Packaging modifications for protecting flavor of extended-shelf-life milk from light

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ABSTRACT

The effectiveness of titanium dioxide (TiO₂)-loaded high-density polyethylene (HDPE) to reduce light-induced oxidation of extended-shelf-life milk (2% total fat) was studied. The objective was to determine differences over time in sensory quality, vitamin retention, and oxidative chemistry as a function of packaging and retail light exposure duration. Effectiveness of packaging for protecting milk quality was assessed by sensory evaluation (triangle tests, untrained panel), changes in volatile compounds, thiobarbituric reactive substances (TBARS), and riboflavin concentration. Milk (2%) was stored in HDPE packages consisting of TiO₂ at 3 levels (low: 0.6%; medium: 1.3%; high: 4.3%) at 3°C for up to 43 d. Light-protected (translucent, foil-wrapped) and light-exposed (translucent) HDPE packages served as controls. The high TiO₂-HDPE package provided protection similar to light-protected control package through d 22 of light exposure, with less consistent performance by the medium TiO₂ package. The TBARS increased in all treatments during storage. Under the experimental conditions used, a TBARS value of 1.3 mg/L could be considered the limiting sensory threshold for differentiating oxidized milk from light-protected milk. Riboflavin concentration decreased 10.5% in the light-protected control and 28.5% in the high TiO₂ packaged milk past 29 d of light exposure, but losses were greater than 40% for all other packages. The high TiO₂ package protected riboflavin concentration from degradation and controlled aldehyde concentration throughout the test period.

Key words: oxidation, sensory, riboflavin, extended-shelf-life milk

INTRODUCTION

Milk and milk products are susceptible to light-induced oxidation reactions, which can negatively af-

fect odor and flavor attributed to increased oxidation-derived volatile compound production and leading to reduced shelf-life. Photooxidation of milk occurs under the presence of light (artificial, sunlight) and in both UV and visible light wavelength regions (Webster et al., 2009). This process is of particular concern in milk because it occurs quickly and influences consumer perception of milk flavor (Heer et al., 1995; Chapman, 2002; Chapman et al., 2002; Duncan and Webster, 2010).

Although no direct evidence exists that light-oxidized off-flavors contribute to decreased sales of fluid milk, the relationship of light-oxidized flavor to milk acceptability and emotional response has recently been reported (Walsh et al., 2014). Light-exposed milk (2% milkfat; 8 h of light exposure) was rated lower in acceptability, corresponding to “neither like nor dislike,” than milk that did not receive light-exposure, which was rated as “like moderately” (Walsh et al., 2014). With continued light exposure (up to 168 h), milk acceptability scores decreased to “dislike moderately.” The experience of drinking high-quality milk without light-oxidized flavor is characterized by positive emotional terms such as content, good, calm, satisfied, pleased, and happy (Walsh et al., 2014). In contrast, emotional terms that suggested withdrawal and had negative connotations, including disgusted and worried, were frequently selected for the disliked light-exposed milk (Walsh et al., 2014). Such responses illustrate that light exposure during retail storage can be detrimental to milk acceptability and increase negative emotional responses.

Specialty and single-serve milk products are often processed for extended shelf life (ESL), allowing for distribution across greater distances and limited processing schedules of products that have lower turnover or are seasonal products. Longer storage periods increase the risk of light exposure with the subsequent risk of altering milk quality before purchase. Milk is commonly displayed under fluorescent or light-emitting diode (LED) lighting in retail dairy cases in supermarkets, convenience stores, as well as vending machines. Whereas fluorescent lighting is most common in retail dairy cases, LED lighting use in open dairy retail cases is rapidly increasing, from about 15% in 2010 to almost

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40% in 2014 (Jack Sjogren, Hillphoenix, Chesterfield, VA, personal communication). Both LED and fluorescent lighting sources deliver light energy in UV and visible wavelength regions that cause excitation of photosensitive molecules, specifically riboflavin (**Rb**), in milk.

