Effect of High-Pressure–Moderate-Temperature Processing on the Volatile Profile of Milk

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The effects of high hydrostatic pressure on volatile generation in milk were investigated in this study. Raw milk samples were treated under different pressures (482, 586, and 620 MPa), temperatures (25 and 60 °C), and holding times (1, 3, and 5 min). Samples submitted to heat treatments alone (25, 60, and 80 °C for 1, 3, and 5 min) were used for comparison. Trace volatile sulfur compounds were analyzed using solid-phase microextraction (SPME) and gas chromatography (GC) with pulsed-flame photometric detection (PFPD), whereas the rest of the volatile compounds were analyzed using SPME-GC with flame ionization detection (FID). Multivariate analysis of variance (MANOVA) and principal component analysis (PCA) were used to study the effect of pressure, temperature, and time on volatile generation. Relative concentration increases of 27 selected volatile compounds were compared to an untreated sample. It was found that pressure, temperature, and time, as well as their interactions, all had significant effects ($P < 0.001$) on volatile generation in milk. Pressure and time effects were significant at 60 °C, whereas their effects were almost negligible at 25 °C. The PCA plot indicated that the volatile generation of pressure-heated samples at 60 °C was different from that of heated-alone samples. Heat treatment tended to promote the formation of methanethiol, hydrogen sulfide, methyl ketones, and aldehydes, whereas high-pressure treatment favored the formation of hydrogen sulfide and aldehydes.

KEYWORDS: Milk; high-pressure processing; volatile; volatile sulfur compounds

INTRODUCTION

Thermal processing is the prevailing method to achieve microbial safety and shelf-life stability of milk. Although high-temperature–short-time (HTST) pasteurization (typically at 72 °C for 15 s) is typically used commercially to process milk, the product shelf life is only 20 days at refrigeration temperatures. Ultrahigh-temperature (UHT) processing (135–150 °C for 3–5 s) produces a product that is stable at room temperature for up to 6 months; however, this process can induce strong "cooked" off-aroma notes in milk (*1*), thus limiting its marketing in the United States and many other countries (*2*). Numerous studies have identified volatile sulfur compounds, aldehydes, and methyl ketones as the most important contributors to this "cooked" off-aroma defect (*3–9*), and reliable quantification methods have been developed to analyze these off-aroma compounds (*8–11*).

New technologies are needed to process milk without compromising its flavor. Several nonthermal processing technologies have been explored to achieve microbial safety and minimize off-flavor formation. Microfiltration using cross-flow membrane separation has showed promising results in eliminat-

ing bacteria from milk and increasing shelf life without the development of off-flavors (*12, 13*). However, high levels of milk fat could foul the membrane and place some restrictions on the use of microfiltration as an alternative technique for milk processing. High hydrostatic pressure processing (HPP), a new technology to the food industry (*14*), can destroy microorganisms by high hydrostatic pressure without heat (*15–17*). This technology has been gaining commercial acceptance in the manufacture of food products with "fresh" flavor that are not possible with other preservation technologies (*18, 19*). To retain the "fresh" milk flavor, HPP has been studied as a potential alternative for the pasteurization of milk. A microbiological reduction similar to that of pasteurized milk has been achieved using pressure treatments of 400 MPa for 15 min or 500 MPa for 3 min at room temperature (*20*). At moderate temperature (55 °C), HPP (586 MPa for 5 min) can significantly extend the shelf life of milk beyond 45 days, which far exceeds that of pasteurized milk (*21*).

HPP has been reported to change some properties of the foods. HPP can reduce the size of casein micelles in milk at pressures above 230 MPa, resulting in a decrease in whiteness and turbidity and an increase in the viscosity of milk (*22*). High pressure can also affect the crystallization properties of milk fat. The crystallization behavior of milk fat can be altered because the high pressure will shift the phase transition

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temperature (*23*). It is generally assumed that HPP at low temperature will retain the flavor of the product; however, Hofmann et al. (*24*) reported that HPP could change the formation of Maillard-derived compounds at high temperature. Information on flavor generation under high pressure is still very limited. The objective of this study was to investigate volatile generation in milk under high pressure and moderate temperature and to compare the volatile formation with that formed under atmospheric pressure conditions at comparable temperature.

