Volatile Compounds and Chemical Changes in Ultrapasteurized Milk Packaged in Polyethylene Terephthalate Containers

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ABSTRACT: The volatile compounds profile and chemical stability of ultrapasteurized 2% milk bottled in amber polyethylene terephthalate (PET) were studied along with its shelf life of 60 d at 5 °C. Identification of volatile compounds using a solid-phase microextraction (SPME) technique with gas chromatography-mass spectrometry was performed in situ in the commercial product. Chemical stability was determined by measuring dissolved oxygen, free sulfhydryl groups, ascorbic acid, and headspace oxygen. The classes of volatile compounds identified were ketones, aldehydes, hydrocarbons, fatty acids, alcohol, and miscellaneous compounds. Volatile compounds found consistently during the storage period were 2-heptanone, methoxy-phenyl-oxime, decane, 1-ethyl-2,4-dimethylbenzene, D-limonene, 2-nonanone, octanoic acid, dodecane, and 4-(1,1,3,3,-tetramethylbutyl) phenol. Possible products of lipid oxidation, such as hexanal, octanal, and nonanal, were also positively identified. Chemical stability of PET-bottled milk was demonstrated by the insignificant changes in pH or TA, with the exception of ascorbic acid concentration, which degraded in the product at 60 d of refrigerated storage. Sensory evaluation of the milk samples revealed that there is no noticeable oxidized or rancid off-flavor at 60 d of storage.

Keywords: oxidation, PET, SPME, ultrapasteurized milk, volatile compounds

Introduction

Flavor is an important attribute that determines the acceptability and shelf life of milk. Ultra-high-temperature (UHT) milk has a different flavor compared with high-temperature, short-time (HTST) pasteurized milk. The flavor of UHT milk has been described as "cooked" or "caramelized," while HTST milk has a bland and sweet flavor (Badings 1991). Even if milk is within microbiologically and chemically acceptable limits, its shelf life is limited to the time it looks and tastes appealing to the consumer. The physicochemical stability of UHT milk is determined by its age-gelation phenomena, but its flavor stability is limited by the development of bitterness, which results from protein hydrolysis (Datta and Deeth 2001). Volatile compounds formed by protein and/or fat reactions will affect milk flavor and its profile of characteristic volatile compounds. Volatile compounds are not only responsible for the characteristic flavor of a food, but also for its off-flavors. Flavor stability, therefore, is also a main factor in the quality of bottled milk.

Volatile milk flavor components resulting from processing have been characterized by Contarini and others (1997). The authors reported that heat treatment increased volatile compounds and the storage of UHT milk at 4 °C decreased the development of flavor defects.

Ultrapasteurized (UP) milk, processed in the lower range of UHT time/temperature processing, has similar flavor characteristics as UHT milk; however, it is believed that the cooked note of its flavor is minimal relative to regularly processed UHT milk (Chapman and others 2001). Cooked flavor has been mainly attributed to the free sulfhydryl groups of denatured β-lactoglobulin (Simon and others 2001) and other volatile sulfur compounds (Steely 1994). In turn, the disappearance of cooked flavor has been related to the amount of dissolved oxygen in the milk as well as in the headspace of the bottle, and to the temperature at which the milk is stored (Hill 1988).

Polyethylene terephthalate (PET) is a versatile plastic material normally used in the bottling of soft beverages, and it has been introduced as a packaging material for fluid milk in the United States. Polyethylene terephthalate offers several advantages as a food-packaging material, including transparency, light weight, resistance, and recyclability (Jenkins and Harrington 1991). Moreover, pigmented PET enhances its versatility by protecting the food from light, which in turn, helps to protect food flavor against light-induced lipid oxidation (van Aardt and others 2001). One potentially negative attribute of PET packaging, however, is that its oxygen permeability may be a factor in the development of oxidized off-flavors in UP milk over time. Milk contains a number of known antioxidant compounds, including naturally occurring ascorbic acid and free sulfhydryl groups, formed during thermal processing that may or may not play an important role in the shelf life and stability of UP-PET-bottled milk.

