Aroma Analysis of Light-Exposed Milk Stored With and Without Natural and Synthetic Antioxidants

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ABSTRACT

The effect of antioxidants, added in a single initial dose or in weekly additions to extended shelf life milk, was evaluated over 6 wk of lighted storage at 4°C. Light-induced oxidation was measured by determining pentanal, hexanal, heptanal, and 1-octen-3-ol contents. Weekly addition of a combination of butylated hydroxyanisole and butylated hydroxytoluene (100 mg/kg of milk fat, each) maintained heptanal content of milk at levels comparable to light-protected milk, whereas an initial single addition of α -tocopherol significantly decreased hexanal content over the first 4 wk of storage. Odor-active compounds associated with light-induced oxidation included 2,3-butanedione, pentanal, dimethyl disulfide, hexanal, 1-hexanol, heptanal, 1-heptanol, and nonanal. The addition of butylated hydroxyanisole and butylated hydroxytoluene in a single initial addition resulted in decreases in pentanal and hexanal odor, but not in heptanal and 1-heptanol odor, whereas the addition of α -tocopherol and ascorbyl palmitate decreased pentanal and heptanol odor, but not hexanal and heptanal odor.

(Key words: light-oxidized flavor, lipid oxidation, milk, antioxidant)

Abbreviation key: ASCP = ascorbyl palmitate, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, ESL = extended shelf life, GC-O = gas chromatography-olfactometry, SPME = solid-phase microextraction, TOC = α -tocopherol.

INTRODUCTION

Milk fat contains approximately 2.3% palmitoleic acid (C16:1n7), 24.6% oleic acid (C18:1n9), and 2.8% linoleic acid (C18:2n6), with the latter being susceptible

to lipid oxidation. Factors such as light exposure and the presence of oxygen increase the potential for lipid oxidation. Milk is sold commercially in clear, translucent, or opaque containers in dairy display cases with fluorescent lighting. Clear glass, poly(ethylene terephthalate), and translucent high-density polyethylene containers allow light to reach the product and initiate lipid oxidation.

Both ultraviolet and visible light wavelengths contribute to the development of light-induced aroma compounds in milk (Rosenthal, 1992; Bosset et al., 1993). Singlet oxygen, created during the cascade of photochemical reactions, reacts with lipid, protein, and vitamin compounds to initiate the formation of oxidation products with unpleasant off-flavors and aromas (Skibsted, 2000). Free radicals generated by auto-oxidation reactions are unstable and contribute further to the accumulation of secondary metabolites with flavor and aroma impact.

The aroma of fresh milk is mild, with a slight cooked note from the thermal process of pasteurization. The volatile chemistry of fresh milk is complex due to the heterogeneous nature of the system (Friedrich and Acree, 1998), and not all analytically measurable volatiles contribute to the aroma of milk. Using gas chromatography-olfactometry (GC-O), Moio et al. (1994) listed 9 odor-active compounds in pasteurized milk. These compounds included heptanal, indole, nonanal, 1-octen-3-ol, dimethylsulfone, hexanal, 2-nonanone, benzothiazole, and D-decalactone. Dimethylsulfone provided the most intense odor followed by hexanal.

Ultra-high temperature processing increases refrigerated shelf life of milk to 6 or more wk, providing an extended shelf-life (ESL) product for convenience marketing and longer distribution times. However, the extended storage time of ESL milk increases the opportunity for light-induced oxidation. Eight odor-active compounds have been identified (using GC-O) in UHT-processed milk. 2-Heptanone, which was described as fruity, spicy, and cinnamon, and was not reported in pasteurized milk, had the highest aroma intensity in

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UHT milk, whereas dimethylsulfone (sulfurous odor) had much lower odor intensity. 2-Undecanone, described as floral and rose-like, was uniquely observed in UHT milk as well. Heptanal, nonanal, and 1-octene-3-ol were not reported in UHT milk (Moio et al., 1994; Friedrich and Acree, 1998).

Aroma-active compounds in light-activated milk have been described, using GC-O, as green/fish oil, sour grass, sweet, mushroom, cut-grass, boiled potato, cheesy, pungent, and sulfurous (Friedrich and Acree, 1998). These odors relate to heptanal, pentanal, heptanol, 1-octene-3-ol, hexanal, dimethyl disulfide, 2,3-butanedione, and other sulfur containing compounds, respectively (Kim and Morr, 1996; Cadwallader and Howard, 1998).

Light-activated odors in milk can be limited by proper storage, such as packaging in light- and oxygen-impermeable packaging, or by adding antioxidants. Synthetic antioxidants commonly used in the food industry include butylated hydroxyanisole (**BHA**) and butylated hydroxytoluene (**BHT**). These antioxidants are lipid soluble and terminate free-radical chain reactions by donating hydrogen atoms or electrons to free radicals and converting them to more stable structures (Frankel, 1998). The legal limit for the addition of BHA and BHT to most foods in the United States is 200 mg/kg of fat. When added in combination, a total of 200 mg/kg of fat is allowed (CFR, 2001).