MATERIALS AND METHODS

Chemicals. 3-Methylbutanal, 2-methylpropanal, ethyl acetate, 3-methylbutanol, 2-furaldehyde, heptanal, octanal, nonanal, decanal, *trans*-2-hexenal, 2-heptanone, 2-nonanone, 2-undecanone, 3-heptanone, 3-octanone, 4-decanone, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), dimethyl sulfoxide (DMSO), dimethyl sulfone (Me_2SO_2), and isopropyl disulfide (IPDS) were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI); 2,3-butanedione (diacetyl) and hexanal were purchased from Sigma (St. Louis, MO); 2-octanone was from Fluka Chemical Corp. (Milwaukee, WI); 2-pentanone, 2-hexanone, 2-decanone, and *trans*-2-nonenal were from K&K Laboratories (Jamaica, NY); 2-methylbutanal was from Polyscience Inc. (Niles, IL); dimethyl sulfide (DMS) and ethyl methyl sulfide (EtMeS) were from TCI America (Portland, OR). Carbon disulfide (CS_2) was from EMD Chemicals Inc. (Gibbstown, NJ).

Milk Samples. Raw homogenized milk with 3.2% fat was obtained locally (Lochmead Farms, Junction City, OR), and sodium azide (0.02%) was added immediately to inhibit microbial activity. The same batch of milk was used for the entire experiment to avoid differences in sample composition. Pasteurized milk samples with 3.2% fat content from two different commercial brands (PA and PB) were purchased locally, stored at 4 °C, and analyzed before their expiration date (2 weeks from manufacturing date). UHT-treated milk samples (3.2% fat) from two different commercial brands (UHT UA and UHT UB) were purchased in Mexico, stored at 4 °C after arrival in the United States, and analyzed before their expiration date (6 months from manufacturing date).

High-Pressure Treatments. Raw milk samples were placed in individually sealed polyethylene bags. Treatments from a 2 × 3 × 3 experimental design (treatments 10−27 in **Table 1**) for temperature (25 and 60 °C), pressure (482, 586, and 620 MPa), and time (1, 3, and 5 min) were run in triplicate. A 2.2 L high-pressure vessel (Engineered Pressure Systems Inc., Haverhill, MA) equipped with a temperature controller and a high-pressure fluid pump (model P100-10FC, Hydro-Pac Inc., Fairview, PA) was used to process the milk. All samples were equilibrated at 25 °C before loading into the high-pressure vessel. Loading time (1 min) and unloading time (1.5 min) were kept constant for all runs. The average pressure-ramp time was 40 s. Time between runs was 15 min. Immediately after treatment, samples were placed in an ice bath and then stored at −38 °C until analyzed. All analyses were performed within 20 days after the treatments.

Heat Treatments. An experimental design (treatments 1−9 in **Table 1**) was run in triplicate under atmospheric pressure to simulate the temperature conditions the sample would experience during a high-pressure treatment at 620 MPa at temperatures of 25, 60, and 80 °C and holding times of 1, 3, and 5 min. The treatment conducted at 80 °C was included in the design as an extra level of reference, although it was not used in the high-pressure treatment. These treatments took into account the temperature increase inside the pressure vessel generated during pressurization due to sample compression. To simulate the thermal component of the high-pressure process, samples contained in sealed polyethylene bags were preheated in a water bath at 25, 60, and 80 °C, respectively, for 3.2 min, which represents the total time required in the high-pressure treatments to load the sample (1 min), increase the pressure (0.7 min), and unload the sample (1.5 min) at the preset initial temperature. Immediately after that, the samples were transferred to another water bath and kept at 44, 79, and 99 °C, respectively, for 1, 3, and 5 min, which represents the additional

