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Influence of Dissolved Gases and Heat Treatments on the Oxidative Degradation of Polyunsaturated Fatty Acids Enriched Dairy Beverage

HÉLÈNE J. GIROUX, GENEVIÈVE ACTEAU, HASSAN SABIK, AND MICHEL BRITTEN*

Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec, Canada J2S 8E3

The combined effect of dissolved gas composition and heat treatment on the oxidative degradation of a dairy beverage enriched with 2% linseed oil was studied. The dairy beverage was saturated with air, nitrogen, or a nitrogen/hydrogen mixture (4% hydrogen) before pasteurization or sterilization. Saturation with either nitrogen or a nitrogen/hydrogen mixture decreased the dissolved oxygen concentration in dairy beverages ($\Delta = 7.7$ ppm), and the presence of hydrogen significantly reduced the redox potential ($\Delta = 287$ mV). Heat treatments also reduced the oxygen content and redox potential, sterilization being more effective than pasteurization. Both pasteurization and sterilization induced the oxidative degradation of the beverages. On average, the propanal concentration increased by a factor of 2.3 after pasteurization and by a factor of 6.2 after sterilization. However, during storage, sterilized beverages resisted light-induced oxidation better than unheated or pasteurized beverages. Furthermore, saturation with nitrogen or a nitrogen/hydrogen mixture significantly reduced oxidative degradation and provided some protection against color changes during storage.

KEYWORDS: Dairy beverage; dissolved gas; heat treatments; lipid oxidation; polyunsaturated fatty acids

INTRODUCTION

Fish, algae and linseed oils are particularly rich in long-chain omega-3 polyunsaturated fatty acids and have been reported to exert many health benefits (*1, 2*). However, the use of these functional ingredients in foods has been limited by their great susceptibility to oxidation (*3*). The hydrogens at the α-position of the double bonds (allylic hydrogens) react with oxygen to form free radicals and isomeric hydroperoxides (*4*). The hydroperoxides are easily decomposed into secondary products, principally aldehydes, carbonyls, ketones, and hydrocarbons responsible for off-flavors at very low concentrations (*5*).

Many factors affect the rate of lipid oxidation in food such as light exposure, storage temperature, oxygen content, and presence of transition metals such as iron or copper (*5*). Methods to prevent the oxidative deterioration of dairy products enriched with polyunsaturated fatty acids are limited to temperature control (*6*), selection of packaging materials with low oxygen permeability and light transmission (*7*), use of metal chelators (*8*), or addition of antioxidants (*9*). However, food additives are perceived as nonfriendly ingredients by consumers and are subject to U.S. Food and Drug Administration regulations.

Thermal processing is commonly used in the dairy industry to ensure milk safety and shelf life. However, heat treatments give rise to numerous chemical reactions, such as Maillard reaction, hydrolysis, and vitamin degradation, that affect the organoleptic and nutritional properties of milk (*10–12*). Nevertheless, it has been shown that heat treatment could enhance the antioxidant activity of milk (*13*). This effect could be attributed to protein unfolding, which exposes thiol groups, especially in β-lactoglobulin (*10*). Thiols can act as hydrogen atom donors and inactivate compounds sensitive to oxidation (*14*). The antioxidant effect of some advanced Maillard reaction products has also been shown (*12*). With the increasing interest in nonthermal preservation technologies (*15*) and the use of highly sensitive ingredients, preventing the oxidative degradation in processed foods will become a challenge.

Dissolved oxygen concentration and redox potential can be decreased by saturation with a neutral gas (nitrogen, argon, helium) or a mixture of neutral and reducing gases such as hydrogen. This process has been used to improve the microbial quality, color retention, and ascorbic acid stability of pasteurized orange juice during storage (*16*), to increase the viability of freeze-dried microorganisms (*17*), and to modify the sensory properties of fermented milk products (*18, 19*). Correlation between oxidized flavor and dissolved oxygen content (*20*) or redox potential (*21*) of milk products has been observed. Saturation with such gases could then be a promising method to prevent the deterioration of flavor and organoleptic attributes by inhibiting oxidation reactions.

The objective of this study was to evaluate the combined effect of dissolved gas composition and heat treatment on the oxidative degradation of a dairy beverage enriched with 2% linseed oil during storage in darkness or fluorescent light exposure. The dairy beverage was saturated with air, nitrogen,

* Corresponding author [telephone (450) 773-1105; fax (450) 773-8461; e-mail brittenm@agr.gc.ca].

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or a nitrogen/hydrogen mixture (4% hydrogen) before pasteurization or sterilization treatment.