Although multiple factors affect the volatile compound profile of milk flavor, those that result in flavor changes are the most vital for the determination of milk quality. The objective of this study was to characterize the headspace volatiles of 2% UP-PET-bottled milk and to investigate a possible relationship between volatile compounds formed in situ in the headspace of UP-PET-bottled milk and off-flavors resulting from oxidative reactions during refrigerated storage using a solid-phase microextraction (SPME) technique.

Materials and Methods

Milk processing

Batches of standardized cows' milk at 2% fat were processed at a

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local dairy processing plant (Smith's Dairy Products Co., Richmond, Ind., U.S.A.) in a DASI Steam Infusion System (APV steam infusion processor; APV Crepaco Inc., Chicago, Ill., U.S.A.). Milk was ultrapasteurized at 141 to 142 °C with a holding time of less than 4 s after homogenization at 13 MPa for the 1st and 3.4 MPa for the 2nd stage (16.4 MPa total) at 69 to 70 °C.

Amber PET bottles (237 mL) with ultraviolet protective dye and a shrink-wrap label were used for milk packaging. The bottles were automatically sanitized with Matrixx™ (Ecolab Inc., St. Paul, Minn., U.S.A.). Filling of PET bottles with milk (3 °C) was performed under aseptic conditions by a rotary net weight filler (Model R24V12/1080; Serac, La Ferté Bernard Cedex, France). Screw-top plastic lids with breakaway safety rings were used to close the bottles. Milk samples refrigerated at 4 °C were transported to The Ohio State Univ. (Columbus, Ohio, U.S.A.) dairy laboratory. Bottle lids were interchanged with modified caps under aseptic conditions immediately.

The modified caps were fitted with rubber septa (Fisher Scientific, Hanover Park, Ill., U.S.A.), inserted into regular caps through a 23-mm-dia opening (Figure 1). The modified caps allowed for multiple samplings of headspace volatiles without exposing the bottled milk to the environment. A subset of glass vials filled with milk from the same batch was prepared at the same ratio of headspace volume in contrast to milk volume of the PET bottles. The milk in the glass vials was used as a control to test possible differences between PET- and glass-bottled milk. Milk samples were held at 5 °C for 60 d in a commercial refrigerator (White Westinghouse, Cleveland, Ohio, U.S.A.), and were withdrawn at 1-mo intervals.

Microbial test

Standard plate counts (SPC) and a presumptive coliform test were performed in milk samples upon arrival at the laboratory and at each sampling time to assure the integrity of the samples. The microbial tests were carried out according to the Standard Methods for the Examination of Dairy Products (Marshall 1992), using a standard methods agar for SPC and a violet red bile agar for coliform bacteria (BBL; Becton Dickinson, Sparks, Md., U.S.A.). Samples were analyzed in triplicate each with 3 replications.

Isolation of volatile compounds

Isolation of the volatile compounds was performed using a SPME technique. A 75-µm carboxen-poly dimethyl siloxane (PDMS) built fiber (Supelco, Bellefonte, Pa., U.S.A.) was conditioned for maximum performance at 280 °C for 30 min before being placed in the subsequent sample. The fiber was inserted through the rubber septum of the modified-cap, and was exposed to the headspace of the sample (Figure 1). The sample bottles were placed in a water bath (45 °C) for 40 min to optimize equilibration of volatile compound absorption on the fiber. The fiber was retracted into the SPME assembly after equilibration. The compounds trapped in the carboxen-PDMS fiber were desorbed in a gas chromatograph (GC) (HP 6890; Hewlett-Packard, Wilmington, Del., U.S.A.).

Identification of volatiles

A Hewlett-Packard 6890 GC equipped with a flame ionization detector, a glass injection liner (0.75 mm i.d.), and an HP-5 (cross-linked 5% PH-ME siloxane) column (30 m × 0.25 mm i.d., 0.25 µm film) (J & W, Folsom, Calif., U.S.A.) was used. The oven temperature was held at 40 °C for 5 min and increased from 40 to 160 °C at 6 °C/min and from 160 to 220 °C at 8 °C/min. The temperatures of the injector and detector were 250 °C and 300 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The volatile compounds isolated by the SPME fiber were desorbed at 250 °C for 2 min in a splitless mode. To identify key volatile compounds present in the samples with those reported in UHT milk in the literature, retention times of authentic compounds were analyzed in duplicate under the same chromatographic conditions as described earlier. The authentic compounds of 2-pentanone, 2-heptanone, 2-nonanone, hexanal, octanal, nonanal, and other chemicals were acquired from Sigma-Aldrich (Milwaukee, Wis., U.S.A.).

