Report:

Using Integrated Data to Inform Expiration Dating for PET-Packaged Fluid Dairy Beverages in Lighted Retail Conditions

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Microbiological and chemical analyses, and data collection were performed by Dr. Jian Wu and Kim Waterman (lab support staff). The study was designed based on the discussion with Brian Lynch from PI Labs and by SD, YY and JW in consultation with Justin Loda, Tessa Anwyll and Minh Le (graduate students) from Department of Statistics. Data analysis and interpretation were performed by JW with assistance from the Statistics team. Report preparation was completed by JW, SD, and YY.

IMPACT STATEMENTS / EXECUTIVE SUMMARY

This milk shelf-life study was designed to compare PET milk packaging (provided by Practically Impossible Labs) and commonly used HDPE milk packaging in refrigerated lighted retail display case storage conditions. The intention was to assess quality changes in milk related to microbial quality, nutrients (Vitamin B_2/riboflavin), and measures of oxidation (dissolved oxygen and changes in odor) as related to packaging and storage conditions. The light intensity in each display case simulated real-world retail lighting (low: 670 lux; high: 3,790 lux) as well as an extreme lighting scenario (>10,820 lux) using high intensity LED lighting (3500K).

Why is this important?

- **Nutrient protection.** Light exposure of milk in retail dairy cases can cause degradation of the nutritional value of milk. Milk is rich in nutrients that are sensitive to light. Vitamin B_2 (riboflavin) reacts to light and causes degradation of other vitamins (Vitamin A, Vitamin D) in milk. Vitamins are essential nutrients that must be consumed from dietary sources in order to support life and enhance health. Appropriate packaging can help protect nutrients from light degradation during retail storage and deliver the nutrients to the consumer when the product is consumed.
- **Flavor protection.** Light exposure of milk in retail dairy cases can cause changes in milk flavor when exposed to light for as little as two hours. These off-flavors alter consumer experience with fresh fluid milk and create negative impressions of the product. Protection of milk flavor from light is essential because flavor is one of the most important quality parameters for consumers. Failure to deliver a good tasting product means consumers may not repeat purchase the product or purchase as much. Appropriate packaging is needed to provide protection of fresh milk flavor. Many consumers like fresh milk flavor but often do not experience it since most milk packaging allows light to degrade the milk flavor before purchase.

What did we learn about PET and HDPE packaging in protecting milk quality?

Highlights:

1. The PET bottle preserved a higher level of riboflavin (vitamin B_2) than the HDPE bottle;
2. When exposed to moderate to strong light, PET bottles retained higher dissolved oxygen level in milk samples, which may indicate a lower degree of oxidation;
3. When exposed to strong light, bacterial count in PET bottled milk samples was higher, but this may be from the residing bacteria in PET bottle before milk filling;
4. Designs of the display cases, storage time and temperature, and package types were all important contributing factors for the microbiological growths and shelf-life.

Summary Brief.

Low-fat (2%) pasteurized milk were filled in half-gallon (1.89L) PET and HDPE bottles at Westover Dairy (Lynchburg, VA) and bottled milk samples were transported to Virginia Tech and stored in 3 refrigerated, lighted display cases over an extended period of 25 days. The light intensity in each display case simulated real-world retail lighting (low: 670 lux; high: 3,790 lux) as well as an extreme lighting scenario (>10,820 lux) using high intensity LED lighting (3500K). Positioning of filled milk packages was randomized within each display cases, though all packaging was placed on front rows of the display cases. One PET- bottled and one HDPE-bottled milk were taken from each display case on day 1, 8, 14, 18, 20, 21, 23, and 25 for several biochemical and microbiological analyses, including microbial count, light-sensitive nutrient (riboflavin) degradation, dissolved oxygen level, and volatile compound profile (analyzed by electronic nose). The purpose of conducting these analyses was to evaluate how well the PET bottle, as milk packaging, preserves milk quality during lighted retail storage conditions, and if the PET bottle preserves better milk quality and results in longer shelf-life of fluid milk, comparing to common HDPE bottles.

The results showed some interesting trends. The first important finding was that, compared to HDPE bottles, **PET bottles retained significantly higher riboflavin level in reduced fat (2%) fluid milk, regardless of light intensity during lighted, refrigerated storage.** Riboflavin is a plant-sourced vitamin that is originally from the plant food sources consumed by cows. It is activated when exposed to light, and the excited status of riboflavin triggers and accelerates oxidation and degradation of other nutrients and creates off-flavors in milk which deteriorates milk quality and shortens shelf-life of milk. However, this significantly different riboflavin level between PET- and HDPE-bottled milk was based only on statistical analyses, and it is yet to say the difference is practically large to be considered. **Light intensity was the key factor that affected overall retention of riboflavin.** High light intensity during storage (3790 lux and 10820 lux) still caused serious degradation of riboflavin in milk regardless of packaging materials. Riboflavin level decreased rapidly during the first week of storage and about 20% riboflavin was left after 2 weeks. In contrast, milk stored under modest lighting (670 lux) still retained about 60% by the end of the experiment (Day 25).

PET material provides better oxygen barrier properties, compared to HDPE packaging, and thus decelerated the progress of light-induced oxidation. Under higher light intensities (3790 lux and 10820 lux), the measured dissolved oxygen level was even higher in PET bottles comparing to HDPE bottles, indicating that less oxygen was used during the light-initiated changes in milk in PET bottle; in other words, less light oxidation occurred in PET-bottled milk.