MATERIALS AND METHODS

Dairy Beverage Preparation. Milk protein concentrate (MPC) Alapro 4560 (New Zealand Milk Products, Wellington, New Zealand) containing 55% (w/w) protein and 32% (w/w) lactose in dried matter was dispersed in deionized water to a protein concentration of 3.58% (w/w). MPC dispersion was supplemented at a level of 0.001% (w/w) Fe (as $FeSO_4$), and sodium azide (0.02%) (w/w) was added as microbial growth inhibitor. The dispersion was stirred for 60 min and stored at 4 °C overnight, and the pH was then adjusted to 6.7 with 2 N HCl. The dairy beverage was enriched with pure linseed oil (Orphée, La Maison Orphée Inc., Quebec City, QC, Canada) at a 2% (w/w) final concentration. Protein and lactose concentrations after pH adjustment and oil addition were, respectively, 3.5 and 2.0% (w/w). The mixture (40 °C) was pre-emulsified using an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany) fitted with an S25KV-25F dispersing tool for 3 min at 8000 rpm and homogenized with a single-stage Emulsiflex-C5 homogenizer (Avestin, Ottawa, ON, Canada). Homogenization pressure was set to 3000 psi for the first two passes and to 500 psi for the third pass.

Saturation with Gases. Following the homogenization, the beverage was divided into three portions and gassed with air, nitrogen (N_2), or nitrogen 96%/hydrogen 4% mixture (N_2/H_2) for 30 min at a flow of 100 mL/min using a 10 μm stainless steel sparging diffuser (Chromatographic Specialties Inc., Brockville, ON, Canada). Antifoam Mazu DF 204 (BASF Corp., Mount Olive, NJ) was added to the beverages before gassing at a concentration of 0.01% (v/v).

Heat Treatment. Immediately after gassing, beverage aliquots of 12.5 mL were placed in screw-cap glass test tubes (14 mL) specially designed to resist the heat treatment pressure. The headspace was purged with the gas used for the saturation treatment for 20 s, and the tubes were tightly sealed. The beverages were batch-pasteurized at 63.5 °C for 30 min or autoclave-sterilized at 110 °C for 10 min and cooled to room temperature. Samples receiving no heat treatment were also prepared according to the same procedure.

Storage. The unheated and pasteurized dairy beverages were stored at 4 °C, whereas the sterilized beverage was kept at room temperature. The samples were stored in darkness or exposed to fluorescent light (warm white, 60 W fluorescent lamps, General Electric, Cleveland, OH) for 6 days and analyzed after 1, 2, 3, or 6 days. For fluorescent light exposure, the tubes were horizontally spread at 30 cm from the light source. For each storage time, analyses were performed on different tubes to prevent sample and headspace contamination.

Analytical Methods. *Dissolved Oxygen.* Dissolved oxygen concentration was determined using an Orion dissolved oxygen meter (Orion 850Aplus, Thermo Electron Corp., Beverly, MA) equipped with an Orion probe (model 083005D). The electrode zeroing was performed with a 6% sodium bisulfite solution as described in the supplier's manual.

Redox Potential. Redox potential was measured with a combined Pt-ring electrode (Metrohm, model 6.0451.100, Herisau, Switzerland) connected to a pH-meter (Corning, model 140, Acton, MA) set to mV mode. Calibration was performed against a Metrohm redox standard solution of 250 ± 5 mV (at 20 °C versus an Ag/AgCl/KCl 3 M reference electrode). An equilibrium period of 10 min was allowed before the reading was taken.

Free Thiol Groups. Free thiol groups were determined using the method of Solano-Lopez et al. (*22*). In our experimentation, the pH of the borax buffer was adjusted to 8.0. The concentration of free thiol groups was reported as micromoles per gram of protein.

Color. A HunterLab spectrocolorimeter (Labscan 2 tristimulus, Hunter Associates Laboratory, Inc., Reston, VA) set for illuminant D-65 and an illumination area measuring 4.5 cm in diameter was used to measure the L^*, a^*, and b^* Hunter scale parameters. The L^* value corresponds to the lightness and ranges from 0 (black) to 100 (white). The a^* and b^* values have no specific numerical limits. The a^* value (+/−) represents the redness (red to green), and the b^* value (+/−) represents the yellowness (yellow to blue). The chroma difference, ΔC, was calculated as a function of storage time according to eq 1 (*23*) by using the data of the unheated samples as initial value