Several molecules in milk are responsive to light, acting as photosensitizers, including Rb, protoporphyrin, hematoporphyrin, chlorophyll a and b, and unidentified tetrapyrroles (Webster et al., 2009, 2011; Duncan and Chang, 2012). Photosensitizers initiate oxidation after being activated by light. Photosensitizers are important because they cause the destruction of milk components usually unaffected directly by light (Boff and Min, 2002). Excitation of Rb, the most studied photosensitizer in milk (Sattar et al., 1976; Wold et al., 2005; Webster et al., 2009), occurs when exposed to light at 250, 270, 370, 400, 446, and 570 nm wavelengths (Kyte, 1995). Some LED lights have visible light spectrums with greatest intensity at the wavelengths of greatest concern. Detrimental retail case lighting effects may be alleviated by selecting appropriate packaging materials to minimize the transmission of light (Duncan and Hannah, 2012).

In the United States, milk is commonly packaged in waxed paperboard, high-density polyethylene (**HDPE**) or polyethylene terephthalate (**PETE**). The HDPE transmits 57 to 60% of light wavelengths between 300 and 700 nm (van Aardt et al., 2001; Duncan and Hannah, 2012). The packaging material frequently used for single-serve milk products, PETE, transmits up to 75 to 85% of visible light. Packaging material can have a protective effect on milk quality through blocking or reducing the transmission of certain light wavelengths (Webster et al., 2009). It is important, then, to develop packaging materials that are consumer friendly yet block the most damaging wavelengths to milk quality.

One possible packaging innovation that has shown usefulness in protecting against photooxidation is titanium dioxide (TiO_2). Since its commercial production in the early 20th century, TiO_2 has been widely used as a leading white pigment in paints, toothpaste, and packaging. Titanium dioxide is a photo-responsive material and its importance is steadily increasing in the polymer and plastic industry (DuPont, 2007). Due to its ability to scatter light and absorb UV light energy, TiO_2 has been added at different concentrations to HDPE and PETE (Robertson, 2006). Moysssiadi et al. (2004) showed that a multilayer HDPE package pigmented with TiO_2 and carbon black protected milk better than a monolayer HDPE package pigmented with TiO_2 , clear PETE and PETE pigmented with TiO_2 when stored under fluorescent light at 4°C for 7

d. They found the multilayer HDPE package protected milk quality and suffered only a 28% Rb loss. The particle size of TiO_2 can be altered to affect color of light wavelengths transmitted (DuPont, 2007). Innovation in the use of TiO_2 particle size and refraction properties may improve packaging materials for milk quality protection.

Almost 30 yr ago, White (1985) evaluated consumer ($n = 393$) response to pigmented HDPE packaging options [opaque white, cream colored, yellow, and a natural (translucent)] for milk; opaque white packaging was most frequently selected as the preferred package if no additional cost was incurred. This study also indicated that most respondents (75%) did not think product visibility was a concern, suggesting that pigmented packaging could be successful. Respondents were equally concerned about the effects of light on nutrition and flavor. This early study suggests that consumers are willing to consider packaging modifications that protect milk flavor and nutrient quality. However, little guidance is available to the industry to identify the level of TiO_2 that is needed for milk quality protection.

The overall purpose of this research was to quantify performance of TiO_2 -dosed HDPE packaging on preserving milk sensory quality and oxidative stability through prevention or control of photochemically induced reactions.

The objective of this research was to determine changes in sensory characteristics, vitamin (Rb) retention, and oxidative stability of 2% milk packaged in HDPE bottles with different TiO_2 modifications up to 36 d of refrigerated (3°C) storage under fluorescent lighting, simulating retail storage.

MATERIALS AND METHODS

Packaging

The HDPE bottle types were differentiated using commercially available titanium dioxide pigments contained within the bottle resin, yielding bottles with different levels of light protection. Four levels of TiO_2 in HDPE (total of 5 packaging treatments) were tested, including 0% (translucent) serving as controls (light exposed: no light barrier; light protected: foil overwrap) and the 3 experimental TiO_2 -modified packaging treatment levels (low: 0.6%; medium: 1.3%; high: 4.3%). Bottle dimensions were 7.16" height $\times$ 3.29" width $\times$ 2.1" depth and a volume of 528 mL. We could readily see the milk in the control HDPE packages (translucent), but the visual appearance of the experimental TiO_2 -loaded packages was opaque white and prevented visualization of the milk.