According to electronic nose analysis, which tested the smell of the milk as it changed over time and as a reaction to light, the volatile compound profile between PET-bottled milk and HDPE-bottled milk were not significantly different; during storage, however, both samples were getting more and more different than fresh milk, which

makes sense that the overall sensory quality of milk changes over time, but not significantly affected by packaging materials. This test does not necessarily reflect what a customer might decide about the smell or taste of the milk but does illustrate that the aroma quality of fresh milk changed over time.

The result of growth of microorganisms in milk was a mixed-bag. Due to completely different designs of milk display cases, the light intensities and actual in-bottle temperatures of the milk samples varied among 3 cases, and it seems that small difference (2.0 °C, 5.2 °C and 7.0 °C) in storage temperature may have resulted in vast differences in microbial count in milk, especially after 14-18 days of storage among all display cases. It is important to communicate with milk retailers that lighting and temperature setting are critical to milk quality, that a modest light (670 lux at milk bottle) and lower storage temperature (2.0 °C) slowed down the microbial growth as well as milk oxidation.

It is also valuable to mention that clean and sanitized packaging is critically important to microbial quality of milk. The microorganisms residing in the PET bottles before any milk filling may have greatly affected the eventual microbial count in milk. It is highly likely that a few bacterial cells stuck on the bottles and later mixed into milk will lead to a heavy growth during storage. From the results, a hit-or-miss pattern can be found among milk samples, that some samples had very high microbial count (10^{-8} per mL) while microbial count in other samples under same storage conditions were below detection limit (10^{-2} per mL). Three PET bottles were sampled and 2 of 3 were found bacterial positive (~10 per mL). There was no data for bacterial count for HDPE. The PET bottles did not completely fit the automated filling and capping line; some PET bottles needed to be capped manually, which may also contribute to unintentional contamination and slightly affect the initial oxygen level in packaged milk.

Detailed Project Report

OBJECTIVES

The purpose of this study was to evaluate microbial quality, nutrients preservation and expiration dating of fluid dairy products using Polyethylene terephthalate (PET) packaging in comparison to High Density Polyethylene (HDPE) packaging.

INTRODUCTION

High Density Polyethylene (HDPE) is a commonly used material for fluid milk packaging applications. A typical HDPE milk bottle has a wide range of temperature tolerance (-50 ~ +140 °F), relatively impact-resistant, flexible, and is an excellent moisture barrier. However, HDPE offers nearly no oxygen barrier properties and is translucent, which is a double-edged sword that allows nearly complete light transmission and offers no protection for light-sensitive nutrients. Natural HDPE does allow customers to see the content inside, which is a recognized advantage. Typical blow-molding operations for HDPE yields a package with a handle, which is an advantage over other

beverage packaging materials. Polyethylene terephthalate (PET or PETE) is a transparent, and mechanically strong material that is widely used for packaging of fluid beverage products, such as juice, carbonated drinks, etc. PET has excellent mechanical strength and impact-resistance, is an excellent moisture and oxygen barrier. Compared to HDPE bottles, an obvious advantage is that PET bottles are highly transparent with a clarity similar to glass, which allows customers to look through the bottle and see the content. The glossy appearance and high contact clarity are also attractive to consumers. PET beverage bottles are also more impact-resistant and rigid, which minimizes cracking and breaking during production and transportation. However, one disadvantage with PET materials is that the molding process is different than HDPE, which makes it difficult to form an integrated handle on the bottle, a feature that has recognized value to consumers purchasing milk. This limitation has restricted PET application for fluid milk packaging to packages 1 liter or smaller in volume.

There is a significant body of literature recognizing that milk freshness and shelf-life is compromised with exposure to light (Wishner 1964; Bosset et al. 1994; Van Aardt et al. 2001; Choe et al. 2005). When exposed to light, milk oxidation occurs. However, most retailers store milk bottles in lighted display cases to assist the display of the products, but few retailers are aware of milk deterioration caused by light exposure (Wang et al. 2018).

Recently, a half-gallon PET bottle with integrated handle was developed by Practically Impossible Labs. This newly developed bottle with a handle has the potential to be a good replacement for the HDPE milk bottle. However, the quality protection ability of this specific PET bottle on milk products previously has not been evaluated. This study was designed to compare the new PET bottle with standard HDPE bottle on their influences on the shelf-life and quality of fluid milk. Freshly processed reduced fat (2%) milk was packaged into PET and HDPE half-gallon (1.89 L) bottles and stored in 3 retail cases with different light intensities for a total of 25 days, during which total bacterial population, psychrotrophic bacterial population, dissolved oxygen concentration, volatile compound profile, and riboflavin content were analyzed.