The trend toward healthier living and eating has drawn more attention to natural antioxidants, such as α -tocopherol (**TOC**; vitamin E) and ascorbic acid (vitamin C). α -Tocopherol is fat-soluble and limits free radical oxidation reactions by the same mechanism as BHA and BHT. Ascorbic acid is water-soluble and acts as a synergist with TOC either by acting as a hydrogen donor to the phenoxy radical of tocopherol, thereby regenerating it, or by reacting with free oxygen and removing it in a closed system (Frankel, 1998). Ascorbyl palmitate (**ASCP**), a fat-soluble analog of ascorbic acid, is also used widely.

Several approaches for antioxidant incorporation in milk have been used in an attempt to reduce lipid oxidation. Charmley and Nicholson (1993) showed with sensory analysis that oxidation flavor decreased significantly with increased TOC levels in milk (from 17.6 to 37.8 $\mu\text{g/g}$ of milk fat after 2 wk) from dairy cows fed a diet supplemented with 3000 IU of D,L- α -tocopheryl acetate daily and administered intramuscular injections of 3000 IU of D- α -tocopherol. Sensory analysis was completed by 3 trained panelists by marking oxidation intensity on an unstructured line scale with fresh, non-oxidized milk representing no oxidation and milk which had been spiked with copper wire for 24 h as highly oxidized. Jung et al. (1998) added ascorbic acid (from

200 to 1000 mg/kg) directly to milk and, using dynamic headspace analyses and gas chromatography, concluded that formation of dimethyl disulfide decreased. Sensory evaluation found that milk containing ascorbic acid (and therefore less dimethyl disulfide) improved in flavor when ascorbic acid was present. Minimization of dimethyl disulfide formation by ascorbic acid suggested that the disulfide was formed by singlet oxygen oxidation of methionine.

Raw milk contains TOC at approximately 13 to 30 mg/kg of milk fat and ascorbic acid at <20 mg/kg. Natural antioxidants are generally recognized as safe when used in accordance with food manufacturing practices and therefore not limited in most foods (CFR, 2001). The addition of TOC, ascorbic acid, and ASCP to milk is permitted. The presence of these substances must be noted on the label (M. Albee Mattow, International Dairy Foods Association, personal communication, 2003). Although no legal limit exists for the addition of TOC and ascorbic acid, care should be taken when adding these antioxidants to food because high concentrations can cause pro-oxidation (Frankel, 1998).

Marketing ESL milk as a single-serve beverage in convenience stores and other markets can increase sales profit. More shelf-life issues will arise due to the higher surface area available to light-induced oxidation of ESL milk in single-serve clear or transparent packaging. Although microbial spoilage is usually indicative of the end of shelf life in HTST milk, aroma and flavor changes in ESL milk are mostly related to chemical reactions. It would be valuable to evaluate the effect of antioxidants on odor-active compounds associated with light-induced oxidation in milk, especially over an extended shelf life. Research is necessary to show the effect of antioxidants on milk fat and protein oxidation, in terms of inhibition of specific off-aromas (cut-grass, green, sweet, sulfurous, mushrooms, etc.) and aroma compounds such as pentanal, hexanal, heptanal, dimethyl disulfide, and 1-octen-3-ol.

The objectives of this study were 1) to compare the aroma profile of light-exposed and light-protected ESL milk for 6 wk when treated with single and weekly additions (100 mg/kg of milk fat per antioxidant) of (a) TOC and ASCP, and (b) BHA and BHT; and 2) to describe the extent of oxidation in control milk and milk treated with antioxidants (as described in objective 1) by comparing pentanal, hexanal, heptanal, and 1-octen-3-ol concentration.

MATERIALS AND METHODS

Milk Processing

Fresh raw milk (80 L) was obtained from the Virginia Tech dairy farm. Milk was prewarmed to 55°C and

separated into cream and skim milk using a pilot plant separator (Elecram separator, model 1G, 292xG, Bonanza Industries, Inc., Calgary, Canada). Low fat milk was standardized at 2% milk fat by adding cream and skim milk in appropriate proportions. Milk was separated into 7 aliquots. Approximately 32 L was used as control (unspiked) milk, and a combination of BHA and BHT was added to three 8-L aliquots to represent 3 replications, and a combination of TOC and ASCP was added to the remaining 3 aliquots (8 L each) for 3 replications. Milk aliquots were homogenized and pasteurized separately. Homogenization was done at 13.6 MPa (10.2 MPa—first stage; 3.4 MPa—second stage) on a 2-stage homogenizer (Type DX, Cherry Burrell Corp., Delavan, WI). Milk was pasteurized at 128°C for 2 s using a UHT tubular heat exchanger (UHT/HTST Laboratory 25 HV, Microthermics Inc., Raleigh, NC). Milk was subsequently cooled to less than 25°C and stored at 4°C in sterile glass containers covered with aluminum foil to prevent light exposure until sample bottles were filled.