Milk Processing

We limited light exposure of milk from the point of raw milk collection, through processing and packaging until packaged milk was placed into the controlled lighting conditions. Fresh raw milk was obtained from the Virginia Tech dairy farm and processed in the Food Science and Technology dairy pilot plant within 24 h of collection. Raw milk was stored at 4°C until processing. Before pasteurization, milk was prewarmed (55°C), separated into cream and skim milk using a pilot plant separator (model 1G, 6400 rpm, Bonanza Industries Inc., Calgary, Canada), and standardized to $2.0 \pm 0.1\%$ fat (Babcock method, AOAC 989.04; Bradley, 2000). Milk was homogenized in a 2-stage homogenizer [10,339 kPa (1,500 psi) for the first stage; 3,446 kPa (500 psi) for the second stage; Type DX, Cherry Burrell Corp., Delavan, WI] and UHT pasteurized at 131.1°C for 2 s (UHT/HTST Lab-25 DH pasteurizer, MicroThermics, Raleigh, NC). Milk was chilled for 24 h, then filled into clean 528-mL (16-oz) HDPE bottles and sealed with a screw cap lid. Prior to filling, bottles were sanitized with 100 mg/kg of chlorine solution, rinsed with deionized dH₂O, and drained. Approximately 470 mL, leaving 58 mL of headspace, was dispensed to each bottle using a Wheaton Unispense II (Wheaton Instruments, Millville, NJ). Milk was clean-filled into packages under a positive laminar flow hood (Atmos-Tech Industries, Ocean, NJ). During the bottling process, filled bottles were stored in iced coolers to control temperature until transferred to the retail storage case.

Storage Conditions

Samples were stored in refrigerated conditions. For the first replication (block A), a refrigerated retail case (Friedrich Floating Air, Friedrich 60–10–1056, San Antonio, TX) was used to simulate retail conditions. The second replication (block B) was completed in a walk-in cooler (Tonka Inc., Hopkins, MI) because the refrigerated case failed under the conditions of summer heat.

The refrigerated retail case was glass fronted with 5 doors and 3 shelves in which 2 fluorescent light bulbs ran the length of the dairy case over each shelf at 1/5th and 4/5th the distance from the front of the shelf. Six fluorescent light bulbs were situated vertically at the front of the dairy case at the ends of the case and in between each door junction. From the top of the bottle to the bulbs at the top of each shelf was a distance of 11.4 cm. The retail case was equipped with fluorescent cool white, 32-W light bulbs (Alto II, Panasonic, Maple Grove, MN) simulating retail conditions. Bottle placement within the dairy case was planned so that all treatments were distributed randomly to

reduce effects of different lighting intensity within the case. Temperature and light intensity were measured routinely. The average temperature of the refrigerated case was $2.7^{\circ}\text{C} \pm 0.8$. Over the 5-wk storage study, light intensity measurements were taken in 3 general locations on each treatment for each day of analysis. Light intensity averaged 2,186 lx, with a range from 396 to 3,970 lx, depending on sampling location. The randomization strategy for placing and removing of bottles was performed using JMP 10.0.0 Statistical Discovery Software (SAS Institute Inc., Cary, NC). Because of the broad range of light intensity, multiple bottles of each treatment and each control were randomly selected on each day of analysis for evaluation. For replication 1, samples were removed from the lighted case on d 1, 3, 8, 15, 22, and 29 for both sensory and chemical testing; in addition, chemical analyses (only) were completed on d 36 and sensory evaluation (only) was completed on d 43.

The walk-in cooler was equipped with 2 fluorescent lighting fixtures (2 bulbs each; 1.2 m length) positioned horizontally over each shelf to mimic the retail case; lights were positioned approximately 13 cm above milk packages. Samples were stored at an average lux and temperature of $2,421 \pm 551$ lx and $3.0 \pm 0.9^{\circ}\text{C}$, respectively. Replication 2 samples were tested on the same dates (through d 36) as the first replication; only chemical analyses were completed in this replication.

For each day of analysis, bottles from each treatment were withdrawn from the refrigerated storage unit and evaluated as described below. For the first replication (block A), 10 bottles were randomly selected. Two of these bottles were tested for analytical analyses; the remaining product from the tested bottles plus the other 8 bottles were commingled for sensory testing. For the second replication (block B), 2 bottles were randomly selected for analytical assays; no sensory testing was completed in this replication. A total of 4 bottles (2 from block A; 2 from block B) were evaluated for each treatment on each day of evaluation. Values for each packaging treatment for each day were averaged for each method of analysis.