MATERIALS AND METHODS

Filling Milk into Treatment Packages

HDPE and PET bottles were filled with high-temperature-short-time (HTST) pasteurized fluid milk (2%; Vitamin A and D added) inline in the Westover Dairy (Lynchburg, VA) plant and capped inline (for HDPE) or manually (for PET). Immediately after filling and capping, milk samples were put into coolers with ice packs, and transported to Dept. of Food Science and Technology (FST), Virginia Tech (Blacksburg). Milk samples were immediately transferred to 3 lighted, refrigerated milk display cases in FST pilot plant. Display case 1 is an open-shelf case with lighted with horizontal LED tube light (18 Watt, 110V, temperature 3500 K) located directly above sample milk bottles; display case 2 is a glass-doored case with vertical LED tube light (3500K) located along the door frame; display case 3 is a walk-in cooler, with glass door

over milk shelf and the walk-in door at the back (Figure 1). The light intensity of each case was measured on the shoulder of every individual milk bottles and the mean light intensity values for case 1, 2 and 3 were 10820 lux, 670 lux, and 3790 lux, respectively. The contrasting lighting conditions among three cases represented various real-world milk display scenarios as well as a worst-case scenario with extremely strong light exposure. The light intensity was affected by the power of the light source (LED) and the distance between light source and milk bottles. Milk samples for this study were all arranged in the front row of the shelves in all three cases to make sure they were fully exposed to light and samples within the same case received the same light intensity. Besides milk samples used for this study, in all three cases milk storage shelves were filled with half-gallon bottles of water. The purpose was to provide airflow and more stabilized temperature as appropriate for the case design.

Figure 1. Retail display cases for milk bottles. a: display case 1, open-shelf, 10820 lux; b: display case 2, glass-doored, 670 lux; c: display case 3, walk-in, 3790 lux.

Microbiological Quality

Microbiological quality of milk samples was analyzed based on literature method with minor modification (Murphy, 2009). Briefly, Milk bottle was inverted 5-10 times and shaken before opening the lid for sampling. One milliliter of milk sample was plated directly onto Petrifilm (3M®, Thomas Scientific) in duplicate, or properly diluted in 0.1% sterile peptone water before plating. The duplicated Petrifilms were separately incubated at 32 °C and 7.2 °C, respectively, to promote growth of total aerobic microorganisms and

psychrotrophic microorganisms. Petrifilms incubated at 32 °C were counted after 48 hours and recorded as the Aerobic Plate Count (APC), while those incubated at 7.2 °C were counted after 10 days as Psychrotrophic Plate Count (PPC). The APC and PPC are indicators that demonstrate the microbiological quality of fluid milk. Federal regulations stipulate that freshly pasteurized fluid milk must have less than 20,000 Colony Forming Units (CFU) per mL (FDA 2017); however, there are no regulatory standards for end of shelf-life bacterial counts or for associated code dating of fluid milk.

Dissolved Oxygen Test

Immediately after sampling for microbiological quality test, dissolved oxygen (DO) was tested using a hand-held DO meter (LDO101, Hach, Loveland, CO). DO was tested after microbiological sampling to avoid contamination, and before all other tests to minimize influence from atmosphere.

Electronic Nose Analysis

Analysis was conducted using the method from Wang et al. (2018) with minor modification. Briefly, ten milliliters of milk were transferred into a 20-mL glass vial, capped with rubber septum, wrapped by aluminum foil and heated in water bath at 45 °C for 30 min. Then the headspace of the sample was analyzed using a conducting polymer electronic nose (Cyranose 320® model, Sensigent, Baldwin, CA). Analysis was repeated 5 times for each sample. Data was processed using the PCnose® software (10.11.0.76), and analyzed in JMP® Pro 14.0.

Riboflavin Retention Analysis

Riboflavin content of milk was analyzed using fluorescence spectrophotometer (Shimadzu RF-1501, Shimadzu Scientific Instrument Inc., Columbia, MD) following an AOAC method (AOAC, 1995) modified by Webster et al. (2009). Briefly, 10 mL milk was transferred into a 15-mL polypropylene conical tube and the pH was adjusted to 5.5. The acidified milk sample was autoclaved at 121 C for 30 min and cooled to room temperature, then adjusted to final pH of 4.5 and centrifuge at 4000 rpm for 10 min before filtered through 0.45 filter. The absorbance value of filtrate was tested at excitement wavelength of 520 nm and emission wavelength of 450 nm. Data of stored samples were compared with fresh milk and a percentage of retention levels was calculated. The whole operation was conducted with minimal exposure to light.

Experimental Design and Statistical Analysis

Two packaging materials, HDPE and PET, were tested using half-gallon size milk packaging. Three batches of fluid milk were produced, within each batch 60 bottles (30 PET and 30 HDPE) were picked up from the Westover Dairy plant and stored in three refrigerated display cases at Virginia Tech on 07/10/2019, 07/17/2019 and 07/24/2019. The light intensity on the milk bottles in display case 1 (open-shelf case, Figure 1a) ranged from 9590 to 11980 lux (avg. 10820 lux), which simulated a worst-case scenario that bottles are stored under strong artificial light. Light intensity in display case 2 (glass-doored, Figure 1b) ranged from 499 to 829 lux (avg. 670 lux), which represented a real-world scenario with modest lighting. Light intensity in case 3 (walk-in, Figure 1c) ranged from 2660 to 4360 (avg. 3790 lux), which represented a real-world scenario of milk storage with comparatively strong lighting. On Day 0, 1, 8, 14, 18, 20, 21, 23 and 25, one

HDPE and one PET bottle were sampled from each of the 3 display cases for all analyses stated above. Results were analyzed in JMP® Pro 14.0 using Randomized Complete Block Design (RCBD) and Matched Pairs student-T test, and P-value < 0.05 was considered as the level of significance.