Preparation of Antioxidant-Spiked Milk Samples

Butylated hydroxyanisole, BHT, TOC, and ASCP were obtained from Fisher Scientific (Cincinnati, OH). The 4 antioxidant treatments were (1) an initial single addition of TOC and ASCP (each at 100 mg/kg of milk fat), (2) an initial single addition of BHA and BHT (each at 100 mg/kg of milk fat), (3) a weekly addition of TOC and ASCP (each at 100 mg/kg of milk fat), and (4) a weekly addition of BHA and BHT (each at 100 mg/kg of milk fat). Antioxidant treatments that included a single initial dose were spiked during processing on d 0. Treatments 3 and 4 were spiked with the antioxidant doses on wk 1, 2, 3, 4, and 5. Each week, 100 mL of low fat UHT milk (Parmalat, Wallington, NJ; obtained from a local grocery store) was used to make a concentrated antioxidant solution for each antioxidant combination and for each replication. From each treated sample to be spiked, 1 mL of milk was withdrawn and discarded. A subsequent addition of 1 mL of the concentrated antioxidant solution was added to achieve an addition concentration of 100 mg/kg of milk fat of TOC and 100 mg/kg of milk fat of ASCP for treatment 3 and 100 mg/kg of milk fat of BHA and 100 mg/kg of milk fat of BHT for treatment 4. Because TOC is a viscous fluid, milk was prewarmed to 50°C before antioxidants were added and blended with a hand blender (White Westinghouse, Tulsa, OK) for approximately 30 s.

Storage and Light Exposure

Aliquots (38 mL) were delivered into 40-mL clear glass bottles fitted with Teflon-coated septa (Supelco,

Bellefonte, PA). Control milk samples were exposed to 2 levels (light-protected, light-exposed) of light. Light-protected sample bottles were covered with aluminum foil to prevent any light exposure, whereas light-exposed samples were not covered with foil. All samples (control and antioxidant-treated samples) were exposed to light of 1100 to 1300 lx (fluorescent 40-W Econ-o-watt lights), as measured at the top of sample bottles, for 12 h/d, each day. All samples were stored at 4°C for 6 wk in a refrigeration unit (Tonka, Hopkins, MN). One sample of each treatment and each replication was randomly chosen for analysis on d 0, wk 1, 2, 3, 4, 5, and 6 of storage.

To ensure that milks were of similar microbiological quality and to verify that type of antioxidant addition did not affect the microbiological shelf life of the product, milk samples from each treatment were evaluated for standard plate count and coliform bacteria count according to standard methods using Petrifilm (3M, St. Paul, MN) on d 0, wk 1, 2, 3, 4, 5, and 6 of storage (Marshall, 1993).

Volatile Analysis

Traditional extraction and isolation techniques for volatiles, such as distillation and solvent extraction, can distort the balance of odor-active and nonodorous compounds or generate artifacts (Friedrich and Acree, 1998). Headspace analysis (static, dynamic) is costly and time-consuming and captures only the most abundant volatiles in the headspace. Purge-and-trap analysis, a form of dynamic headspace analysis, includes a concentration step but the intent is to isolate a single component instead of a balance of compounds representing the volatile mixture. Solid-phase microextraction (SPME) is a relatively new volatile extraction technique that offers a simple, cost-effective method for extracting volatiles in the headspace (Marsili, 1999).

SPME. Aliquots (21 mL) of milk were pipetted into 40-mL clear glass bottles fitted with teflon septa (Supelco). A 75- μ m carboxen poly(dimethyl siloxane)-coated SPME fiber (Supelco) was exposed to the milk headspace with the end of the fiber approximately 1 cm above the milk surface for 22 min at 45°C with magnetic stirring of the sample (van Aardt et al., 2001).

Gas chromatography conditions. Volatile compounds were desorbed in the injector port of a system consisting of an HP 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector. The injector temperature was 280°C, and all injections were made in the splitless mode. Separation was completed on a 30-m $\times$ 0.25-mm i.d. $\times$ 0.25- μ m film thickness capillary column (DB-5ms; J&W Scientific, Folsom, CA) with helium carrier gas

linear flow rate of 35 cm/s. The oven temperature was programmed from 35 to 180°C at a rate of 15°C/min, and from 180 to 260°C at a rate of 20°C/min with initial, intermediate, and final hold times of 0.5 min. The flame ionization detector was maintained at 300°C.