Gas chromatography-mass spectrometry (HP 5971A Mass Selective Detector, Hewlett-Packard, Pittsburgh, Pa., U.S.A.) equipped with a 59822B ionization controller was also used in electron impact ionization mode to identify the volatile compounds in the headspace of the sample bottle. Mass spectra were obtained at 70 eV of ionization voltage and 230 °C of ion source temperature. Mass scan range was *m/z* 25 to 200 with a 1.0 scan/s of scan rate. Volatile compounds were identified by the combination of Natl. Inst. of Standards and Technology (NIST)-98 GC-MS spectrum library and gas chromatographic retention times of authentic compounds. The chromatographic analyses were performed in duplicate each with 3 replications.

Chemical analyses

Chemical determinations were done the same day after chromatographic analyses. Headspace oxygen (HSO) was determined using GC according to Min (1991). Dissolved oxygen (DO) was measured using a dissolved oxygen meter with a FEP Teflon membrane (YSI 50B; Yellow Springs Inc., Yellow Springs, Ohio, U.S.A.). Ascorbic acid (AA) was determined by the AOAC (1995) method. Determinations of pH and titratable acidity (TA) were conducted as described in the Standard Methods for the Examination of Dairy Products (Marshall 1992). Total free sulfhydryl groups (FSH) were determined by the method described by Koka and others (1968), with minor modifications published by Stapelfeldt and others (1997). Modifications included the use of higher pH buffer solutions (borax buffer, pH 9.5), higher concentrations of ammonium sulfate (6 g/10 mL milk), and extended agitation times (up to 1.5 min after each ammonium sulfate addition) compared with the original method. Chemical determinations were performed in triplicate each with 3 replications.

Sensory evaluation

A sensory evaluation was performed to correlate any change of a particular attribute over the storage time with specific volatiles detected in samples by the chromatographic analysis. A panel of 6 members was trained to identify the milk flavor attributes—acid,

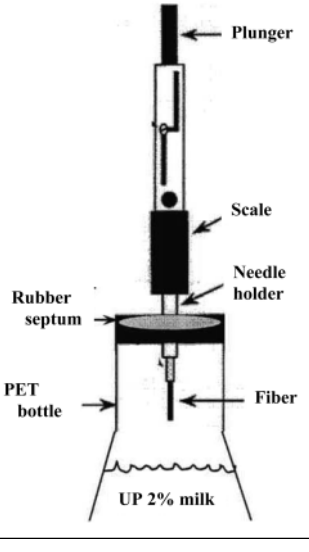

Figure 1—Sampling of headspace volatiles of 2% ultrapasteurized (UP) polyethylene terephthalate (PET)-bottled milk in situ with a solid-phase microextraction fiber.

bitter, fermented, oxidized, and rancid. The sensory panel evaluated milk samples the same day the chemical analyses were performed to correlate analytical with subjective inputs. Cooked flavor was not scored as a flavor attribute because it was present in the sample due to the UP processing. Triplicate samples were rated on a hedonic scale from 1 (none) to 9 (extremely strong) over a 60-d period.

Experimental design and statistical analysis

Experiments for chemical analysis were performed according to completely randomized design. Data were analyzed using general linear model procedure with SAS (1999) software. Means of 3 replicates were compared for significant difference ($\alpha = 0.05$) using Duncan's multiple range test. Sensory test was analyzed using the β-binomial model of the ART software (Inst. for Perception, Richmond, Va., U.S.A.).

Results and Discussion

Standard plate counts at 1, 30, and 60 d were all less than 10 colony-forming units/mL and coliforms were not detected during the milk storage, which indicated the absence of microbial contamination (data not shown).