Table 1. Experimental Design for Pressure, Temperature, and Time Treatments of Milk Samples

treatment	temperature[a] (°C)	pressure (MPa)	time (min)
1	25 (43.6)		1
2	25 (43.6)		3
3	25 (43.6)		5
4	60 (78.6)		1
5	60 (78.6)		3
6	60 (78.6)		5
7	80 (98.6)		1
8	80 (98.6)		3
9	80 (98.6)		5
10	25	482	1
11	25	482	3
12	25	482	5
13	25	586	1
14	25	586	3
15	25	586	5
16	25	620	1
17	25	620	3
18	25	620	5
19	60	482	1
20	60	482	3
21	60	482	5
22	60	586	1
23	60	586	3
24	60	586	5
25	60	620	1
26	60	620	3
27	60	620	5
past. A[b]			
past. B[b]			
UHT A[c]			
UHT B[c]			

[a] Refer to text for treatment detail. [b] Pasteurized commercial milk samples. [c] Ultrahigh-temperature-processed commercial milk samples.

temperature increase that milk samples would experience under high pressure due to compression heating estimated on an average increase of 3 °C per 100 MPa (*25*). Immediately after each treatment, samples were placed in an ice bath and then stored at −38 °C until analyzed.

Analysis of Thermally Derived Volatile Compounds. Dimethyl sulfide and thermally derived volatile compounds were analyzed using headspace solid-phase microextraction and gas chromatography with flame ionization detection (HS-GC/FID) (*8*). The milk sample (20 g) was extracted with a 2-cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco Co., Bellefonte, PA) at 35 °C for 1 h. Volatile compounds were analyzed using an HP 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with an FID and an HP-5 capillary column (50 m × 0.32 mm i.d., 0.52 μm film thickness, Hewlett-Packard). *trans*-2-Hexenal, 3-heptanone, 3-octanone, 4-decanone, and *trans*-2-nonenal were used as internal standards. Calibration curves for the volatile compounds were constructed on the basis of the standard addition technique.

Analysis of Trace Volatile Sulfur Compounds. Hydrogen sulfide, methanethiol, dimethyl disulfide, dimethyl trisulfide, carbon disulfide, dimethyl sulfoxide, and dimethyl sulfone were analyzed using headspace solid-phase microextraction and gas chromatography with pulsed-flame photometric detection (HS-SPME/GC-PFPD) described previously (*9*). The milk sample (10 g) was extracted with a 85 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco) at 30 °C for 15 min and analyzed with a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a DB-FFAP fused-silica capillary column (30 m × 0.32 mm, 1.0 μm film; Agilent Technologies, Inc., Palo Alto, CA) and a PFPD detector. Calibration curves for seven sulfur-containing compounds were constructed in milk by the standard addition technique. Ethyl methyl sulfide and isopropyl disulfide were used as internal standards. Triplicate analysis was performed on each sample.

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Statistical Analysis. The concentration of each of the 27 volatile compounds was compared to that of the raw milk sample (3.2% fat), and a normalized percentage change was calculated as follows:

$$\% \text{ change} = \frac{\text{concn in treated milk} - \text{concn in raw milk}}{\text{concn in raw milk}} \times 100$$

Multivariate analysis of variance (MANOVA) and principal component analysis (PCA) were conducted using S-Plus 6.2 (Insightful Corp., Seattle, WA) for the normalized percentage of change of 27 compounds as the multivariate response.

RESULTS AND DISCUSSION

Effect of Thermal Processing on Volatile Generation in Milk. During high-pressure processing, there is a temperature increase upon pressurization due to adiabatic heating (*26*). The magnitude of this change depends on the compressibility of the substance and its specific heat. At the pressures typically encountered during high-pressure processing (400–1000 MPa), milk increases ∼3 °C for every 100 MPa of pressure increase (*25*). The temperature decreases instantly when the pressure is released. Because thermal degradations of lipids, proteins, and sugars contribute most to the volatile formation in milk, the adiabatic heating effect was taken into account for all samples treated under atmospheric pressure, so the corresponding treatments submitted to high pressure could be directly compared. Treatments 1−9 (**Table 1**) simulated the temperature increase experienced inside the pressure vessel at 620 MPa.

Twenty-seven volatile compounds including aldehydes, ketones, esters, and sulfur compounds were quantified in this study. Because the concentrations of volatile compounds varied widely and were affected by treatment conditions, the normalized percentage change for each compound was also investigated.