RESULTS AND DISCUSSIONS

Retail lighting display cases have different configurations and vary in lighting conditions (lighting intensity, color temperature). Packaging for fluid milk intended to provide nutrient and sensory quality protection must be able to provide protection under a wide variety of lighting conditions. Wang et al. (2018) characterized retail lighting, illustrating that light intensity can range from approximately 600 lux to greater than 3500 lux, based on a limited survey of retail dairy case conditions in Virginia. In this study, we tested conditions that bracketed those conditions, plus an extreme (experimental; remotely possible in a retail case) condition with high light intensity of approx. 11000.

Microbiological quality of milk product

Population of microorganisms in milk samples directly reflects the quality of milk. Freshly pasteurized milk must be below 20,000 CFU/mL but there is no defined 'end of shelf-life' bacterial count for regulatory or prediction of quality. Microbiological quality of milk during storage was represented by aerobic plate count (APC, the plate count after 48 h incubation at 32 °C) and psychrotrophic plate count (PPC, the plate count after 10-day incubation at 7 °C). APC and PPC were logarithmically transformed and plotted over sample storage time (days) as shown in Figure 2a and 2b, respectively. The RCBD model was fit, analyzed, and results were listed in Table 1 and Table 2.

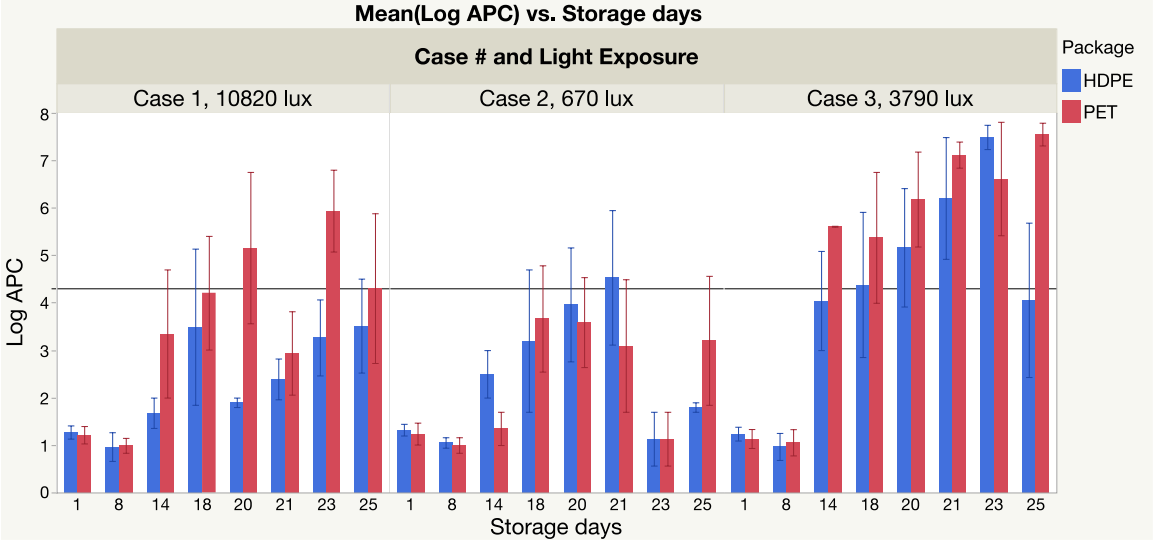

Figure 2a. Aerobic Plate Count (APC) results of milk samples. Error bar represents standard error. The horizontal line (Log APC = 4.3) represents the allowed maximum APC in freshly pasteurized Grade-A milk (FDA 2017).

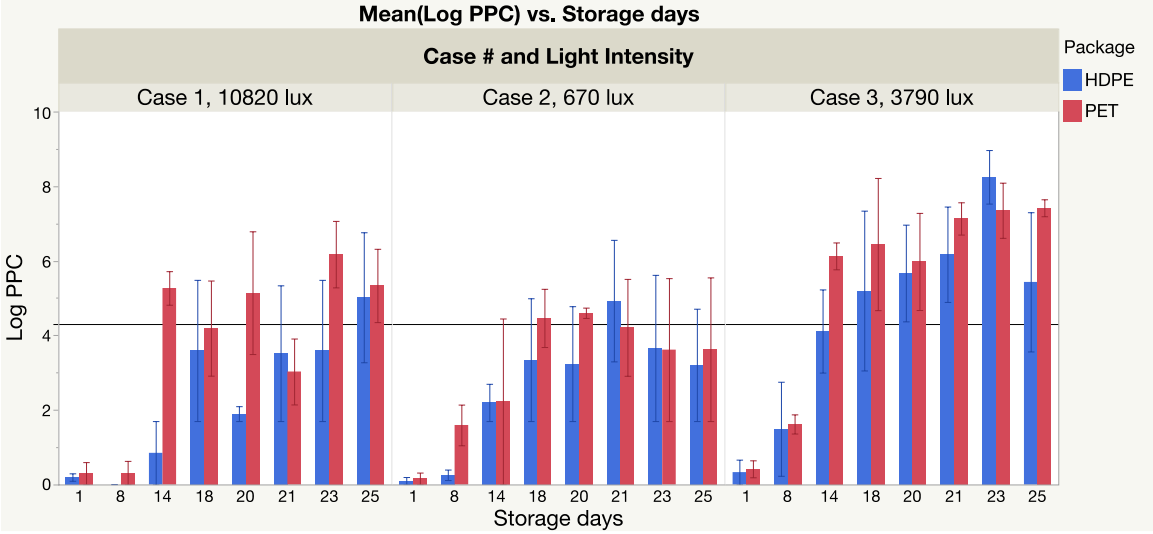

Figure 2b. Psychrotrophic Plate Count (PPC) results of milk samples. The horizontal line (Log APC = 4.3) represents the allowed maximum APC in freshly pasteurized Grade-A milk (FDA 2017).