Pentanal, hexanal, heptanal, and 1-octen-3-ol concentrations, determined in triplicate, were calculated by comparing area counts to those of a calibration curve (McNair and Miller, 1998). Stock solutions of these compounds in methanol:water (1:99) consisted of concentration levels of 0.01, 0.1, 1, 10, 100, and 10,000 mg/kg. An external standard of 2-methyl-3-heptanone in water was used to determine an absolute calibration factor for each day of analysis (Marsili, 1999). This was done by normalizing external standard, and subsequently, volatile compound area counts from wk 1 to 6 according to the external standard area counts from d 0.

Training of panelists for GC-O. Six people (who regularly participate in GC-O studies), consisting of students and staff at the Department of Food Science and Technology at Virginia Tech in Blacksburg, VA, were trained in four 20-min sessions before GC-O analysis of treated samples. Two aroma training kits were obtained: (1) Beer Aroma Recognition kit, (2) Beer Taint Recognition kit (Brewing Research International, United Kingdom). These kits contained a variety of chemical compounds representing odors commonly associated with lipid oxidation (van Aardt, 2004). During training, panelists were asked to sniff each aroma, practice placing a verbal descriptor with the aroma, and then compare with the identified aroma for each reference standard. Intensities were rated on a 5-point scale (1 = slight odor to 5 = extreme odor), and tabulated as “+” (odor intensities 0 to 1), “++” (odor intensities 2 to 3), and “+++” (odor intensities 4 to 5). Three panelists who consistently correctly identified aroma compounds were chosen to participate in GC-O of samples.

GC-O. The GC-O analysis was done weekly on 1 replication per treatment. Three trained panelists evaluated samples. Volatile compounds analyzed by GC-O were extracted and concentrated as described above. Compounds were desorbed in the injector port of a GC-O system consisting of an HP 5890A GC (Hewlett-Packard Co.) equipped with a flame ionization detector and a sniffing port (ODOII; SGE, Inc.). Chromatography conditions and temperature program were as described above. The sniffing port was maintained at 300°C, and was supplied with humidified medical grade air at 10 to 15 mL/min. Column eluent was split 1:1 between the flame ionization detector and sniffing port using deactivated fused silica capillaries (1 m length $\times$ 0.32 μ m i.d.). Chromatograms were graphed on an HP integrator (HP 3396A, Hewlett-Packard Co.). Volatile odor compounds were identified after comparing retention

times with known retention times of flavor compounds from preliminary gas chromatography-mass spectrometry results of light-exposed milk samples.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (HP 6890, 5973 Mass Selective Detector, Hewlett-Packard Co.) was done to identify volatile compounds in light-exposed milk samples. Separation was completed on a 15-m $\times$ 0.25-mm i.d. $\times$ 0.25- μ m film thickness capillary column (HP-5; Hewlett-Packard Co.). Oven temperature program and conditions were the same as described above.

Lipid Extraction and HPLC Analysis of Antioxidants

Milk from the same sample vials used for GC-O was ultracentrifuged (Beckman L2-65B, Beckman Instruments, Inc. Palo Alto, CA) for 30 min at 15,000 rpm under refrigeration (10 to 15°C). The top fat layer was extracted with the Bligh and Dyer (1959) fat extraction method to obtain pure milk fat. Pure milk fat was combined with 1 mL of HPLC-grade methanol in a 2-mL clear glass crimp vial (HP 5181-3375, Hewlett-Packard Co.) for further analysis on HPLC. The methanol extracts were placed in an Agilent G1313A (Agilent, Palo Alto, CA) autosampler and analyzed on an Agilent 1100 Series HPLC system using a Zorbax Eclipse XDB-C8 column (5 μ m, 4.6 mm i.d. $\times$ 15 cm, a diode array detector, and peak detection at 295 nm (band width 20 nm). The mobile phase consisted of 95:5 methanol: water at a flow rate of 1 mL/min. The thermostat temperature was 50°C.

Statistical Analysis

Volatile compounds were quantified by calculating means of concentrations ($n = 3$) and standard deviations. One-way ANOVA was used to test the null hypothesis (H_0 : $\mu_{\text{control-light}} = \mu_{\text{control-no light}} = \mu_{\text{tocopherol, ascorbyl palmitate (single addition)}} = \mu_{\text{tocopherol, ascorbyl palmitate (weekly addition)}} = \mu_{\text{BHA, BHT (single addition)}} = \mu_{\text{BHA, BHT (weekly addition)}}$) for each week of storage, with 147 degrees of freedom ($n - 1$) and 6 treatment levels as mentioned in the null hypothesis. Two-way ANOVA was used to show significant interactions between time (wk) and treatment (antioxidant addition). This type of an interaction is observed visually in slope changes in graphs. Tukey's least significant difference (LSD) was used to compare means. Significant differences were defined at $P < 0.05$ (SAS Institute, 1998).