As shown in **Table 2**, the concentrations of aldehydes and ketones were at the microgram per kilogram level, with hexanal, nonanal, and decanal dominating for most of the treatments. The concentration of both straight-chain and branched-chain aldehydes increased with the severity of the applied heat treatment (**Table 2**). At 25 °C, the concentrations of aldehydes in all treatments were the same as those in the raw milk, demonstrating that none of the aldehydes was generated at this temperature. When the temperature was raised to 60 °C, the concentrations of nonanal in the heated samples were triple those in the raw milk, whereas the concentrations of octanal and decanal were double. Other aldehydes also increased but at different degrees. Hexanal, on the other hand, had lower concentrations than that in the raw milk. More dramatic increases of aldehydes were observed at 80 °C. In all cases, nonanal had the highest relative increase upon heating, followed by octanal and decanal. At both 60 and 80 °C, longer holding time increased the formation of aldehydes. Straight-chain aldehydes can result from the autoxidation of unsaturated fatty acids and spontaneous decomposition of hydroperoxides promoted by heat (*27*). At low temperature, the increase of $C_{2-7,9}$ aldehydes is thought to be the main cause for the stale off-flavor in milk (*8, 28, 29*). At high temperature, they probably also contribute to the "cooked" off-flavor because the concentrations of most aldehydes were higher than their sensory thresholds (*29*). Branched-chain aldehydes are from Strecker degradation of amino acids.

Methyl ketones were also dramatically affected by thermal treatment (**Table 2A**), which is in agreement with a previous study (*7*). Similar to aldehydes, methyl ketones were not formed at 25 °C. When samples were heated at 60 °C, the concentrations of 2-nonanone increased approximately 4 times, whereas 2-undecanone and 2-hexanone increased 2 times. At 80 °C, 2-decanone and 2-heptanone also had dramatic increases. The concentrations of 2-octanone were not affected by thermal treatments. Although methyl ketones are naturally present in raw milk, most of them are formed thermally by the oxidation of fatty acids (*30*) or by the decarboxylation of β-ketoacids naturally present in milk fat (*27, 31*).

2,3-Butanedione and 2-furaldehyde also showed an increasing trend in concentration with temperature and heating time (**Table 2B**). Both of them can be formed through the thermal degradation of carbohydrates. Ethyl acetate had slight increases only at 80 °C, and it is formed by esterification of ethanol with acetic acid catalyzed by heat (*32*). 3-Methylbutanol was also monitored; this compound is naturally present in raw milk and is produced mainly by the microbial reduction of 3-methylbutanal (*33*). However, all of these compounds, with the exception of 2,3-butanedione, have very high sensory thresholds (*29*), suggesting that they are of little importance for the development of off-flavors in heated milk.

The concentrations of sulfur-containing compounds presented the widest variation. Dimethyl sulfoxide and dimethyl sulfone were the most abundant, reaching milligram per kilogram levels, whereas CS_2, DMDS, and DMTS were present only at nanogram per kilogram levels. It has been reported that volatile sulfur compounds are mainly responsible for the development of the "cooked" off-flavor in heated milk (*2, 34−36*). A recent study based on odor activity value (ratio of concentration to sensory threshold) further suggested that MeSH, DMS, DMTS, and H_2S are probably the most powerful off-flavor compounds in heated milk (*8, 9*).