$$\Delta C = C_{t=x} - C_{t=0} \quad (1)$$

where

$$C = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

Lipid Oxidation. Two volatile secondary oxidation compounds (propanal and hexanal) were selected as indicators of linseed oil oxidation and extracted from the dairy beverages by solid-phase microextraction (SPME). Three grams of sample was sealed in a 10 mL amber vial with 1 ppm of internal standard (4-methyl-2-pentanone, CDN Isotopes Inc., Pointe-Claire, QC, Canada). The SPME fiber (85 μm Carboxen/PDMS, Supelco, Oakville, ON, Canada) was inserted into the headspace of the vial for 15 min at 45 °C. The SPME operations were automated using an MPS2 multipurpose sampler (Gerstel Inc., Baltimore, MD). Volatile compounds were desorbed by inserting the fiber into the injection port of a Varian CP-3800 gas chromatograph (Palo Alto, CA) in splitless mode for 3 min at 300 °C. The GC system was fitted with a Varian CP-Sil 8CB-MS capillary column (30 × 0.25 mm; 25 μm film thickness) and a Saturn 2000 mass spectrometry detector (Varian Inc.). Helium was used as carrier gas at a flow rate of 1.0 mL/min. The column oven was set initially at 35 °C for 3 min, heated to 80 °C at a rate of 6 °C/min, increased to 280 °C at a rate of 20 °C/min, and then held at 280 °C for 2 min. The total time of analysis was 20 min. The mass spectrometer was operated in the mass range from 40 to 200 at a scan rate of 1.00 s/scan. Calibration curves were done using standards of hexanal and propanal (Sigma-Aldrich, Oakville, ON, Canada). The quantification was realized by selective ion monitoring (SIM) mode. The selected ions were 57 for propanal and 83 and 99 for hexanal. Analyses were performed in duplicate.

Statistical Analysis. The dairy beverages were prepared in triplicate according to a split-plot design in which the main plot was the heat treatment and the subplots were the dissolved gas composition, the storage condition, and the storage time. Variance analysis was used to determine whether the factors and their interactions had a significant effect on the measured parameters (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Dissolved Oxygen. The initial oxygen concentration in the dairy beverage was 8.1 ± 0.1 ppm. After gassing with air, dissolved oxygen content increased to 9.0 ± 0.1 ppm (**Table 1**). Saturation with either N_2 or N_2/H_2 decreased the dissolved oxygen content of the dairy beverages to 1.3 ± 0.1 ppm. Heat treatments also decreased the oxygen concentration in the beverages, suggesting that oxygen was involved in heat-induced reactions with other beverage components. Expulsion of oxygen in the headspace of the samples, as a result of lower oxygen solubility at higher temperatures, could have also reduced oxygen concentrations in heated samples. After sterilization, oxygen concentration in the three beverages was lower than 1 ppm. However after pasteurization, dissolved oxygen content in the beverage saturated with air remained higher than in the beverages gassed with N_2 or N_2/H_2.

Figure 1 presents the changes in dissolved oxygen concentration in unheated, pasteurized, and sterilized dairy beverages during storage. For samples exposed to fluorescent light (solid lines), heat treatment and dissolved gas composition significantly affected ($P < 0.01$) the changes in oxygen concentration with time. Unheated (**Figure 1a**) and pasteurized (**Figure 1b**) beverages showed similar behaviors during storage. Dissolved oxygen content rapidly decreased ($P < 0.01$) during the first 3 days, reaching concentrations lower than 0.3 ppm. It appears that almost all of the available oxygen was consumed in this short period of time, independently of initial concentration. For the sterilized beverages (**Figure 1c**), no significant oxygen consumption ($P > 0.05$) was observed during storage and concentrations remained higher than 0.5 ppm. Furthermore, in

Table 1. Physical Characteristics of Dairy Beverages before Storage[a]

treatment	gas	O_2 (ppm)	redox (mV)	free SH (μmol/g)	color			propanal (ppm)	hexanal (ppm)
					L^*	a^*	b^*		
unheated	air	9.0 a	147 a	0.41 a	88.90 a	−2.89 ab	7.15 ab	0.28 a	0.011 a
	N_2	1.3 bd	141 a	0.45 a	88.80 a	−2.88 ab	7.26 ab	0.26 a	0.011 a
	N_2/H_2	1.3 bd	−140 b	0.45 a	88.75 a	−2.91 ac	7.34 ac	0.23 a	0.012 a
pasteurized	air	6.7 c	146 a	0.40 a	89.38 b	−2.76 b	7.07 b	0.71 b	0.019 b
	N_2	1.0 e	103 c	0.42 a	89.34 b	−2.83 bc	7.17 bc	0.55 b	0.019 b
	N_2/H_2	1.1 de	−99 d	0.42 a	89.35 b	−2.73 b	7.25 bc	0.52 b	0.018 ab
sterilized	air	0.9 e	−26 e	0.39 a	87.63 c	−1.10 d	10.04 d	1.91 c	0.029 c
	N_2	0.8 e	−53 f	0.63 b	87.71 c	−1.32 e	9.63 e	1.38 d	0.027 c
	N_2/H_2	0.8 e	−93 g	0.61 b	87.61 c	−1.38 e	9.58 e	1.52 d	0.028 c
SE		0.1	3	0.05	0.05	0.04	0.08	0.07	0.002

[a] Values with the same letter within a column are not significantly different at $P > 0.05$.