Identification of volatile compounds

A number of volatile compounds of 2% UP-PET-bottled milk samples stored under refrigeration were identified by GC and GC-MS. There were 16, 22, and 26 compounds identified at 1, 30, and 60 d, respectively. The classes of volatile compounds included ketones, aldehydes, hydrocarbons, fatty acids, alcohol, and miscellaneous compounds (Figure 2). There were 9 volatile compounds present at all 3 sampling times. These were 2-heptanone, methoxy-phenyl-oxime, decane, 1-ethyl-2,4-dimethyl-benzene, D-limonene, 2-nonanone, octanoic acid, dodecane, and 4-(1,1,3,3,-tetramethylbutyl) phenol. The most abundant volatile compounds found were methyl ketones and hydrocarbons (Table 1). Similarly, it has been reported that methyl ketones are the main group of volatile compounds found in whole UHT milk (Contarini and others 1997; Valero and others 2001). The volatile compounds identified in 2% PET-bottled milk were compared with those identified in the same milk packed in glass vials over a 60-d period (Table 2). Among methyl ketones, 2-pentanone was detected at 1 and 30 d of storage in PET-bottled samples only. Headspace volatiles identified consistently in glass- and PET-bottled samples during the length of the 60 d study were 2-heptanone, 2-nonanone, and