The APC and PPC for both PET and HDPE bottled milk reached the regulatory 'pasteurized fresh milk' bacterial limit of 20,000 CFU/mL within 14-21 days; this also varied among display cases. Several factors may have influenced bacterial growth, including case capacity, bottle placement, open-shelf or close-door design, etc. The different designs of display cases may result in air flow patterns within the cases, and eventually impact the in-bottle temperature of milk samples. A parallel continuous temperature monitoring demonstrated that in-bottle temperature in Display case 1, 2, 3 were 5.2, 2.0 and 7.0 °C. These values were all acceptable storage temperatures for fluid milk under which the spoilage bacteria growths were controlled, the difference may still considerably contribute to the growth rate of in-bottle bacteria. **Display case 2 features glass doors, low in-bottle temperature (2.0 °C) and low light intensity (670 lux), and growth was slowest in this case**. In contrast, display case 1 has the highest light intensity (10,820 lux) and an open-shelf design with 5.2 °C in-bottle temperature; case 3 is a walk-in cooler with moderate light intensity (3,790 lux) but highest in-bottle temperature (7.0 °C). It is also with considerably larger capacity, in which the milk bottles only took up a small portion of the cooler space while most in-cooler space was vacant. Comparing bacterial growths from display case 1 and 3 (Figure 2a and 2b), it seems that **higher temperature (in case 3) was the more dominant factor for bacterial growth rather than higher light intensity (in case 1).** However, since there are many design factors were involved among the display cases, it is more reasonable to consider the whole effect from display cases on bacterial growths.

Table 1. List of effect of factors for aerobic plate count of milk samples.

Source	DF	Sum of Squares	F Ratio	Prob > F
Storage days	7	249.50181	13.6503	<.0001*
Display cases (light intensity/temperature)	2	127.48007	24.4107	<.0001*
Package	1	14.11716	5.4065	0.0223*

Table 2. List of effect of factors for psychrotrophic plate count of milk samples.

Source	DF	Sum of Squares	F Ratio	Prob > F
Storage days	7	487.14877	15.7257	<.0001*
Display cases (light intensity/temperature)	2	122.97933	13.8947	<.0001*
Package	1	24.70451	5.5824	0.0203*

From the results of statistical analysis, the primary factors of **storage days** and **light intensities/temperatures, as well as package types** all contributed significantly to the overall difference of Aerobic Plate Count and Psychrotrophic Plate Count among all milk samples. It is obvious that bacterial growth is significantly influenced by milk storage time which is also the incubation time of microorganisms in milk; it is also reasonable that the different designs of display cases with varied bottle placements, lighting conditions and capacities also significantly contributed to APC.

Looking more closely to the influences from packaging materials, a Matched Pairs student T-test was conducted on the microbial populations between PET and HDPE bottles in each display case, with **the purpose of eliminating the effects from storage days and any display case difference**. The results were summarized in Table 3 as below:

Table 3. Results of Matched Pairs tests.

	Aerobic Plate Count			Psychrotrophic Plate Count		
Display Case	Package	Mean ± SEM	Prob > |t|	Package	Mean ± SEM	Prob > |t|
Case 1* (10,820 lux; 5.2°C)	PET	3.52 ± 0.48	0.0035*	PET	3.65 ± 0.55	0.0169*
	HDPE	2.34 ± 0.31		HDPE	2.40 ± 0.55	
Case 2* (670 lux; 2.0°C)	PET	2.32 ± 0.37	0.7531	PET	3.10 ± 0.49	0.2589
	HDPE	2.43 ± 0.38		HDPE	2.64 ± 0.53	
Case 3* (3,790 lux; 7.0°C)	PET	5.06 ± 0.57	0.0428*	PET	5.28 ± 0.61	0.1259
	HDPE	4.20 ± 0.56		HDPE	4.60 ± 0.66	

*Display case 1 is the open-shelf case; display case 2 is the glass-doored case; display case 3 is the walk-in case. See Figure 1a-1c.

It can be seen that **in display cases with higher light intensities and higher temperatures, plate counts are significantly higher in PET bottled milk samples**, although the starting microbial population was assumed to be the same. Together with the information from Figure 2, it can also be seen that there were **large variabilities (**standard error approx. 2 log CFU/mL, in other words, 1% - 10,000% of mean plate count**) in both APC and PPC**. These variabilities may be caused by the lack of strict sanitation of bottles before filling, that the culturable microorganisms were randomly present or absent in each individual bottle. A brief microbial test of empty PET bottles

was conducted, and the initial plate count in the PET bottles were 10 CFU/mL for 2 of 3 bottles tested.