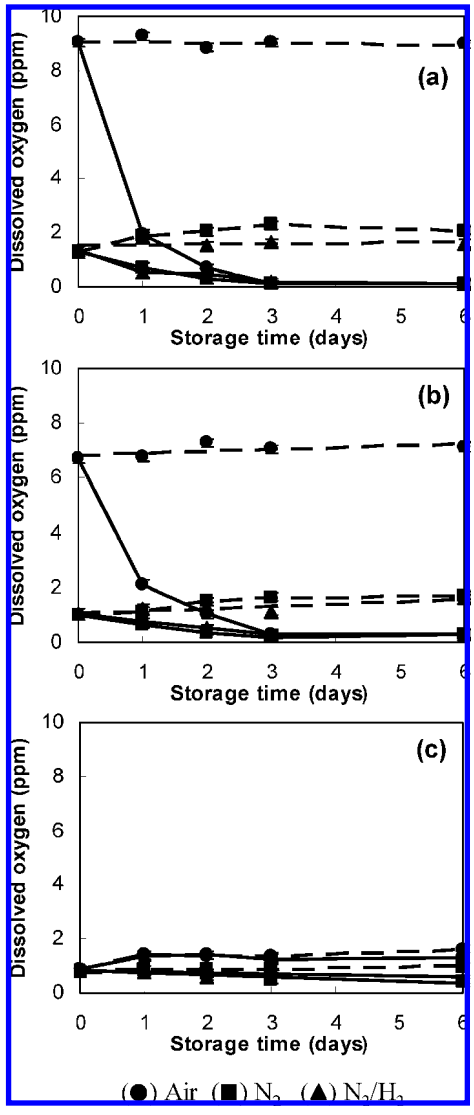

Figure 1. Changes in dissolved oxygen concentration in unheated (**a**), pasteurized (**b**), and sterilized (**c**) dairy beverages enriched with linseed oil during storage in darkness (- - -) and fluorescent light exposure (—).

air-saturated beverage, a slight but significant increase in oxygen concentration was observed after 1 day of storage ($P < 0.01$). This increase could be attributed to the re-equilibrium of oxygen expulsed in the headspace of the sample during sterilization treatment. No oxygen consumption was observed in dairy beverages stored in darkness (**Figure 1**, dashed lines), suggesting that no autoxidation took place during storage.

Redox Potential. Initial redox potential of the dairy beverages was 143 ± 2 mV. Saturation with air or N_2 had no significant effect ($P > 0.05$) on the redox potential, whereas saturation with N_2/H_2 decreased the redox potential to a value of −140 ± 6 mV (**Table 1**). Heat treatments also decreased the redox potential of the beverages saturated with air or N_2. The drop in redox potential with heat treatments is a well-known phenomenon. Heating at temperatures above 60 °C causes the denaturation of milk proteins. The unfolding of globular proteins and the exposure of thiol groups of cysteine residues are responsible for the decrease in the redox potential (*10*). Heating also promotes Maillard reactions between lactose and amino groups of milk proteins with the production of enediol-type reductants (*10*). These reactions depend on heating time and temperature. The results showed that sterilization was more effective than pasteurization in decreasing the redox potential of the beverages saturated with air or N_2. Redox potential values of the samples saturated with N_2/H_2 increased from −140 ± 6 to −99 ± 9 and −93 ± 1 mV after pasteurization and sterilization, respectively (**Table 1**). The rise in redox potential after heating of dairy beverages treated by electroreduction (to decrease the redox potential) had previously been observed by Giroux et al. (*24*) and suggests that the redox potential of the reductants produced by heat treatment is higher than the redox potential of the N_2/H_2-gassed beverage. Despite this increase, the redox potentials of the pasteurized and sterilized beverages were, respectively, 245 and 67 mV lower in the N_2/H_2-gassed than in the air-gassed beverages.

The changes in redox potential values of the unheated, pasteurized, and sterilized dairy beverages during storage are presented in **Figure 2**. For samples exposed to fluorescent light (solid lines), heat treatment and dissolved gas composition significantly affected ($P < 0.01$) the changes in redox potential values with time. Unheated (**Figure 2a**) and pasteurized (**Figure 2b**) beverages showed similar behaviors during storage. The redox potential of the air- and N_2-gassed beverages decreased ($P < 0.01$) by about 35−60 mV after 6 days of storage. This decrease could be related to the decrease of the dissolved oxygen concentration (**Figure 1a**,**b**). Storage time had no significant effect ($P > 0.05$) on the redox potential values of the same beverages stored in darkness. The redox potential of the N_2/H_2-gassed beverages stored in the dark or exposed to fluorescent light significantly increased ($P < 0.01$) after 1 day of storage and remained relatively constant thereafter. Some redox systems are very slow to react (*10*), and the potential measured immediately after N_2/H_2 treatment might not be at equilibrium. Moreover, the concentration and the nature of species endogenous to dairy products seem to provide low poising capacity and could not maintain a stable reducing environment. Poising