RESULTS AND DISCUSSION

Extent of Lipid Oxidation: Volatile Content

Pentanal, hexanal, heptanal, and 1-octen-3-ol were used to measure the extent of light-induced oxidation

Figure 1. Pentanal content of control and antioxidant-treated milk after 12 h/d of light exposure (1100 to 1300 lx) for 6 wk. Treatments included: Control milk (unspiked) (□); control milk (unspiked, light-protected) (■); control milk with UHT milk added, control for weekly additions (○); control milk with UHT milk added, control for weekly additions (light-protected) (●); single addition of 100 mg/kg (of milk fat) of butylated hydroxyanisole (BHA) and 100 mg/kg of butylated hydroxytoluene (BHT; Δ); single addition of 100 mg/kg of tocopherol (TOC) and 100 mg/kg of ascorbyl palmitate (ASCP; ×); weekly addition of 100 mg/kg of BHA and 100 mg/kg of BHT dissolved in a small quantity of UHT milk (◇); weekly addition of 100 mg/kg of TOC and 100 mg/kg of ASCP dissolved in a small quantity of UHT milk (+).

of antioxidant-treated milk. These volatile aldehydes and carbonyl compounds are primarily responsible for development of off-flavors/off-odors related to the quality and oxidative stability of milk fat (Frankel, 1998). Pentanal and hexanal have been found to change significantly in pasteurized, homogenized milk when exposed to sunlight (Jenq et al., 1988). Moio and coworkers (1994) found (using GC-O) that 1-octene-3-ol was responsible for much of the aroma of water buffalo milk. Two-way ANOVA ($P < 0.05$) showed significant interactions between time (week) and treatment (antioxidant addition). This can be seen visually by observing slope changes in Figures 1 to 3. Pentanal content of control and antioxidant-treated milk did not differ significantly when exposed to light (1100 to 1300 lx) for 12 h/d throughout 6 wk of storage (Figure 1).

In addition, 1-way ANOVA showed few significant differences between treated and control milks for each week of storage. Table 1 shows the increase in hexanal concentration for milk treated with antioxidants and exposed to light for 12 h/d for 6 wk. From as early as 1 wk of storage under fluorescent lights, significant differences existed in hexanal content of light-protected and light-exposed milk. An initial single addition of a combination of TOC (100 mg/kg) and ASCP (100 mg/kg) significantly reduced hexanal content in milk, compared with light-exposed control milk throughout the first 4 wk of storage. However, the constant weekly addition of these 2 antioxidants resulted in hexanal

levels similar to that of light-exposed control milk throughout storage. The single and weekly additions of BHA and BHT also controlled hexanal content to some degree over the first 4 wk of storage. The human aroma threshold for hexanal in low fat milk is 0.339 mg/kg (Norton, 2003). In our study, hexanal concentration increased to a maximum of 50.11 ± 5.77 mg/kg after 6 wk of light-exposed storage, which indicates that hexanal could affect milk aroma. Data from GC-O demonstrated that panelists could identify the characteristic "cut grass" odor that represents hexanal after 2 wk of storage (data not shown). Hexanal concentration at 2 wk of storage was greater than aroma threshold (5.22 to 6.48 mg/kg) in light-protected control milk and was 3 to 4 times higher in light-exposed milk samples.

Heptanal content of milk increased in light-exposed control and antioxidant-treated milk and not in light-protected control milk over 6 wk of storage (Figure 2). However, the weekly addition of a combination of BHA and BHT reduced heptanal content of milk to such a degree that, except for wk 5, no significant difference was observed when compared with light-protected control milk. No other antioxidant treatment evaluated in this study yielded that degree of protection against the effect of light. The initial single dose of a combination of BHA and BHT or a combination of TOC and ASCP limited light-induced oxidation for the first 4 wk of storage, but not thereafter. The observed increase in heptanal content at wk 5 might indicate that the antioxidant

Figure 2. Heptanal content of control and antioxidant-treated milk after 12 h/d of light exposure (1100 to 1300 lx) for 6 wk. Treatments included: Control milk (unspiked) (□); control milk (unspiked, light-protected) (■); control milk with UHT milk added, control for weekly additions (○); control milk with UHT milk added, control for weekly additions (light-protected) (●); single addition of 100 mg/kg (of milk fat) of butylated hydroxyanisole (BHA) and 100 mg/kg of butylated hydroxytoluene (BHT; Δ); single addition of 100 mg/kg of tocopherol (TOC) and 100 mg/kg of ascorbyl palmitate (ASCP; ×); weekly addition of 100 mg/kg of BHA and 100 mg/kg of BHT dissolved in a small quantity of UHT milk (◇); weekly addition of 100 mg/kg of TOC and 100 mg/kg of ASCP dissolved in a small quantity of UHT milk (+).