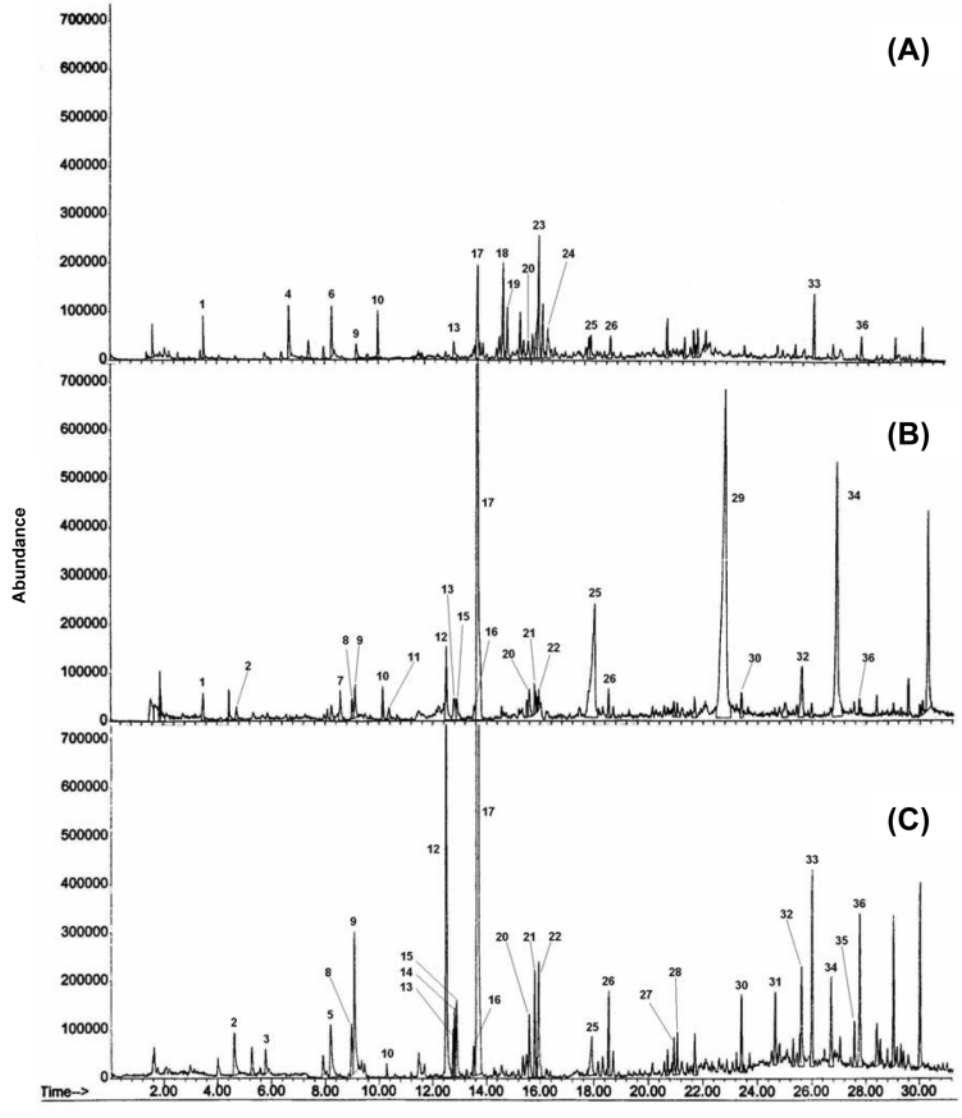

Figure 2—Gas chromatography–mass spectrometry chromatograms of 2% ultrapasteurized polyethylene terephthalate-bottled milk at 1 d (A), 30 d (B), and 60 d (C) of refrigerated storage (numbering is the same as in Table 1).

Table 1—Volarile compounds identified by gas chromatography (GC) and mass spectrometry (MS) in 2% ultrapasteurized polyethylene terephthalate-bottled milk during refrigerated storage[a]

Peak	Retention time (min)	GC	MS	Compound	Storage time (d)[b] 1	30	60
1	3.51	+	+	2-Pentanone	+	+	nd
2	4.64		+	Toluene	nd	+	+
3	5.79	+	+	Hexanal	nd	nd	+
4	6.68		+	2,4-Dimethyl-heptane	+	nd	nd
5	8.21		+	1,3-Dimethyl-benzene	nd	nd	+
6	8.27		+	4-Methyl-octane	+	nd	nd
7	8.58		+	2-Furanmethanol	nd	+	nd
8	8.99		+	bicycle[4,2,0]octa-1,3,5-triene	nd	+	+
9	9.09	+	+	2-Heptanone	+	+	+
10	10.15		+	Methoxy-phenyl-oxime	+	+	+
11	10.38		+	2(5H)-Furanone	nd	+	+
12	12.50		+	β-Myrcene	nd	+	+
13	12.78		+	Decane	+	+	+
14	12.82		+	Ethyl ester hexanoic acid	nd	nd	+
15	12.90	+	+	Octanal	nd	+	+
16	13.54		+	1-Ethyl-2,4-dimethyl-benzene	+	+	+
17	13.98	+	+	D-Limonene	+	+	+
18	14.94		+	7,9-Dimethyl-hexadecane	+	nd	nd
19	15.10		+	3,7-Dimethyl-decane	+	nd	nd
20	15.60	+	+	2-Nonanone	+	+	+
21	15.80		+	Undecane	nd	+	+
22	15.95	+	+	Nonanal	nd	+	+
23	16.25		+	3,6-Dimethyl-decane	+	nd	nd
24	16.61		+	3-Methyl-decane	+	nd	nd
25	17.92	+	+	Octanoic acid	+	+	+
26	18.54		+	Dodecane	+	+	+
27	20.93		+	2-Decanone	nd	nd	+
28	21.06		+	Tridecane	nd	nd	+
29	22.84	+	+	n-Decanoic acid	nd	+	nd
30	23.42		+	Tetradecane	nd	+	+
31	24.65		+	5-Methyl-2,4-diisopropylphenol	nd	nd	+
32	25.63		+	Tetrahydro-6-(2-pentenyl)-2H-pyran-2-one	nd	+	+
33	26.00	+	+	Butylated hydroxytoluene	+	nd	+
34	26.70	+	+	Dodecanoic acid	nd	+	+
35	27.57		+	Hexadecane	nd	nd	+
36	27.75		+	4-(1,1,3,3,-tetramethylbutyl)phenol	+	+	+

[a]Average of 3 replicates.
[b]+, a volatile compound identified by GC and/or MS; nd, not detected.