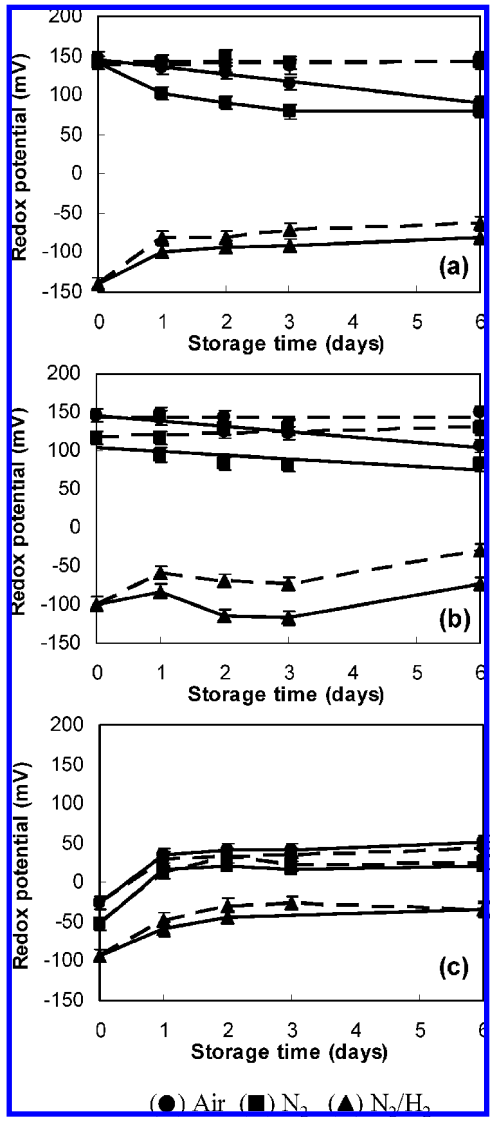

Figure 2. Changes in redox potential of unheated (**a**), pasteurized (**b**), and sterilized (**c**) dairy beverages enriched with linseed oil during storage in darkness (- - -) and fluorescent light exposure (—).

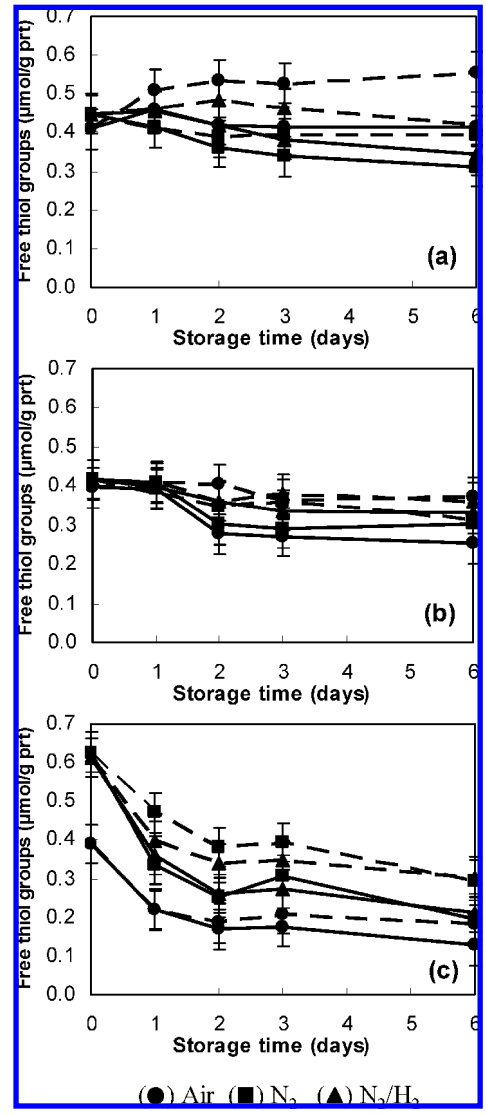

Figure 3. Changes in concentration of free thiol groups of unheated (**a**), pasteurized (**b**), and sterilized (**c**) dairy beverages enriched with linseed oil during storage in darkness (- - -) and fluorescent light exposure (—).

capacity (analogous to buffering capacity) relates to the stability of milk redox potential when exposed to oxidizing or reducing conditions. In addition to oxygen, the main active species that determine the redox potential of milk are ascorbate and riboflavin, found in low concentrations in milk. Exposed thiols and Maillard reaction conjugates produced by thermal process could also affect the redox potential of heated milk (*10*).

For the sterilized beverages (**Figure 2c**), the storage condition (fluorescent light exposure or darkness) had no significant effect ($P > 0.05$) on redox potential values. Previous results had shown that storage conditions had no significant effect on the dissolved oxygen content of sterilized beverages either (**Figure 1c**). The redox potential of the three beverages significantly increased ($P < 0.01$) after 1 day of storage but remained stable afterward, suggesting again the slow equilibrium and the low poising capacity of the redox systems. The increase in redox potential appears not to be associated with a change in dissolved oxygen concentration, because the values were stable to about 1 ppm for the corresponding storage period (**Figure 1c**). The redox potential value of the beverage saturated with N_2/H_2 was −35 mV at the end of the storage period.

