not protect milk from oxidation. On the contrary, it appeared to increase off-flavor development. Milk exposed to this light presented higher old oil aroma, plastic aroma and cardboard aftertaste than all other treatments. The wavelength profile used is likely affecting other photosensitive compounds instead of riboflavin.

Antioxidant supplementation was found to have a protective effect against milk oxidation. Sensory thresholds and colorimetry analysis demonstrated that this strategy

mitigated off-flavor development and color changes in milk respectively. However, antioxidants seem to modify milk flavor in a negative way. Milk samples with antioxidants presented higher old oil aroma, probably from the tocopherols added. In addition, consumers did not like these samples as much as the control.

Protective packaging offered milk the most effective protection from light oxidation.

The flavor profile, as well as consumer responses were very similar to controls.

Trained panelists did not detect off-flavors in LED exposed milk packed in PET bottles. More importantly, these samples received the highest ratings in overall, aroma and flavor liking when consumers rated them. Interestingly, consumer preference for the appearance of the container was not in agreement with consumer response to the milk's flavor. A packaging design resembling the HDPE package could be a solution for this issue.

6. Conclusion

LED and fluorescent light, both cause milk oxidation nevertheless, LED seems to initiate more oxidation. Further research is needed to understand the mechanisms by which the light-activated flavor is developed, the influence of each milk component, the wavelengths to avoid it, and the nutritional losses that occur during this process. Overall, packaging materials proved to be the best approach to avoid light-induced milk damage, however, either a transparent protective packaging material is needed, or consumers will need to be educated to learn to accept this new appearance in the milk aisle.

A. Chapter 1 of appendix

Consumer Analysis Questionnaires

Display Pictures



LED 2 Picture



Packaging picture



LED with antioxidants picture



Fluorescent Picture



LED Picture

How much do you like or dislike the overall APPEARANCE of this product? (Please disregard the shape of the package and the color of the cap)

- | | | | | | | | | |
|-----------------------|-----------------------|-----------------------|-----------------------|--------------------------------|-----------------------|-----------------------|-------------------------|-----------------------|
| Like
Extremely | Like
very
much | Like
Moderately | Like
Slightly | Neither
like nor
dislike | Dislike
Slightly | Dislike
Moderately | Dislike
very
much | Dislike
Extremely |
| <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

If this product were available to you in a store where you usually shop, at a price that you typically pay, and from the brand that you typically buy, would you say you would...? (Select one response)

- Definitely would purchase
- Probably would purchase
- May or may not purchase
- Probably would not purchase
- Definitely would not purchase

If you have any additional thoughts or comments, please let us know below.

The following questions will ask about your impressions of the sample's *appearance, aroma, flavor, and mouthfeel*.

Before you taste the sample...

Thinking about the overall APPEARANCE of this test sample, would you say you...?
(please select one response)

- | | | | | | | | | |
|-------------------------|-------------------------|-----------------------|-----------------------|--------------------------------------|---------------------------|-----------------------------|----------------------------|----------------------------|
| Like
it
extremely | Like it
very
much | Like it
moderately | Like it
slightly | Neither
like nor
dislike
it | Dislike
it
slightly | Dislike
it
moderately | Dislike
it very
much | Dislike
it
extremely |
| <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

What, if anything, did you particularly LIKE about this sample's APPEARANCE?

What, if anything, did you DISLIKE about this sample's APPEARANCE?

Now please taste this sample...

Taking everything into consideration (appearance, aroma, flavor and mouthfeel), what is your OVERALL OPINION of this test sample? Would you say you... (please select one response)

- | | | | | | | | | |
|-------------------------|-------------------------|-----------------------|-----------------------|--------------------------------------|---------------------------|-----------------------------|----------------------------|----------------------------|
| Like
it
extremely | Like it
very
much | Like it
moderately | Like it
slightly | Neither
like nor
dislike
it | Dislike
it
slightly | Dislike
it
moderately | Dislike
it very
much | Dislike
it
extremely |
| <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

What, if anything, did you particularly LIKE about this sample OVERALL?

What, if anything, did you DISLIKE about this sample OVERALL?