Microbial Analysis

Standard plate counts of aerobic organisms were performed based on standard methods to ensure milk samples were properly pasteurized and maintained satisfactory microbial quality over the storage period (Laird et al., 2004). Samples were plated using Petrifilm Aerobic Count Plates (3M, St. Paul, MN). Aerobic count plates were incubated at $32 \pm 1^{\circ}\text{C}$ for 48 ± 3 h. Microbial tests were conducted on d 0 and 2 d before sensory tests.

Degradation of Rb by Fluorometric Analysis

The Rb concentration in milk was analyzed using the fluorometric method (AOAC method 970.65; Bradley, 2000; Webster et al., 2009) and was measured on a Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Scientific Instrument Inc., Columbia, MD). Samples were prepared by adding 0.01 *N* hydrochloric acid to milk (10 mL) to pH between 5.0 and 6.0. Hydrochloric acid (10 *N*, 0.1 mL) was added and the sample was autoclaved under pressure (117 kPa) for 30 min at 121 to 123°C. Samples were cooled and pH adjusted between 6.0 and 6.5 using 0.025 *N* sodium hydroxide. Hydrochloric acid (0.1 *N*) was immediately added to stop further precipitation around pH of 4.5. Each sample was then filtered using 25 mm syringe with a 0.2- μ m filter (Grace, Deerfield, IL) and 10-mL Norm-Ject syringe (Henke Sass Wolf Inc., Tuttlingen, Germany) before being measured using the spectrofluorophotometer.

Secondary Oxidation By-Products by Thiobarbituric Acid Reactive Substance Analysis

Thiobarbituric acid reactive substances (TBARS) were used to ascertain the development of malondialdehyde and other aldehydes, which are secondary oxidative products of fatty acids. This oxidative product reacts with thiobarbituric acid and creates a reddish/pink color, which can be read spectrophotometrically. The TBARS method was adapted according to Spanier and Taylor (1991). Samples (1 mL) from each treatment and control were diluted 1:4 ratio by adding 4 mL of distilled H₂O. Diluted sample was vortexed for approximately 20 s, and 1 mL was pipetted into a disposable centrifuge tube (15 mL) with SDS solution (4 mL) and EDTA solution (0.1 mL). Samples were mixed via vortex for 20 s and incubated in a water bath (95°C, 60 min), cooled to room temperature in an iced water bath, then mixed with pyridine and butanol solution. Samples were centrifuged for 20 min at room temperature at 1,600 $\times g$. The top organic layer (2.5 mL) was carefully transferred into a 1.5-mL polystyrene cuvette and absorbance read at 532 nm using a spectrophotometer (Spectronic 21D, Milton Roy, Ivyland, PA).

Volatile Chemistry by GC-MS

To help identify volatile compounds developed during storage, milk samples were analyzed for headspace volatiles. Replication 1 samples were analyzed within 24 h of sampling. Replication 2 samples were frozen at -70°C, thawed slowly in the refrigerator, and analyzed. Volatile compounds from milk were adsorbed on a solid-

phase microextraction fiber (SPME; 85 μ m carboxen-polydimethyl siloxane SPME fiber, Supelco, Bellefonte, PA) and separated using gas chromatography (GC). An HP 5890 GC with 5972 series mass selective detector HP5MS (Hewlett Packard, Palo Alto, CA) was used to identify volatile headspace compounds. Samples (8 mL) from each treatment and control were pipetted into 20-mL amber vials. Volatiles were adsorbed onto a clean polydimethyl siloxane SPME fiber. The fiber was exposed (4 mm) to sample headspace for 20 min with heating to 45°C (RCT basic heater with an ETS-D4 Fuzzy Controller, IKA Werke, Wilmington, NC) and agitation (250 rpm). Volatiles were desorbed from the fiber onto a DB-5 capillary column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; J&W Scientific, Folsom, CA), separated and analyzed using the following conditions: helium gas flow: 1.8 mL/min; injector temperature: 280°C; and detector temperature: 280°C. An initial run temperature rate of 35°C was held for 0.5 min and then the temperature was increased by 15°C/min to 180°C and held for 0.5 min. The temperature then was increased by 20°C/min to 260°C with the final temperature held for 0.5 min. The total run time was 15.17 min and the program ran in split-less mode. Chromatograms were plotted using HP ChemStation software (Hewlett Packard, Palo Alto, CA). External standards of pentanal and hexanal in dH₂O were used to identify oxidation products.