Free Thiol Groups. The initial concentration of free thiol groups in the dairy beverage was 0.40 ± 0.01 μmol/g of protein. Saturation with air, N_2, or N_2/H_2 had no significant effect ($P > 0.05$) on the free SH concentration in unheated and pasteurized beverages (**Table 1**). Sterilization promotes protein unfolding and the exposure of the thiol groups hidden in the core of the protein (*10*). However, the sterilized beverage saturated with air did not show a free SH content as high as those of the beverages saturated with N_2 and N_2/H_2, likely because of the oxidation of the newly exposed SH during heating. Saturation with N_2 or N_2/H_2 reduced the dissolved oxygen content of the beverages (**Table 1**) and confers a protection against SH oxidation during sterilization, but no relationship seems to be established with their corresponding redox potential values.

Figure 3 presents the changes in the concentration of free thiol groups of unheated, pasteurized, and sterilized dairy beverages during storage in darkness and fluorescent light exposure. Heat treatment, dissolved gas composition, and storage condition had significant effects ($P < 0.01$) on the concentration changes of free thiol groups with time. Free thiol concentration significantly decreased ($P < 0.01$) as a function of exposure time to fluorescent light. The decrease was greater in the sterilized samples that contain a higher initial concentration of free thiol groups than in the unheated and pasteurized samples.

It has been shown that free thiol groups in UHT milk are oxidized during storage if there is sufficient oxygen present (*25*). In this study, no correlation between the free thiol concentration and the dissolved oxygen content or redox potential value of the samples was observed. Storage time had no significant effect ($P > 0.05$) on the concentration of free thiol groups in the beverages stored in darkness with the exception of the sterilized beverages that showed a lower decrease than the beverages exposed to fluorescent light.

Color. Heating treatment significantly affected ($P < 0.01$) the color of dairy beverages. Heat treatments induce nonenzymatic browning reactions in milk (*11*). The final products of Maillard reactions (melanoidins) are responsible for heat-induced color changes (*12*). The formation of brown pigments increases with the increase in heating temperature, and the sterilized beverages showed a significant ($P < 0.01$) loss of lightness (L^* value) and increase in redness (a^* value) and yellowness (b^* value) (**Table 1**). Our results are in good agreement with those of Rhim et al. (*26*), who reported that heated skim milk ($T >$ 100 °C) exhibited more redness and yellowness and less lightness. The pasteurized beverages showed little color changes compared to unheated beverages. It has been shown that only the early phase of the Maillard reaction takes place in pasteurized milk, and in that phase, no color modification is observed (*27*). However, Rhim et al. (*28*) observed initial whitening on heating of skim milk caused by the denaturation and subsequent aggregation of the soluble protein in milk. This phenomenon may have been the cause for the increase of the L^* value after pasteurization (**Table 1**). Dissolved gas composition had no significant effect ($P > 0.05$) on the L^* value of the three beverages or on the a^* and b^* values of the unheated and pasteurized beverages (**Table 1**). Nevertheless, dissolved gas composition significantly affected ($P < 0.01$) a^* and b^* values of the sterilized beverages. Saturation with N_2 or N_2/H_2 decreased dissolved oxygen concentration and redox potential value of the dairy beverages and seemed to provide some protection against browning during sterilization.

The changes in L^*, a^*, and b^* values of the unheated, pasteurized, and sterilized dairy beverages were determined during storage. Heat treatment and dissolved gas composition had no significant effect ($P > 0.05$) on the L^* values during storage in darkness or with fluorescent light exposure. However, heat treatment and storage condition significantly affected ($P < 0.01$) the changes in a^* and b^* values with storage time, whereas dissolved gas composition had no significant ($P > 0.05$) effect. Upon fluorescent light exposure, the a^* value of the beverages increased and the b^* value decreased as a function of storage time (**Figure 4**). Similar findings were reported by Mestdagh et al. (*29*) for sterilized milk. It has been shown that browning reactions continue during storage (*12, 26*) and could explain the a^* value increase (red color increase) (**Figure 4a**). The gradual decrease in b^* value (decrease in yellow color) (**Figure 4b**) could be caused by the degradation of the yellowish compounds of the milk such as riboflavin, β-carotene, and vitamin A (*29*). Storage time had no significant effect ($P >$ 0.05) on a^* and b^* values for unheated and pasteurized beverages stored in darkness, but a slight variation ($P < 0.01$) was observed for the sterilized sample. During the total storage period, the a^* and b^* values of the sterilized beverage exposed to fluorescent light or kept in the dark remained significantly higher ($P < 0.01$) than both those of the unheated and pasteurized beverages.