It is interesting to note that differences in bacterial count associated with packaging occurred at the high and extreme light intensity conditions and higher storage temperature (5.2 and 7.0 °C). However, we suspect that there were differences in the bacterial load associated with the packaging prior to filling, thus contributing to these differences. It is also possible that the higher temperature in display cases contribute to the replication of residual bacteria in the packages, and the energy generated from higher light intensities may also contribute to the higher temperature within the retail, further complicating the determination if effects are attributed to packaging or lighting. Additionally, in cases were the light intensity is low, neither of the packaging materials are likely to cause the concern for food safety. Therefore, it is important to communicate to the retail stores and manufacturers the potential significance of the LED light intensity and refrigeration temperature setting (36~45°F) on product quality.

Dissolved Oxygen

When fluid milk is kept in bottles, residual dissolved oxygen (DO) in fluid milk suggests the extent of oxidation in the milk. DO level decreases means that the dissolved oxygen was used in oxidation reactions, which causes degradation of nutrients and potential changes of sensory property. DO level of milk samples are shown in Figure 3. Results of RCBD model and Matched Pairs analyses are listed in Table 4. Overall **storage days and package type** were significant contributing factors to different DO levels in milk; **packaging was a less significant factor** ($P = 0.0872$). When looking at each light intensity, under moderate to high light intensity, DO levels in milk quickly dropped from 11 mg/L to under 1 mg/L, indicating the occurrence of oxidation in milk. **In display case 1 (extreme light intensity) and 3 (high light intensity), PET bottled milk had significantly higher dissolved oxygen level than HDPE bottled milk** ($P < 0.05$) which indicates that the **oxidation rate in PET bottled milk was slower**. In display case 2 with lowest light intensity (670 lux), DO levels were not significantly different. PET provides a much higher oxygen barrier than does HDPE. However, fluid milk contains sufficient dissolved oxygen within the package to allow oxidation to occur until the available oxygen is consumed. Thereafter, in PET, the reaction slows (day 8 and later) as oxygen is depleted and remaining oxygen is retained within. In HDPE, with a very low oxygen barrier, the reaction continues throughout the storage period as oxygen can migrate through the package to support the process. At low light intensity, there is less light energy available to initiate excitation of riboflavin, so the reaction progresses more slowly and the packaging differentiation is not evident.

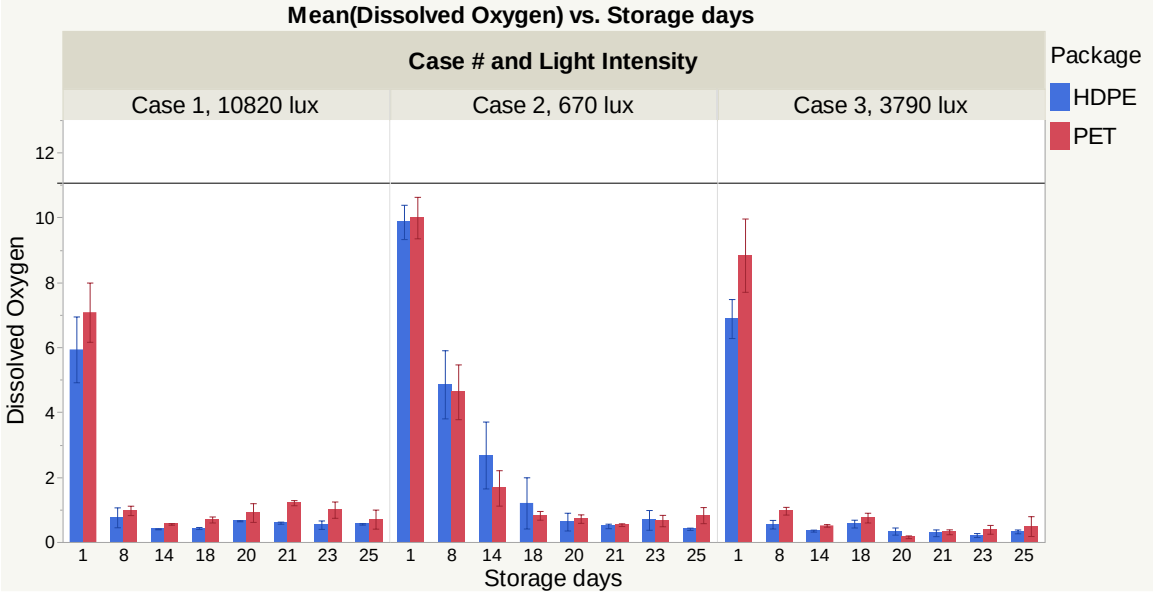

Figure 3. Dissolved oxygen level (mg/L) of milk. The mean and standard deviation of DO level for fresh milk samples (11.08 ± 0.88 mg/L) were shown as the black line and grey zone.

Table 4. Statistical Analyses of Dissolved Oxygen Level (mg/L) in milk.

Source	DF	Sum of Squares	F Ratio	Prob > F
Storage days	7	852.40634	229.0174	<.0001*
Display Case (light intensity/temperature)	2	39.06918	36.7387	<.0001*
Package	1	1.58957	2.9895	0.0872

Matched Pairs T-test			
Display Case	**Package**	**Mean ± SEM**	**Prob > |t|**
Case 1*	PET	1.69 ± 0.46	0.0030*
(10,820 lux; 5.2°C)	HDPE	1.27 ± 0.40	
Case 2*	PET	2.52 ± 0.69	0.6146
(670 lux; 2.0°C)	HDPE	2.60 ± 0.70	
Case 3*	PET	1.60 ± 0.61	0.0313*
(3,790 lux; 7.0°C)	HDPE	1.22 ± 0.47	

*Display case 1 is the open-shelf case; display case 2 is the glass-doored case; display case 3 is the walk-in case. See Figure 1a-1c.