Figure 3. 1-Octen-3-ol content of control and antioxidant-treated milk after 12 h/d of light exposure (1100 to 1300 lx) for 6 wk. Treatments included: Control milk (unspiked) (□); control milk (unspiked, light-protected) (■); control milk with UHT milk added, control for weekly additions (○); control milk with UHT milk added, control for weekly additions (light-protected) (●); single addition of 100 mg/kg (of milk fat) of butylated hydroxyanisole (BHA) and 100 mg/kg of butylated hydroxytoluene (BHT; Δ); single addition of 100 mg/kg of tocopherol (TOC) and 100 mg/kg of ascorbyl palmitate (ASCP; ×); weekly addition of 100 mg/kg of BHA and 100 mg/kg of BHT dissolved in a small quantity of UHT milk (◇); weekly addition of 100 mg/kg of TOC and 100 mg/kg of ASCP dissolved in a small quantity of UHT milk (+).

Table 1. Hexanal content (mean $\pm$ standard error of the mean; mg/kg) of control and antioxidant-treated milk after light exposure (1100 to 1300 lx) for 12 h/d for 6 wk.

Treatment ²	Light	Time (wk)											
		1 ¹		2		3		4		5		6	
		Mean	$\pm$ SEM	Mean	$\pm$ SEM	Mean	$\pm$ SEM	Mean	$\pm$ SEM	Mean	$\pm$ SEM	Mean	$\pm$ SEM
Control	Yes	13.81 ^a	0.57	18.64 ^{ab}	2.48	36.75 ^{bc}	3.80	31.69 ^b	3.93	28.50 ^b	4.09	32.41 ^a	2.62
Control	No	4.42 ^c	0.37	6.48 ^c	0.21	4.06 ^e	0.00	6.43 ^d	0.07	5.70 ^c	1.64	4.06 ^b	0.00
Control (weekly) ³	Yes	13.81 ^a	0.57	23.91 ^a	1.97	45.00 ^a	0.72	40.31 ^a	3.53	41.73 ^a	4.98	50.11 ^a	5.77
Control (weekly) ³	No	5.07 ^c	0.58	5.22 ^c	0.60	6.31 ^e	1.14	4.70 ^d	0.65	4.06 ^c	0.00	4.06 ^b	0.00
BHA/BHT (single)	Yes	10.62 ^b	0.85	15.80 ^b	3.23	30.36 ^c	5.26	22.94 ^c	1.15	27.29 ^b	1.97	30.96 ^{ab}	1.12
TOC/ASCP (single)	Yes	9.79 ^b	1.17	16.36 ^b	2.59	21.34 ^d	2.41	21.60 ^c	1.73	38.47 ^a	0.37	32.41 ^a	2.62
BHA/BHT (weekly)	Yes	10.62 ^b	0.85	18.05 ^{ab}	3.47	29.55 ^c	1.99	19.48 ^c	2.83	21.32 ^b	3.14	38.12 ^a	25.4
TOC/ASCP (weekly)	Yes	9.79 ^b	1.17	21.43 ^{ab}	0.84	39.21 ^{ab}	2.21	37.77 ^{ab}	3.10	38.47 ^a	0.37	46.64 ^a	3.90

^{a-c}Means (n = 3) in the same column with different superscripts are significantly different ($P < 0.05$).

¹Hexanal content on d 0 was 0 mg/kg for all samples.

²Treatments: Control = milk (unspiked) with and without light exposure; Control (weekly) = control milk with UHT milk added at the same levels as antioxidant samples spiked weekly, with and without light exposure; BHA/BHT (single) = single addition of 100 mg/kg butylated hydroxyanisole (BHA) and 100 mg/kg butylated hydroxytoluene (BHT); TOC/ASCP = single addition of 100 mg/kg of tocopherol (TOC) and 100 mg/kg ascorbyl palmitate (ASCP); BHA/BHT (weekly) = weekly addition of 100 mg/kg BHA and 100 mg/kg BHT dissolved in 1 mL of UHT milk; TOC/ASCP (weekly) = weekly addition of 100 mg/kg TOC and 100 mg/kg ASCP dissolved in 1 mL of UHT milk.

³Controls for weekly addition of antioxidants (contain equivalent portions of UHT milk).

content dropped below a critical value for the stability of milk fat. No significant reduction in heptanal content was observed when adding a combination of TOC and ASCP weekly. The human aroma threshold for heptanal in low fat milk is 2.322 mg/kg (Norton, 2003). Like hexanal, heptanal concentration increased to levels above aroma threshold, as high as 349.1 ± 25.7 mg/kg after 6 wk of light-exposed storage, whereas the concentration in light-protected milk remained at approximately 35 mg/kg. Data from GC-O shows that subjects were able to detect the characteristic “green/fish oil” odor of heptanal present in control milk exposed to light after only 1 wk of storage (data not shown). The increase in heptanal concentration over 6 wk of light exposed storage could substantially influence milk aroma and flavor.