Despite the fact that dissolved gas composition had no significant effect on individual color parameters (a^* and b^*),

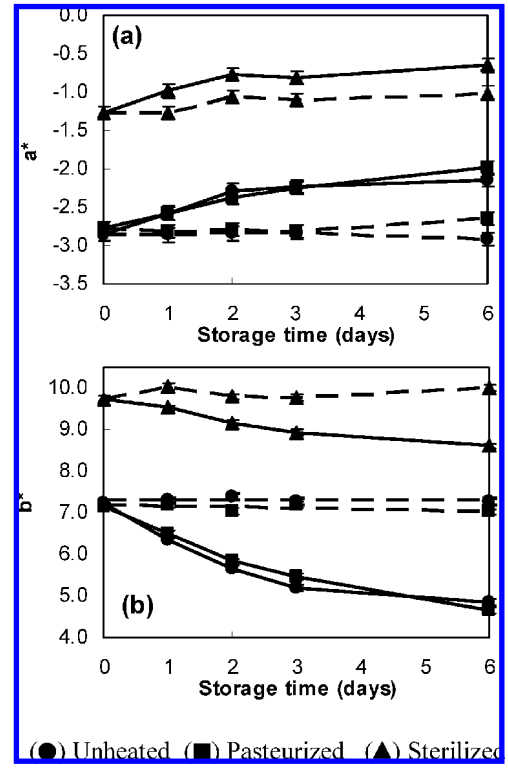

Figure 4. Changes in a^* (**a**) and b^* (**b**) values of dairy beverages enriched with linseed oil during storage in darkness (- - -) and fluorescent light exposure (—). Means of air, N_2, and N_2/H_2 results.

the chroma difference of the dairy beverages exposed to fluorescent light was significantly affected ($P < 0.01$) by this factor. The chroma difference was reduced by 5 and 16% in the samples saturated with N_2 and N_2/H_2, respectively. Saturation with N_2 or N_2/H_2 also seemed to provide some protection against color changes during storage.

Lipid Oxidation. Linseed oil is composed of about 58% linolenic acid (18:3 n-3), 17% linoleic acid (18:2 n-6), and 14% oleic acid (18:1 n-9). The autoxidation or photo-oxidation of these polyunsaturated fatty acids produces many hydroperoxide isomers that are decomposed into a complex mixture of volatile secondary oxidation products (*5*). It has been shown that the propanal originates from linolenate hydroperoxides, whereas the hexanal originates from linoleate hydroperoxides (*5*). These aldehydes are characterized by their intense aroma and flavor impact at very low threshold values (1.6 ppm for the propanal and 0.15 ppm for the hexanal) (*5*) and have been selected to evaluate the extent of lipid oxidation in the dairy beverages enriched with linseed oil.

Initial propanal and hexanal concentrations of the dairy beverages were 0.28 ± 0.01 and 0.014 ± 0.001 ppm, respectively. The values obtained were below the threshold of sensory perception of oxidation. Heat treatment significantly increased ($P < 0.01$) the propanal and hexanal concentrations of the dairy beverages (**Table 1**). Polyunsaturated fatty acids are susceptible to oxidation during heat processing (*30*). Calligaris et al. (*27*) suggested that heat treatment (depending on time−temperature combinations) can promote an increase in the pro-oxidant activity of milk, likely as a consequence of both the loss of natural antioxidants and the formation of novel oxidative molecules in the early stages of the Maillard reaction. Propanal concentration in the beverages was higher than that of hexanal. Propanal originates from linolenic acid, and it has been shown that linolenic acid was 2.4 times more reactive to oxidation than linoleic acid (*5*). In addition, linolenic acid concentration in

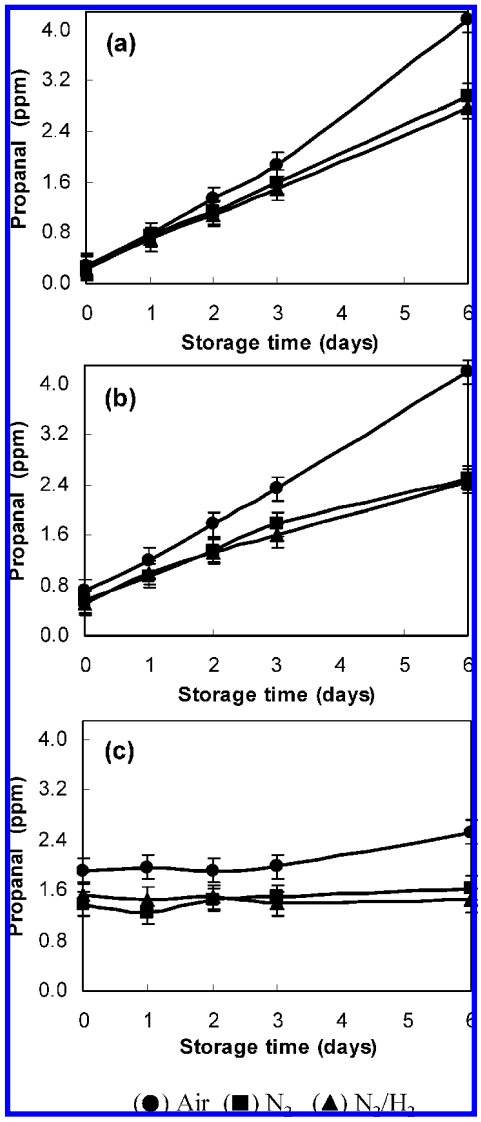

Figure 5. Changes in propanal concentration of unheated (**a**), pasteurized (**b**), and sterilized (**c**) dairy beverages enriched with linseed oil during fluorescent light exposure.