D-limonene. The origin of ketones, 2-pentanone and 2-heptanone, is both as a natural volatile compound in milk and as a consequence of heat treatment formed by β-ketoacid decarboxylation and by β-oxidation of fatty acids, followed by decarboxylation (Grosch 1982). The origin of aromatic and aliphatic hydrocarbons is not known (Toso and others 2002). Limonene has been found by Contarini and others (1997) in pasteurized, UHT, and "in-bottle" sterilized milk. Octanoic acid was identified at all times in the PET-bottled samples, whereas it was identified only at 30 d and 60 d of storage in the glass-bottled sample. Other volatile compounds, possibly products of lipid oxidation, such as hexanal, octanal, and nonanal, were also positively identified in the PET-bottled samples at 30 d and 60 d of storage. However, the absence of these volatiles in the glass-bottled samples seems to correspond to the imperviousness of the sealed glass vials. The presence of hexanal and larger peaks of octanal and nonanal in PET-bottled milk at 60 d of refrigerated storage indicates that lipid oxidation may occur extensively at 60 d. Most volatile compounds identified in this study have been reported to be present in UHT milk stored under different conditions (Jeon and others 1978; Bassette and Jeon 1983; Rerkrai and others 1987; Gaafar 1991; Christensen and Reineccius 1992; Contarini and others 1997; Valero and others 2001). However, low-molecular-weight aldehydes such as ethanal, propanal, furfural, heptanal, and 1-pentanal, were not positively identified. Possible explanations for the absence of low-molecular-weight compounds could be the different techniques used for the isolation of the volatiles, differences in the sensitivities of the methods, and the conditions of the tests (Contarini and others 1997; Valero and others 2001; Potineni and Peterson 2005). Although the formation of new compounds was evident with the appearance of several peaks after 2 mo of refrigerated storage, the number of peaks overall did not vary greatly, which implies that while some compounds are formed, others disappear.

Table 2—Volatile compounds identified by gas chromatography (GC) and mass spectrometry (MS) in 2% ultrapasteurized polyethylene terephthalate (PET)-bottled or glass-bottled milk at 5 °C for 60 d

Compound	PET-bottled milk[a] D-1	D-30	D-60	Glass-bottled milk[a] D-1	D-30	D-60
2-Pentanone	+	+	nd	nd	nd	nd
2-Hexanone	nd	nd	nd	+	nd	nd
2-Heptanone	+	+	+	+	+	+
2-Nonanone	+	+	+	+	+	+
Hexanal	nd	nd	+	nd	nd	nd
Octanal	nd	+	+	nd	nd	nd
Nonanal	nd	+	+	nd	nd	nd
Octanoic acid	+	+	+	nd	+	+
BHT[b]	+	nd	+	nd	nd	nd
D-Limonene	+	+	+	+	+	+

[a]+, a volatile compound identified by GC and/or MS; nd, not detected.
[b]BHT, butylated hydroxyl toluene.

Table 3—Chemical analyses of 2% ultrapasteurized polyethylene terephthalate-bottled milk stored at 5 °C for 60 d[a]

	Storage time (d)		
	1	30	60
Headspace oxygen (mg/L) × 10^2	2.55 ± 0.03a	2.46 ± 0.10b	2.55 ± 0.04a
Dissolved oxygen (mg/L)	9.11 ± 0.12a	7.13 ± 0.49b	5.36 ± 1.24c
Ascorbic acid (mg/L)	12.32 ± 0.35a	11.91 ± 0.84a	0.79 ± 0.27b
Free sulfhydryl (μmol/g NFS[b])	0.73 ± 0.06a	0.13 ± 0.03b	0.10 ± 0.01c
pH	6.68 ± 0.03a	6.72 ± 0.02a	6.72 ± 0.03a
Titratable acidity (% lactic)	0.14 ± 0.01a	0.14 ± 0.01a	0.14 ± 0.01a

[a]Mean values of 3 determinations. Means with different letters within same row are significantly different ($P < 0.05$).
[b]NFS, nonfat solids.