Sensory Analyses

Sensory analysis was performed by triangle tests (Meilgaard et al., 2007) on each day of evaluation (replication 1). Milk (10 packages; commingled) from each TiO₂-modified package treatment was compared with light-protected milk (100% opacity, 20 packages, commingled), testing for similarity to determine if the treatment provided equivalent and adequate protection as the light-protected product. Statistical parameters for similarity were $\alpha = 0.10$, $\beta = 0.05$, with an estimated proportion of discriminators (p_d) of 30%. In addition, following the same sampling plan, milk from each TiO₂-modified package was compared with light-exposed milk to determine if a difference could be detected between samples based on protection of the product by the packaging material. Statistical parameters for difference testing were $\alpha = 0.05$, $\beta = 0.10$, and the estimated p_d of 30%.

Virginia Tech Institutional Review Board approval was obtained before the beginning of the study. Informed consent was obtained from a minimum of 98 panelists who were recruited via email. To avoid sensory fatigue, an incomplete block design was used; panelists were

presented with three 3-sample (triangle) sets on each day of evaluation. Each sample set included 3 numerically coded (3-digit) samples (30 mL; 4°C). Panelists were told that 2 samples were identical and 1 sample was different. Panelists were asked to smell and taste each sample from left to right and record which sample they perceived to be the different sample. Water was provided to rinse between sessions and to limit sensory fatigue; a rest time of 1 min was established between each sample set.

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

Statistical Analysis

A 2-way factorial ANOVA with repeated measures (time; $n = 7$ d of testing), with blocking for the experimental blocks (replication 1 and 2), was used to assess changes in oxidation. Main effects and interactions were tested at $\alpha = 0.05$. Analytical analyses for TBARS and Rb were completed on each day of evaluation on 2 bottles of each treatment with duplicate samples evaluated from each bottle for each method. Volatile analysis was completed on each sample bottle (one evaluation). Statistical analyses were performed using JMP 10.0.0 Statistical Discovery Software (SAS Institute Inc., Cary, NC). Least significant difference contrast tests were used for mean separation when significant differences were found. Sensory data (replication 1) were analyzed based upon statistical parameters mentioned above and equations outlined in Meilgaard et al. (2007) by replication as well as for commingled data.

RESULTS AND DISCUSSION

Packages used in this project were not characteristic shape or wall thickness of a typical HDPE milk package. Therefore, although it is not possible to make a direct comparison of outcomes from this project to commercial packaging, we can learn about the relationship of TiO₂ levels in HDPE for protecting milk flavor and nutrients from light. The TiO₂ levels in our packaging ranged from 0% (translucent control) to 4.6% (high). Moyssiadi et al. (2004) used PET and HDPE packaging at 2% (20 g/kg of TiO₂).

Although the refrigeration conditions were different between replications, blocks were not statistically different. This allows for comparison of packaging treatments over time.

Protection of Sensory Quality

The goal of this research was to determine if the TiO₂ modifications provided better protection than a standard HDPE package (translucent control) and was comparable in protection to an optimum light barrier protection (foil-wrapped control). Therefore, the sensory testing was designed to minimize type I error (difference testing) when comparing the TiO₂ packages against the translucent control package and to minimize type II (similarity testing) error in comparison with foil control. The actual power of the sensory test was high, ranging from 60 to 95%, depending on the test design.

Table 1 describes the packaging success by providing the *P*-values for each package comparison over the product shelf-life. As expected, the difference between the foil and translucent control packages was noted early in the shelf life (d 3; $P = 0.0002$) and was consistently observed thereafter. High TiO₂ protected better than translucent control ($P < 0.05$; assumption that translucent control provides no protection) throughout the shelf-life as illustrated by difference testing. The medium and low TiO₂ packages did not provide greater protection than the translucent control in the first week, but sensory differences were noted by d 8. This suggests that, whereas milk in these packages did undergo oxidation early in the shelf-life, the oxidation rate over the extended storage period was lower in the milk in the low and medium TiO₂ packaging than that of the milk in the translucent control package.