Thinking about the level of WHITENESS would you say it was...? (please select one response)

Not white enough

Somewhat not white enough

Just about the right level of whiteness

Somewhat too white

Much too white

Thinking about the level of VISUAL CONSISTENCY would you say it was...? (please select one response)

Not thick enough

Somewhat not thick enough

Just about the right level of thickness

Somewhat too thick

Much too thick

How would you rate the AROMA of this sample? (please select one response)

Like it extremely

Like it very much

Like it moderately

Like it slightly

Neither like nor dislike it

Dislike it slightly

Dislike it moderately

Dislike it very much

Dislike it extremely

What, if anything, did you particularly LIKE or DISLIKE about this sample' s AROMA?

How would you rate the FLAVOR of this sample? (please select one response)

- | | | | | | | | | |
|---------------------------------|-----------------------|-----------------------|-----------------------|--------------------------------------|---------------------------|-----------------------------|----------------------------|----------------------------|
| Like
it
extremely
much | Like it
very | Like it
moderately | Like it
slightly | Neither
like nor
dislike
it | Dislike
it
slightly | Dislike
it
moderately | Dislike
it very
much | Dislike
it
extremely |
| <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

What, if anything, did you particularly LIKE about this sample's FLAVOR?

What, if anything, did you particularly DISLIKE about this sample's FLAVOR?

Thinking about the level of FRESHNESS, would you say it was...? (please select one response)

Not Fresh

Fresh

Would you say you detected an OFF-FLAVOR or OFF-AROMA of any kind...? (please select one response)

Yes

No



Does the off-odor or off-flavor that you detected fit in any of the below categories? Does it smell/taste more like...?

Mushrooms

Plastic

Hay/grain

Old Oil

Boiled
Eggs

Cardboard

Other



You've indicated that you've detected an off-flavor or off-aroma in this sample, could you please describe what this sample tastes or smells like to you and how intense is this off-flavor.

Thinking about the level of ORAL THICKNESS, would you say it was...? (please select one response)

Not thick enough

Somewhat not thick enough

Just about the right level of thickness

Somewhat too thick

Much too thick

If this product were available to you in a store where you usually shop, at a price that you typically pay, and from the brand that you typically buy, would you say you would...? (Select one response)

- Definitely would purchase
- Probably would purchase
- May or may not purchase
- Probably would not purchase
- Definitely would not purchase

Descriptive Analysis Questionnaire

These are retail milk samples, score each attribute that you perceive. If you don't perceive an attribute give it a score of 0.

When evaluating the following samples for AROMA, please swirl sample, hold close to nose and smell IMMEDIATELY after opening.

AROMA

Sample: 933

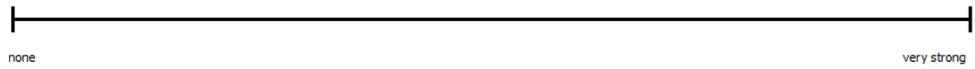
CREAM



MUSHROOM



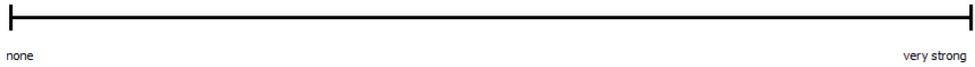
OLD OIL



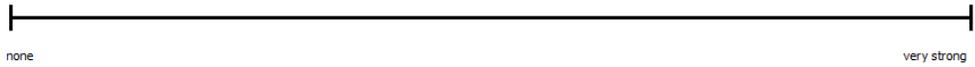
PLASTIC



SULFUR



OTHER AROMA



Please describe any OTHER Aroma you detected:

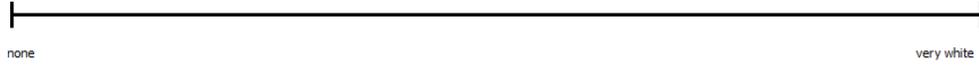
Sample: 933

APPEARANCE

APPEARANCE

Sample: 933

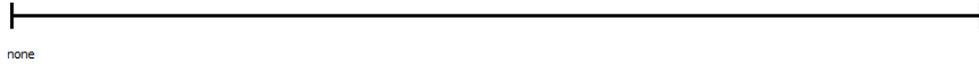
WHITENESS



VISUAL THICKNESS



OTHER APPEARANCE



Please describe any OTHER Appearance you detected:

Sample: 933

TASTE

Sample: 933

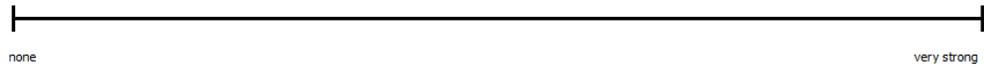
BUTTER



COOKED



SWEET



OTHER TASTE



Please describe any OTHER Taste you detected:

Sample: 933

Please taste sample again.