Retention of Light-sensitive Vitamin (Riboflavin)

Riboflavin (Vitamin B2) is a light-responsive nutrient in fluid milk. It can be activated by light and produce other active oxidants which eventually causes further oxidation in milk thus deteriorates milk quality (Choe et al. 2005). During lighted storage, riboflavin concentration in milk samples were decreasing, and the decomposition was expressed as Riboflavin retention rate, which is calculated as:

Retention = (riboflavin in sample / riboflavin in fresh milk) x 100%

The retention rate of riboflavin in milk during storage indicates the abilities of packages to protect milk from degradation of light-sensitive nutrients. Riboflavin levels were tested and decreased faster when exposed to high light intensity. Milk from display case 1 (10,820 lux) and 3 (3,790 lux) suffered a quick loss of riboflavin, about 30%-40% within 8 days, and below 20% after two weeks (Figure 4). In Case 2 (670 lux) with low-intensity lighting, 60% riboflavin was retained after 3 weeks. Riboflavin level in PET bottled milk was consistently higher than in HDPE bottled milk, and the difference was statistically significant in all 3 display cases (Matched Pairs, $P<0.01$). The results indicate that the **PET bottle retains higher riboflavin level in milk than HDPE bottle.** According to Coltro et al. (2003), HDPE transmits wavelengths throughout UV and visible light spectrum, while PET absorbs wavelengths below 300 nm, which prevents some of the UV light wavelengths from reaching milk product inside.

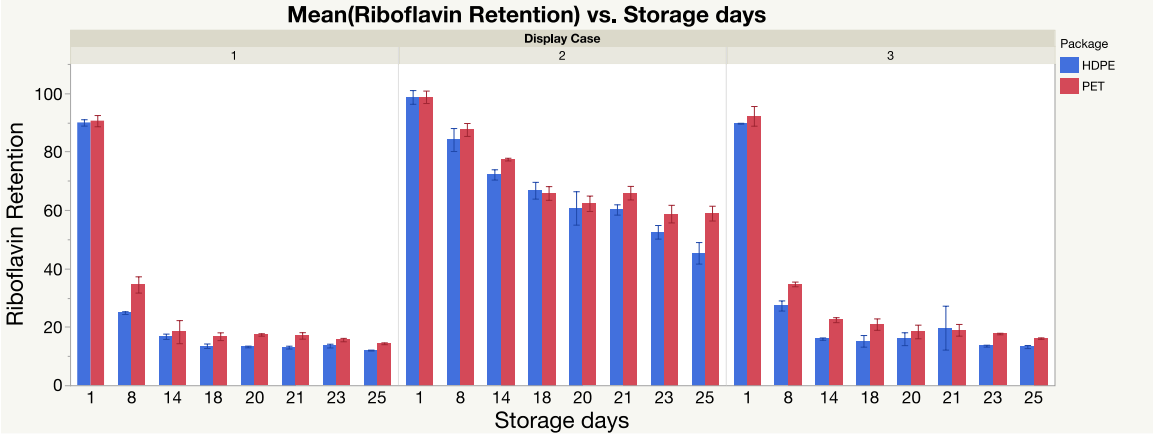

Figure 4. Riboflavin retention (%) during storage.

Riboflavin, which is highly photosensitive, serves as a primary photo-initiator for light-induced oxidation reaction in fluid milk. PET packaging was more effective at protecting riboflavin but did not provide sufficient barrier to the upper UV range (370 nm) and low visible wavelength regions (400, 446 nm), at which riboflavin is also excited (Kyte, 1995; Webster et al., 2011). Studies with addition of TiO_2 to PET have illustrated that riboflavin retention can be similar to optimum light-protected packaging conditions (foil-wrapped; fully light-blocked) but sacrifice the consumer-identified preference for product visibility (Wang et al., 2018). The holy grail is a transparent package (with a handle) that provides riboflavin protection to limit photo-initiated oxidation.

Electronic Nose Analysis of Volatile Compounds

E-nose analysis reveals the changes of volatile compound profile in milk during storage. The changes of volatile compounds in milk were visualized using Discriminant analysis and shown in Figure 5. These graphs demonstrate the similarity or difference among samples – sample points located close together means they were with similar

volatile profiles, and *vice versa*. There was a trend that the volatile compounds in milk with both package types changed, and the difference between profiles of fresh milk and stored milk was increasing over time. Volatile compounds profiles in PET bottled milk and HDPE bottled milk were mostly not significantly different. This characterization does not specifically describe the flavor volatiles of the product but illustrate that the 'smell print' of the milk from the two packages may be slightly different at day 8 but thereafter converges.