The high TiO₂ package provided the best protection of the experimental packages, as indicated by similarity testing against the light-protected control. This package provided protection at a level equivalent to the light-protected control through d 29 and possibly longer; however, a difference was observed on d 43. An exception occurred on d 8, when the tested population discerned the difference between the high TiO₂ and light-protected package, with 31% correctly identifying the difference; the proportion of discriminators on that date was calculated as 10 to 52% of the true population able to find the difference. This marginally surpassed the preset proportion of discriminators (30%) and may have been attributed to a chance disproportionate number of experienced participants in the panel on that day.

With the exception of d 15, the other 2 TiO₂ treatments (low, medium) were statistically different ($P < 0.05$) from light-protected control, indicating that they did not protect the milk from off flavors or aromas as well as full light protection. As previously noted, milk within low and medium packages was different from light-exposed control milk by d 8. Distinguishing that the packaging yields a sensory difference from the

Table 1. Summary of statistical significance of sensory testing (triangle tests) for 2% milk packaged in high-density polyethylene (HDPE) with different TiO₂ experimental levels (low, medium, high) compared with controls (light exposed, LE); light protected (LP) over 43 d of light exposure (2,186 lx) at 3°C¹

Item	d 1	d 3	d 8	d 15	d 22	d 29	d 43
Difference test $p_d = 30\%$; $\alpha = 0.05$; $\beta = 0.10$							
LE vs. low	0.737	0.232	0.002*	0.515	0.009*	0.164	0.776
LE vs. medium	0.220	0.517	<0.001*	0.003*	<0.001*	0.008*	NT
LE vs. high	0.014*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	NT
LP vs. LE	0.314	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Similarity test $p_d = 30\%$; $\alpha = 0.10$; $\beta = 0.05$							
LP vs. low	0.104	0.079	0.126	<0.001*	<0.001*	<0.001*	NT
LP vs. medium	0.121	0.004*	0.014*	0.011*	<0.001*	<0.001*	NT
LP vs. high	0.779	0.232	0.009*	0.164	0.251	0.095	<0.001*
Panelists/test	24–28	27–31	34–36	37–39	35–36	37–38	48

¹Dosage of TiO₂-HDPE modified packaging [low (0.6% TiO₂), medium (1.3% TiO₂), high (4.3% TiO₂)]. Controls: light-protected (HDPE with foil overwrap); light-exposed (HDPE bottles with no TiO₂ or foil). NT = not tested because the difference had already been established. The d-36 evaluation period occurred during university spring break and the local population at that time was not sufficient to complete the testing. Therefore, an additional day (d 43) of testing was included for those tests needing further data. p_d = proportion of discriminators.

* $P < 0.05$.

translucent control milk product does not guarantee that milk flavor is sufficiently protected. However, evaluating for similarity to the milk in the light-protected (foil) control provided that validation. The low and medium TiO₂ packages provided no significant protection; milk from those packages was different from the light-protected milk by the third day. However, the high TiO₂ package provided protection equivalent to the light-protected milk through d 29 with an anomaly on d 8 ($P = 0.0085$).

The sensory work was completed on milk that was commingled from 10 different packages for each treatment. Analytical assessment was not completed on the commingled milk. It is probable that the averages reported for the analytical assessments described below are representative of the packaging treatments but may not be the exact values for the commingled milk that was evaluated by sensory testing.

Vitamin Retention

Riboflavin, also known as vitamin B₂, is a water-soluble vitamin found in large amounts in milk at an average concentration between 1.36 and 1.75 mg/mL (Dimick, 1982; Zygoura et al., 2004). The average initial Rb concentration in milk for this study was below that level (0.73 mg/mL) for both replications; all Rb values throughout the study were at that level or decreased with time and light exposure. Vallet et al. (2013) reported that Rb concentration in milk is affected by bovine nutrition. The milk used in this study was from the Virginia Tech dairy herd, which is often used for nutrition studies; we do not have direct knowledge of the herd nutritional management during the time of this study. Thus, we reported Rb as % retention

(Figure 1) over time, to illustrate the efficacy of the packaging for protecting Rb.