Formation and disappearance of compounds in the gas chromatogram (Figure 2) agree with those reported by Kirk and others (1968) who found that the development of staleness in milk parallels increases in carbonyl peaks and the disappearance of many unidentified compounds. The presence of butylated hydroxyl toluene (BHT) and phenol identified in PET-bottled milk may be related to the packaging material and to the sanitizing chemical, respectively. Butylated hydroxyl toluene is potentially used as an antioxidant additive in the plastic package and has been identified in mineral and spring water bottled in PET by Tombesi and Freije (2002). Acetaldehyde has been reported in heated milk (Jaddou and others 1978) as a product of light-induced oxidation in milk (Cadwallader and Howard 1998) and as a degradation product of PET (van Aardt and others 2001). Acetaldehyde was not identified, possibly due to the packaging material's effectiveness in blocking the entrance of light and its lack of reaction with milk. A previous study on UHT-processed milk postulated that decomposition or partial destruction of sulfur-containing amino acids is the source of volatile sulfides liberated by the heating process in milk (Aboshama and Hansen 1977). Sulfur compounds generally associated with cooked flavor are hydrogen sulfide, dimethyl disulfide, methyl sulfide, methanethiol (Jaddou and others 1978; Christensen and Reineccius 1992), iso-butanethiol, dimethyl trisulfide, methyl isothiocyanate, isothiocyanate, 2,4-dithiapentane, 2,3,4-trithiapentane, and benzothiazole (Steely 1994). In this study, the sulfur compounds were not positively identified in the chromatograms of milk samples. Studies on cooked milk reported the need to use sensitive and selective detectors such as flame photometric detector or sulfur chemiluminescence detector (Steely 1994; Bosset and others 1996) to identify sulfur compounds. Therefore, those selective detectors may have been necessary to identify sulfur compounds not found in the present study. A recent study described a colorimetric assay technique for sulfhydryl group analysis in fluid milk (Owusu-Apenten 2005).

Table 4—Scores for oxidized (auto-oxidation) and rancid (lipolytic) off-flavors in 2% ultrapasteurized polyethylene terephthalate-bottled milk evaluated after 60 d of refrigerated storage[a]

	Oxidized		Rancid	
Panelist	Trial 1	Trial 2	Trial 1	Trial 2
1	1	1	1	1
2	1	1	1	1
3	1	1	2	2
4	3	3	1	1
5	2	1	1	1
6	1	1	1	1

[a]Scores represent an average of 3 replicates. Rating scale: 1 = not detectable; 9 = extremely strong.

Chemical analyses

Table 3 shows the results of the chemical determinations in 2% UP-PET-bottled milk stored up to 60 d. The chemical stability of UP-PET-bottled milk was demonstrated by the insignificant changes in pH or TA over the 60-d period at refrigeration temperatures. The concentration of AA was decreased minimally from 12.32 to 11.91 mg/L during the 1st 30 d of storage but was decreased significantly ($P < 0.05$) to 0.79 mg/L at 60 d. Ascorbic acid content in milk is related to handling practices and processing, as well as packaging material. Losses of AA during milk storage have been reported in the literature for UHT milk storage at various temperatures, durations of storage, and different packaging materials, such as C-enameled tin cans (Jeon and others 1978), brown glass bottles (Fink and Kessler 1986), and Tetra Brik cartons (Andersson and Öste 1992). However, there are no reports about the stability of AA in UP-PET-bottled milk. The decrease in AA concentration after 30 d of storage may indicate that the antioxidant activity that ascorbic acid could be providing in the milk system is severely affected after most of the sulfhydryl groups have reacted. Once FSH groups have been oxidized, then oxygen reacts with AA causing the dramatic drop in concentration at 60 d of storage. Apparently, the antioxidant activity of the FSH groups allows AA to be stable for a longer time in the 2% UP-PET-bottled milk system. The concentration of FSH drops significantly ($P < 0.05$) at 30 d of storage (Table 3). This may indicate that the reduction in the concentration of DO during the 1st 30 d could be due to the reaction of DO with FSH groups. Our results agree with those of Fink and Kessler (1986) regarding the decrease in DO and FSH groups, but not in regards to the decrease in AA. These authors reported that the decrease in DO parallels the disappearance of FSH groups and that of AA. It has been documented that sulfhydryl groups perform an antioxidant role in milk (O'Connor and O'Brien 1995). However, the drop of AA concentration found in the present study during the 1st 30 d of storage does not appear to be related to the DO concentration.