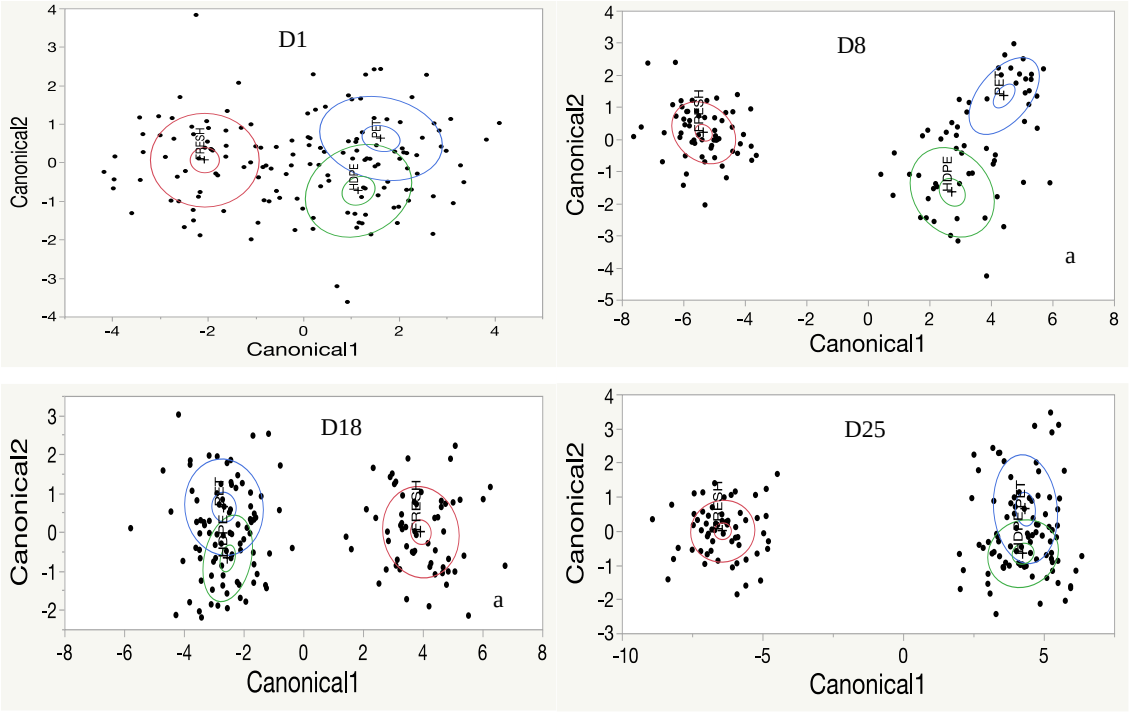

Figure 5. Discriminant analysis similarity maps for Fresh milk control, PET and HDPE bottled milk from Day 1, Day 8, Day 18 and Day 25. Eclipses represent for 95% confident limits; Red: Fresh milk; blue: PET bottled milk; green: HDPE bottled milk

Reference:

1. AOAC International (1995). AOAC official method 970.65. Riboflavin (Vitamin B2) in foods and vitamin preparations-fluorometric method. *AOAC Official Methods of Analysis*, 9-10.
2. Bosset, J. O., Gallmann, P. U., & Sieber, R. (1994). Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products—A review. In *Food packaging and preservation* (pp. 222-268). Springer, Boston, MA.

3. Coltro, L., Padula, M., Saron, E. S., Borghetti, J., and Buratin, A. E. P. (2003). Evaluation of a UV absorber added to PET bottles for edible oil packaging. *Packaging Technology and Science: An International Journal*, *16*(1), 15-20.
4. Choe, E., Huang, R., & Min, D. B. (2005). Chemical reactions and stability of riboflavin in foods. *Journal of food science*, *70*(1), R28-R36.
5. FDA, 2017. Grade "A" Pasteurized Milk Ordinance. Available at: https://www.fda.gov/media/114169/download
6. Kyte, J. (1995). Mechanisms in protein chemistry. New York, NY, USA: Garland Publishing. In Webster, J.B., Duncan, S.E., Marcy, J.E., & O'Keefe, S.F. (2011). Effect of narrow wavelength bands of light on the production of volatile and aroma-active compounds in ultra high temperature treated milk. *International Dairy J*. 21:305-311.
7. Murphy S.C. (2009). Shelf-life of Fluid Milk Products - Microbial Spoilage - the Evaluation of Shelf-life. Available at: https://foodsafety.foodscience.cornell.edu/sites/foodsafety.foodscience.cornell.edu/files/shared/documents/CU-DFScience-Notes-Bacteria-Milk-Shelf-Life-Evaluaton-06-09.pdf
8. Van Aardt, M., Duncan, S. E., Marcy, J. E., Long, T. E., & Hackney, C. R. (2001). Effectiveness of poly (ethylene terephthalate) and high-density polyethylene in protection of milk flavor. *Journal of Dairy Science*, *84*(6), 1341-1347.
9. Wang, A., Dadmun, C.H., Hand, R.M., O'Keefe, S.F., J''Nai, B.P., Anders, K.A. and Duncan, S.E. (2018). Efficacy of light-protective additive packaging in protecting milk freshness in a retail dairy case with LED lighting at different light intensities. *Food Research International*, *114*, pp.1-9.
10. Webster, J. B., Duncan, S. E., Marcy, J. E., & O'keefe, S. F. (2009). Controlling light oxidation flavor in milk by blocking riboflavin excitation wavelengths by interference. *Journal of food science*, *74*(9), S390-S398.
11. Webster, J.B., Duncan, S.E., Marcy, J.E., & O'Keefe, S.F. (2011). Effect of narrow wavelength bands of light on the production of volatile and aroma-active compounds in ultra high temperature treated milk. *International Dairy J*. 21:305-311.
12. Wishner, L. A. (1964). Light-induced oxidations in milk. *Journal of Dairy Science*, *47*(2), 216-221.