Riboflavin degradation is undesirable in food systems because it decreases the nutritional value of the food (Hoskin, 1988). Riboflavin concentration in milk decreased over the 36 d of evaluation in all packages (Figure 1). Riboflavin in milk from the light-protected treatment did not change significantly ($P > 0.05$), with only a 10.5% relative loss of Rb over the 5-wk storage period. Milk packaged in the high TiO₂ package maintained high level of Rb, comparable with the light-protected control through d 22, but had more rapid Rb degradation from d 22 through d 36 (28% decrease overall from d 1; $P < 0.05$). Riboflavin concentration in milk in the high TiO₂-package was significantly higher (<0.05) than in translucent control bottles by d 3. The low and medium TiO₂ packages protected Rb through 15 d of storage ($P > 0.05$; contrast to d 1). The medium TiO₂ packages retained 60% Rb through the storage period (d 36), whereas the low TiO₂ and light-exposed control packages permitted 60 to 76% Rb degradation by d 36.

The degradation of Rb due to light exposure is well studied (Dimick, 1973; Hoskin and Dimick, 1979; Christy et al., 1981; Hoskin, 1988; Moyssiadi et al., 2004; Webster et al., 2009). Webster et al. (2009) reported a loss of total Rb between 52.6 and 67.5% in 2% milk in light-exposed and specialized film packaging treatments within 3 d of refrigerated storage; at the end of the study (21 d), 95 to 98% of the Rb was degraded. In the current study, the relationship between packaging protection of Rb closely relates to the observed sensory protection with the high TiO₂ providing photo-protection of Rb, thus limiting the onset of photo-induced oxidation. In this study we have shown that white

Figure 1. Riboflavin retention in milk (2% milkfat) packaged in TiO₂-loaded [low (0.6% TiO₂); medium (1.3% TiO₂); high (4.6% TiO₂)] high-density polyethylene (HDPE) compared with light-exposed (translucent; natural HDPE with no TiO₂) and light-protected (foil wrapped; natural HDPE with no TiO₂) packaging. Packages were stored at an average light intensity of $2,304 \pm 166$ lx over 36 d (3°C). The average initial riboflavin concentration was 0.73 mg/mL. Mean values ($\pm$ SE) for each packaging treatment on each day were based on 2 replications of 2 bottles each. Color version available online.

opaque package appearance, as perceived visually, is insufficient to indicate appropriate protection of nutrients from the effects of light exposure. This study illustrates that LPA additives, such as TiO₂, in HDPE must be added at a level sufficient to protect Rb during retail storage, thus providing greater protection of milk flavor and nutrients. The TiO₂ added at 0.6% to HDPE did not provide sufficient protection for light exposure longer than 24 h in our study, although it must be considered that the packages used in this study were not the shape or wall thickness of fluid milk packaging.

Control of Oxidation

The TBARS assessment of MDA and other aldehydes has been studied extensively in meat and milk oxidation and shown to increase in concentration as photooxidation of milk occurs (Rosenthal et al., 1993; van Aardt et al., 2005; Campo et al., 2006). Thiobarbituric acid reactive substances have also been shown to correlate with changes in sensory characteristics of light-induced oxidation (Rosenthal et al., 1993). Rosenthal et al. (1993) reported levels of TBARS (0.61 mg/L) for whole milk that was stored in the dark for 7 d. Milk in this study had initial values in the same range (0.68 mg/L). Campo et al. (2006) estimated that a TBARS value

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

As expected, light-protected packaging provided optimal oxidative control (Figure 2); aldehyde production was lowest in this group and did not increase significantly ($P > 0.05$) over time. Milk in the high TiO₂ package had TBARS values similar to ($P > 0.05$) the light-protected control up to d 29. However, by d 36, aldehyde production increased more rapidly than in the milk from the light-protected control package ($P < 0.05$), although still significantly lower than light-exposed milk control. This closely approximates the trends of Rb concentration. The TBARS increased by d 8 in the other packages to values exceeding 1.0 mg/L.