Dissolved oxygen changes from 9.11 to 5.36 mg/L at 60 d storage were similar to the results described by Fink and Kessler (1986) who found a drop in the concentration during 60 d. These authors explained that diffusion of oxygen from the headspace into milk takes place during the 1st wk of storage, but afterward the consumption of oxygen in the milk overtakes the oxygen diffusion into it, lowering its concentration. Once oxidative reactions have been completed, oxygen diffuses into the milk again until equilibrium between the oxygen concentration in the headspace and that of the milk is reached. Dissolved oxygen concentration decreased significantly ($P < 0.05$) at 60 d storage and, as explained before, it is possible that the decrease in concentration is directly related to the AA oxidation.

The oxygen concentration in the headspace decreased and increased at 30 d and 60 d of storage, respectively (Table 3). The decrease in the 1st period may be due to the diffusion of oxygen from the headspace into the milk during the early stage of storage as reported in a previous study (Fink and Kessler 1986). The increase at 60 d could result after equilibrium between oxygen concentration in the headspace and milk. Then, there is a possibility of oxygen transmission to the headspace due to oxygen permeation through the PET as reported by Moyssiadi and others (2004).

Considering the oxygen concentration in the headspace and the DO concentration in the milk, AA stability in 2% UP-PET-bottled milk is excellent during the 1st 30 d of storage. Jeon and others (1978) reported a total loss of AA in UHT milk packed in C-enameled tin cans after 30 d of storage at 3 °C, with a DO concentration of 4.5 ppm. The concentration of DO was about 78.3% of the original value (9.11 mg/L) and AA was about 96.7% of the initial concentration of 12.32 mg/L after 30 d of storage (Table 3). The packaging material and the reactivity of FSH groups may be the factors that allowed a longer stability of AA in 2% UP-PET-bottled milk, up to 30 d of storage.

Sensory evaluation

Table 4 shows the sensory evaluation scores for oxidized (autoxidation) and rancid (lipolytic) off-flavors of 2% UP-PET bottled milk stored at 5 °C for 60 d.

Only 2 panelists scored slightly or moderately detectable oxidized off-flavor. The flavor was undetectable for the rest of the panelists. Oxidized off-flavor or oxidative rancidity in milk results from the action of oxygen on milkfat components that produce short-chain volatile aldehydes and ketones (Bodyfelt and others 1988). The sensory scores could not be correlated with the GC-MS results. Although volatile compounds responsible for oxidized off-flavor, such as aldehydes and ketones, were detected in the chromatogram (Figure 2), the panelists did not identify the off-flavor. This may be due to the chemical stability producing low concentrations of those volatile compounds in milk as discussed previously.

The sensory evaluation revealed no significant hydrolytic rancidity at 60 d of storage (Table 4). A rancid off-flavor in milk can be caused by the release of short-chain free fatty acids (C_4-C_{12}) through the enzyme reaction. This kind of off-flavor is known as hydrolytic rancidity or lipolyzed flavor (Woo and Lindsay 1983). From the chromatograms of GC-MS (Figure 2), the peaks numbered 25, 29, and 34 were identified as short-chain free fatty acids responsible for rancid off-flavor, which are octanoic (C_8), decanoic (C_{10}), and dodecanoic (C_{12}) acids, respectively. These findings suggest that the minimum rancid off-flavor in UP-PET bottled milk stored at refrigeration temperatures may be due to low temperatures and proper hygienic handling before and after heat treatment. Similar observations were reported by Deeth and Fitz-Gerald (1995).

The acid, bitter, or fermented off-flavors remained undetectable at 60 d of storage. However, cooked flavor was detected by all panelists consistently as cooked, caramelized, or scorched. Thus, cooked flavor was not scored.

Conclusions

Flavor of UP-PET-bottled milk remained without noticeable change during the 60 d of refrigerated storage. Insignificant changes in pH and TA during 60 d of storage reflect the chemical stability of UP-PET-bottled milk. The presence of aldehydes, such as hexanal, octanal, and nonanal, indicates lipid oxidation in PET-bottled milk at 60 d of storage. Therefore, PET packaging with ultrapasteurization can be applied to shelf life up to 60 d without compromising the chemical and sensory properties of milk.

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