1-Octen-3-ol content in light-exposed control milk increased only after 2 wk of storage (Figure 3). Again, the weekly addition of a combination of BHA and BHT significantly reduced 1-octen-3-ol content of milk when compared with light-exposed control milk. No other antioxidant treatment evaluated in this study yielded that degree of protection against the effect of light throughout the 6 wk study. Single doses of a combination of BHA and BHT and a combination of TOC and ASCP to milk, at the beginning of storage, reduced 1-octen-3-ol content of milk from 3 to 5 and 3 to 4 wk, respectively. The weekly addition of TOC and ASCP did not decrease 1-octen-3-ol content of milk.

A weekly addition of a combination of BHA and BHT protected milk fat against light-induced oxidation when evaluating pentanal, hexanal, heptanal, and 1-octen-3-ol content of milk. Studies that demonstrate the effect

of tocopherol on the stability of milk fat and off-flavor in milk have evaluated the degree of oxidation with sensory evaluation or chemical analysis (Charmley and Nicholson, 1993; Focant et al., 1998). However, no published research documents the effect of antioxidants on milk fat oxidation, in terms of specific aroma compounds such as pentanal, hexanal, heptanal, and 1-octene-3-ol.

Lipid Oxidation Odors

The chemical composition of aroma and flavor in milk and other dairy products is complex due to the complicated matrix of milk. Gas chromatography-olfactometry is a tool for evaluating odorous compounds that contribute to milk aroma. Table 2 lists the odorous volatile compounds identified in light-exposed control and antioxidant-treated milk. It is important to note that, although compounds might be detected as peaks on a chromatogram, these compounds might not be odor-active, and that human thresholds for these odors could be above the detected concentrations in the milk matrix. The opposite could also be true. Compounds with strong odors even at low concentrations might not yield significant peaks on a chromatogram. Odors contributed by microbiological spoilage were not a factor because aerobic and coliform bacteria counts remained <33 and 0 cfu/mL, respectively, throughout the 6 wk storage period.

Aroma-active compounds in light-protected control milk included only an unidentified compound described as “butter, cooked milk” at a moderate intensity as well as pentanal and n-heptanal at low intensity (Table 2).

Table 2. Odor-active compounds detected by gas chromatography-olfactometry (GC-O) of control and antioxidant-treated milk exposed to light for 12 h/d for 5 wk [odor intensities: + (0 to 1), ++ (2 to 3), +++ (4 to 5)].

Compound ¹	Retention index ²	Odor description	Antioxidant treatment ³				
			Control	Control (light-protected)	BHA/BHT (single)	TOC/ASC (single)	TOC/ASC (weekly)
1 (2-propanone)	0290	Sweet, perfume	+		++	++	
2	0321	Butter, cooked milk	+	++	++	++	+
3 (2,3-butanedione)	0347	Diacetyl, butter	+		+		+
4	0408	Roasted, manure	++				+
5 (pentanal)	0440	Sour, cut-grass	++	+		++	
6 (dimethyl disulfide)	0503	Cooked vegetable, grain	+			++	
7 (hexanal)	0567	Cut-grass	+++		+	+++	+++
8 (1-hexanol)	0639	Grass, earth	+			++	+
9 (2-heptanone)	0687				++	++	+
10 (n-heptanal)	0702	Green, fish oil	+++	+	+++	+++	+++
11 (1-heptanol)	0792	Plastic, floral	++		++		
12 (1-octene-3-ol)	0805	Mushroom				+++	++
13	0836	Burnt floral	+			++	
14	0907	Cooked			++	+++	++
15 (nonanal)	0962	Soapy, floral	+			+++	

¹Compound identified by comparison of its mass spectrum.

²Retention indices from GC-O data.

³Treatments: Control = milk (unspiked); Control (light-protected) = control milk protected from light; BHA/BHT (single) = single addition of 100 mg/kg butylated hydroxyanisole (BHA) and 100 mg/kg butylated hydroxytoluene (BHT); TOC/ASCP = single addition of 100 mg/kg of tocopherol (TOC) and 100 mg/kg ascorbyl palmitate (ASCP); BHA/BHT (weekly) = weekly addition of 100 mg/kg BHA and 100 mg/kg BHT dissolved in 1 mL of UHT milk; TOC/ASCP (weekly) = weekly addition of 100 mg/kg TOC and 100 mg/kg ASCP dissolved in 1 mL of UHT milk.