AFTERTASTE

Sample: 933

BITTER



CARDBOARD



METALLIC



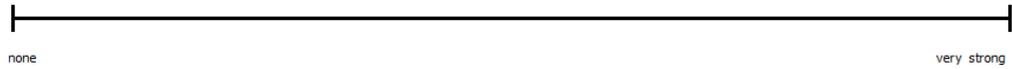
SWEET



SOUR/ACID



OTHER AFTERTASTE



Please describe any OTHER Aftertaste you detected:

Sample: 933

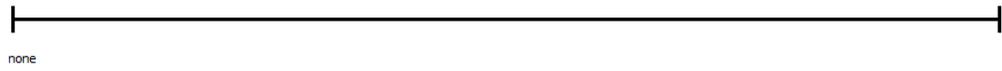
TEXTURE
TEXTURE

Sample: 933

ORAL THICKNESS
ORAL THICKNESS



OTHER TEXTURE
OTHER TEXTURE



Please describe any OTHER Texture you detected:

Sample: 933

Descriptive Analysis Attributes and References

Aroma	
Attribute	Reference
Cream	Upstate Heavy cream
Old Oil	Unsalted Premium crackers, stale
Sulfur	Wegmans eggs, boiled and mashed
Plastic	Clear Mildew Proof shower curtain
Mushroom	Chopped crimini mushroom
Taste	
Attribute	Reference
Cooked	Parmalat UHT milk
Butter	ICB Inc. spray margarine
Sweet	Lactaid 100% and Cornell Dairy skim milk (50:50)
Aftertaste	
Attribute	Reference
Cardboard	Byrne dairy skim milk
Sweet	Lactaid 100% and Cornell Dairy 2% milk (50:50)
Metallic	Ferrous sulfate heptahydrate, Sigma #F8633, 0.01 g/L spring water
Sour	Upstate Farms buttermilk
Bitter	Caffeine, 2 g/L spring water (Sigma-Aldrich)
Appearance	
Attribute	Reference
Whiteness	Sheet of paper WBM-20100
Visual Thickness	Continuum of fat in dairy products
Texture	
Attribute	Reference
Oral Thickness	Continuum of fat in dairy products

Consent form
INFORMED CONSENT

This is a study to investigate the flavor properties of milk samples throughout their normal shelf life. You will be asked to sip and rinse your mouth with various milk samples, then spit them out and make judgments about how they taste using rating scales. None of the solutions are harmful. Water will be provided to rinse your mouth. We don't anticipate any potential risks to be associated with this study. Those with food allergies should abstain from this study. You will be trained, via sampling of milk which is known to differ in flavor profile in defined ways, which you'll be taught to identify. Finally, you will sample the test milks, and will rate them on a number of scales.

The study requires several return visits, and thus requires a commitment to the full length of the study, around 20 days. Throughout this time, you will be required to attend sessions varying from 30 minutes to 2 hours, and will be compensated at a rate of \$10/hr. For the total of 21 training hours, and 6-10 testing sessions, we estimate the total compensation will be \$270-\$310 per panelist for taking part in the study. If you withdraw from the study you will be compensated for the hours of training you attended. There are no other benefits from the study.

There are no right or wrong answers in these tests. It is your perceptions about the stimulus materials that we are interested in. After the experiment, your data will be kept on an encrypted hard drive. In any electronic records, you will be identified only by a code number. Your personal data will never be displayed in any presentation or publication with your identity revealed by name or initials.

Please ask any questions you have about the study at this time. If you have questions at any later time please contact Dr Robin Dando, 254 - 3319, robin.dando@cornell.edu.

By agreeing below, I indicate that I am participating in this study voluntarily. I understand that I have the right to withdraw from the experiment at any time, without penalty. I also indicate that to the best of my knowledge, I have a normal sense of taste and smell, and that I have none of the following conditions: any chronic health problem, respiratory disease such as a cold or asthma, respiratory allergies such as hay fever, and/or food allergies. Note, you may be asked to sample peanuts, or other forms of nut within this test. All my questions about the experiment have been answered to my satisfaction.

Name (Print) _____ Date _____

Signature _____

You may contact the Institutional Review Board (IRB) with any concerns or complaints (irbhp@cornell.edu) or 607-254-5162. **This consent form will be kept by the researcher for at least five years beyond the end of the study.**

Cornell IRB
Approved: 2/27/17

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