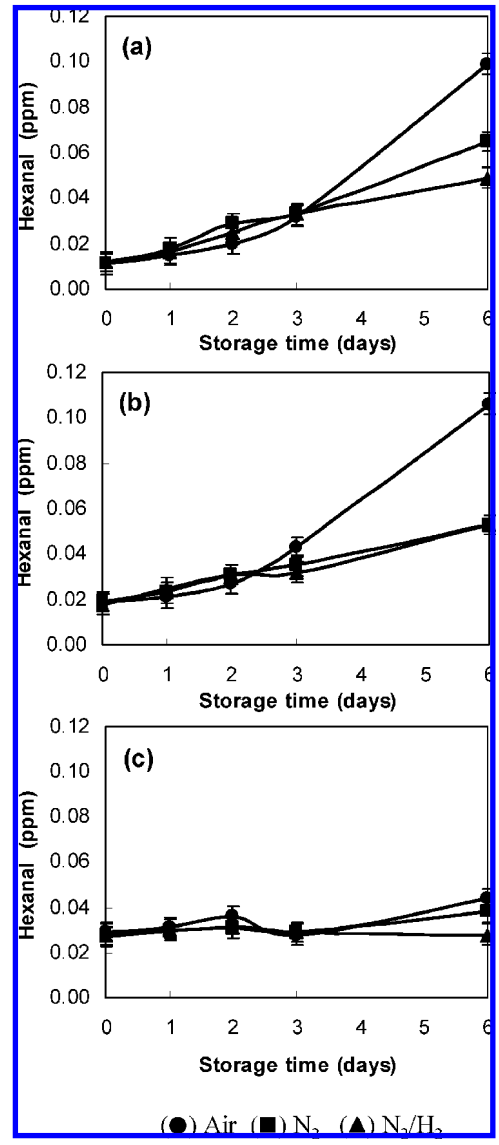

Figure 6. Changes in hexanal concentration of unheated (**a**), pasteurized (**b**), and sterilized (**c**) dairy beverages enriched with linseed oil during fluorescent light exposure.

linseed oil was about 3.4 times higher than linoleic acid concentration. Dissolved gas composition had no significant effect ($P > 0.05$) on propanal and hexanal concentrations of the unheated or pasteurized beverages (**Table 1**). However, saturation with N_2 or N_2/H_2 reduced the propanal concentration of the sterilized beverages by about 25% compared to the air-gassed sample, and this effect could be associated with the low dissolved oxygen content of these samples.

Changes in propanal and hexanal concentrations of the unheated, pasteurized, or sterilized beverages were determined during storage. Storage time had no significant effect ($P > 0.05$) on propanal and hexanal concentrations of the beverages stored in darkness (data not shown). Heat treatment and dissolved gas composition significantly affected ($P < 0.01$) the changes in propanal and hexanal concentrations with time of exposure of the sample to fluorescent light. Beverage concentration in propanal and hexanal significantly increased ($P < 0.01$) as a function of storage time with the exception of the sterilized beverages saturated with N_2 and N_2/H_2, which showed no significant changes ($P < 0.05$) during the total storage period (**Figures 5** and **6**). After 6 days of storage, the increase was significantly higher ($P > 0.01$) in the beverages saturated with air than in the beverages saturated with N_2 or N_2/H_2. Furthermore, the concentrations were greater in unheated and pasteurized beverages than in sterilized beverages, despite the higher initial concentration in sterilized beverages. It has been shown that severe heat treatments may increase milk's antioxidant properties because of protein unfolding and the exposure of thiol groups, as well as the production of reductants in the advanced Maillard reaction (*7*). Sterilized beverages resisted fluorescent light-induced oxidation better than pasteurized beverages, and the results indicated that no oxygen was consumed in sterilized beverages during the total storage period (**Figure 1c**). Lipid oxidation was completely inhibited in sterilized beverages saturated with N_2 or N_2/H_2. These beverages contained a high initial free SH concentration (**Table 1**) responsible for the antioxidant activity during storage. Moreover, saturation with N_2 or N_2/H_2 significantly decreased oxidative degradation in the unheated and pasteurized dairy beverages. Propanal concentrations in the unheated and pasteurized beverages saturated with both N_2 and N_2/H_2 were reduced by about 31 and 41%, respectively, after 6 days of storage whereas the hexanal concentrations were reduced by about 42 and 50%. The protection against oxidative degradation during the exposure to fluorescent light appears to be related to the decrease in

dissolved oxygen concentration and redox potential value resulting from the N_2 or N_2/H_2 saturation and heating treatments and to the heat-induced antioxidant activity. As expected, storage in darkness also constitutes an effective way to prevent oxidative degradation of dairy beverages.

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