In contrast, odor-active compounds were noted in light-exposed control milk. Moderate to strong odors associated with light-exposed control milk included sour and green cut-grass, fish oil, and floral odors, which can be associated with pentanal, hexanal, heptanal, and heptanol, respectively, as well as one unidentified compound described as “roasted, manure”. These odors were noted after only 1 wk of light-exposed storage and became stronger throughout 6 wk of storage. The “butter, cooked milk” aroma compound intensity was lower than that observed in light-protected milk. Although Cadwallader and Howard (1998) observed a combination of similar odor-active compounds when exposing low fat milk to light for a maximum of 48 h, compounds with highest intensities included dimethyl disulfide, 2-methylpropanal, 1-hexen-3-one, and 1-octen-3-one. Jadhav et al. (1996) described 2 components that are involved in the development of “light-activated” flavor in milk. Initially, a burnt activated sunlight flavor develops and predominates for about 2 or 3 d. The second off-flavor, often characterized as metallic or cardboardy, usually develops after 2 d and does not dissipate. This might indicate why Cadwallader and Howard (1998) observed slightly different odors in milk exposed to light for 48 h (due to protein oxidation), vs. our study, which observed lipid oxidation odors in ESL milk after 6 wk of light-exposed storage.

Gas chromatography-olfactometry analysis of milk samples with initial single and weekly additions of BHA

and BHT, and a weekly addition of TOC and ASCP suggest a decrease in intensity of odorous compounds (Table 2). In contrast, GC-O data show that a single addition of TOC and ASCP caused increased oxidation, compared with light-exposed control milk. Although there were no differences in odor intensities that represent pentanal, hexanal, and heptanal, odors that represent acetone, 2,3-butanedione, dimethyl disulfide, 1-hexanol, 2-heptanone, and nonanal increased from light-exposed control milk to light-exposed milk treated with a single dose of TOC and ASCP. The apparent pro-oxidant effect might be due to the antioxidants being consumed, with the subsequent effect being an inability to stabilize milk fat.

Furthermore, it seems that different antioxidant treatments influenced the intensity of certain odorous volatiles (Table 2). A single initial addition of BHA and BHT to milk decreased odor intensity of hexanal, but did not change odor level of heptanal and 1-heptanol. Addition of TOC and ASCP to milk decreased odor intensity of pentanal and heptanol, but not hexanal and heptanal odor intensity. Sensory evaluation found that development of light-induced flavor in milk was suppressed after 10 h of light exposure when an initial single dose of 250 mg/kg of TOC and 250 mg/kg of ascorbic acid was added (van Aardt et al., 2005). This level and combination of antioxidants provided protection against light-induced flavor. Odor-active compounds associated with light-induced changes in milk

after 10 h included 2-heptanone, n-heptanal, 1-octene-3-ol, octanal, and nonanal, and ranged in intensity from 0 to 3 on a 5-point scale. Because light-oxidized flavor in milk is comprised of lipid and protein oxidation products, it is important to know the stage during light exposure that odorous flavor compounds increase to levels above human threshold. Volatile compounds associated with protein oxidation (dimethyl disulfide) were measured when milk was exposed for a short time (10 h) (van Aardt et al., 2005). In this study, off-odors were associated with lipid oxidation because analyses were done after weeks of storage.

Antioxidant levels in control and antioxidant-treated milk were evaluated weekly to document the amount of antioxidants left in the milk throughout storage. Maximum extracted levels of BHT and ASCP did not exceed 8 and 5 mg/kg of milk fat, respectively. However, BHA content of milk with an initial single addition and before weekly addition ranged between 7.4 and 20.4 mg/kg of milk fat. Analysis of BHA content of milk immediately after weekly addition showed levels of up to 75.0 mg/kg of milk fat (wk 1), which was lower than the spiked 100 mg/kg of milk fat. α -Tocopherol content of milk spiked with an initial single addition of 100 mg/kg of milk fat stayed below 50.3 mg/kg of milk fat throughout storage. When spiked weekly, TOC levels increased to a maximum of 123.5 mg/kg of milk fat after 5 wk of storage. Extraction occurred after milk samples were handled for SPME analysis, which may have affected antioxidant recovery. Additional analysis is needed to evaluate the role of antioxidants in milk systems.

CONCLUSIONS

The addition of antioxidants to milk was effective in controlling or limiting the development of increased concentrations of hexanal, pentanal, and 1-octen-3-ol, compounds used to monitor oxidation of ESL milk. Antioxidant addition may help protect ESL milk aroma from oxidative changes resulting from protein and lipid oxidation over 6 wk of light-exposed storage. This study showed that a weekly addition of BHA and BHT to ESL milk protected milk aroma significantly more than initial single additions of BHA and BHT or TOC and ASCP. Potentially, timed addition could be achieved by controlled migration of antioxidants from polymeric packaging. Further research on the addition of antioxidants to polymeric packaging for intentional migration into milk could provide an avenue for protecting ESL milk and improve milk sensory quality for the longer marketing period.

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