We observed that differences in TBARS values closely related to the observed differences in sensory evaluation. All significant statistical contrasts for sensory comparisons had TBARS values of 1.3 mg/L or greater with 2 exceptions very early in the shelf-life. On d 36 aldehyde concentrations in the high TiO₂ package (1.43 mg/L) and light-protected control (1.0 mg/L) were significantly different between the 2 samples. It can be inferred that TBARS values in milk greater than 1.3 mg/L may be indicative of perceptible sensory changes

Figure 2. Thiobarbituric acid reactive substances (TBARS) concentration in milk (2% milkfat) packaged in TiO₂-loaded [low (0.6% TiO₂); medium (1.3% TiO₂); high (4.6% TiO₂)] high-density polyethylene (HDPE) compared with light-exposed (translucent; natural HDPE with no TiO₂) and light-protected (foil wrapped; natural HDPE with no TiO₂) packaging. Packages were stored at an average light intensity of $2,304 \pm 166$ lx over 36 d (3°C). The average initial TBARS value was 0.55 mg/L. Mean values ($\pm$ SE) for each packaging treatment on each day were based on 2 replications of 2 bottles each. Color version available online.

from light-induced oxidation. This does not directly imply a change in sensory acceptability of milk, but may imply a notable change in sensory quality.

Numerous volatile compounds are produced during photooxidation. Pentanal and hexanal are common secondary lipid oxidative compounds found in milk (Mehta and Bassett, 1978; Farrer, 1983; Bekbolet, 1990; Cadwallader and Howard, 1998; Rysstad et al., 1998; van Aardt et al., 2001, 2005; Mestdagh et al., 2005; Webster et al., 2009, 2011; Moore et al., 2012) and are commonly used as indicators of lipid oxidation. In this study, several compounds (pentanal, hexanal, dimethyl disulfide, and 2-propanone) increased in peak area over time (data not shown for pentanal, dimethyl disulfide, and 2-propanone). Hexanal peak area was statistically significantly higher ($P < 0.05$) in light-exposed control than the light-protected treatment by d 15 and this finding remained true through d 36 (Figure 3). Hexanal concentration (based on peak area) in milk was not significantly different among TiO₂ packaging treatments through d 29 of the experiment. However, several treatments were significantly lower in hexanal than the light-exposed control on several days of the experiment. The medium and high TiO₂ treatments

appeared to limit hexanal production, relative to the light-protected control. Webster et al. (2009) also reported that hexanal was a major volatile peak in light exposed milk. Their study used film overwraps that provided light interference targeted at the major wavelengths of Rb. The light interference films limited the development of hexanal compared with light-exposed control milk (no film overwraps), but were not as effective as the light-protected (foil overwrap) control (Webster et al., 2009). However, in our current project, we did observe a longer delay in hexanal development, through 8 d, with the HDPE with TiO₂ compared with the light interference overwrap films used in the study by Webster et al. (2009), which showed increases in hexanal by 8 d.

Statistical contrasts for hexanal (data not shown) were similar to the results of the Rb analysis. No significant differences were observed in hexanal peak area in high TiO₂-packaged milk through d 29 compared with d 1. On d 36, milk in the high TiO₂ packages had higher hexanal production compared with light-protected control milk. The light-exposed control and low TiO₂ package were unable to control hexanal production; the medium TiO₂ packaging was more effective than the

Figure 3. Hexanal concentration in milk (2% milkfat) packaged in TiO₂-loaded [low (0.6% TiO₂); medium (1.3% TiO₂); high (4.6% TiO₂)] high-density polyethylene (HDPE) compared with light-exposed (translucent; natural HDPE with no TiO₂) and light-protected (foil wrapped; natural HDPE with no TiO₂) packaging. Packages were stored at an average light intensity of 2,304 ± 166 lx over 36 d (3°C). Mean values for each packaging treatment on each day based on 2 replications of 2 bottles each. Color version available online.

low TiO₂ packaging but not as effective as the high TiO₂ packaging.

CONCLUSIONS

Not all visually opaque milk packaging effectively protects milk nutrients and flavor over an ESL period. Packaging can be modified to protect milk sensory quality and control photo-oxidation of Rb by manipulating photo-responsive molecules in the packaging. Levels of TiO₂ greater than 1.3% incorporated with HDPE resin during package production may effectively protect milk quality during an extended period of retail lighting exposure. A TBARS of 1.3 mg/L or higher is indicated as a marker of milk sensory quality changes due to light exposure. The dairy industry can use this information to assist with packaging selection to improve fluid milk quality for ESL fluid milk.

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