Most volatile sulfur compounds followed an increasing trend in concentration when temperature and processing time were increased (**Table 2B**). H_2S and MeSH had dramatic increases upon heating. At 60 °C, the concentration of MeSH increased 3−4 times depending on the holding time, whereas H_2S increased 3−7 times. This increase was even more dramatic at 80 °C; an increase of 8−9-fold was observed for MeSH. An increase of 10−15-fold was noted for H_2S, raising its concentration comparable to that of MeSH. Although H_2S had the highest concentration increase upon heating, its contribution to the off-flavor of heated milk is probably not as important as previously suggested (*28, 36, 37*). H_2S has a sensory threshold of 10 µg/kg in water (*29*); on the basis of its calculated odor activity value, its contribution to "heated" off-flavor is probably limited to samples subjected to only the most severe temperature−time treatments (60 °C for 5 min or 80 °C). Even with the most severe temperature−time treatment, it had odor activity values of only 2−3. The concentration of MeSH in all treatments far exceeded its sensory threshold (0.2 µg/kg in water) (*29*), and its odor activity value increased from 15 in raw milk to as high as 140 in heated milk (80 °C/5 min), which further confirms that MeSH may be a much more important off-flavor contributor than H_2S (*9*). DMS had a slight increase in concentration upon heating, and its concentration in all treatments was higher than its sensory threshold (2 µg/kg in water) (*29*). Calculated odor activity values (OAV 2−4) suggest that it may contribute to the off-flavor of heated milk but to a much lesser degree than MeSH. Although increasing upon heating, concentrations of DMTS in all treatments were below its sensory threshold; thus, its contribution to the off-flavor of heated milk is probably very limited, if any. The concentrations of DMDS were also below its sensory threshold.

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Table 2. Concentrations (Micrograms per Kilogram) of (**A**) Straight-Chain Aldehydes and Methyl Ketones in Milk and (**B**) Volatile Sulfur and Other Compounds in Milk[a]

(A) Straight-Chain Aldehydes and Methyl Ketones

treatment[b]	2-pentanone	2-hexanone	hexanal	2-heptanone	heptanal	2-octanone	octanal	2-nonanone	nonanal	2-decanone	decanal	2-undecanone
1	0.73	0.66	14.40	1.04	1.13	4.31	1.49	0.77	3.40	0.51	9.68	0.31
2	0.87	1.05	12.42	1.04	0.81	3.91	1.33	0.76	3.42	0.50	5.99	0.36
3	0.66	1.08	13.48	1.08	1.21	4.09	1.49	1.10	4.59	0.57	8.47	0.36
4	0.84	1.74	10.33	1.12	1.55	4.06	2.31	3.03	9.01	0.67	16.11	0.33
5	0.96	1.56	11.48	1.22	1.53	3.84	2.35	3.22	12.74	0.80	16.83	0.64
6	1.99	1.68	11.87	1.57	1.78	3.90	2.73	3.80	13.67	0.76	19.01	0.63
7	1.85	1.74	13.23	1.94	1.86	4.35	3.29	4.61	17.09	1.05	21.60	1.09
8	1.69	1.72	15.24	3.23	2.14	4.79	3.74	6.29	20.75	1.05	21.79	1.37
9	1.77	1.63	16.98	5.24	2.61	4.82	5.13	8.64	26.56	1.12	23.69	1.57
10	0.43	0.40	9.74	1.08	1.02	4.21	1.00	0.50	2.70	0.40	4.28	0.28
11	0.54	0.62	10.63	1.01	1.09	5.00	1.18	0.58	2.97	0.41	4.52	0.46
12	0.48	0.50	8.09	1.06	1.05	3.87	0.92	0.54	3.46	0.51	5.20	0.43
13	0.59	0.54	8.64	1.35	1.01	5.07	1.06	0.36	2.32	0.38	5.28	0.33
14	0.55	0.48	6.46	1.02	0.92	4.08	1.00	0.63	3.01	0.64	5.89	0.32
15	0.41	0.45	6.19	1.01	0.75	2.96	0.74	0.52	2.57	0.42	3.83	0.21
16	0.54	0.51	8.70	1.09	1.01	4.34	1.03	0.60	2.85	0.58	7.32	0.39
17	0.49	0.51	9.47	1.04	1.06	4.89	1.00	0.59	3.72	0.80	8.82	0.49
18	0.43	0.42	7.88	1.24	0.86	3.71	0.88	0.45	2.34	0.34	4.87	0.27
19	0.94	1.21	11.85	2.71	1.17	3.15	1.47	2.07	5.59	0.91	15.65	0.51
20	0.91	1.41	14.94	2.05	1.59	4.44	1.57	2.07	5.95	1.10	16.64	0.47
21	1.01	1.09	12.04	1.92	1.40	4.35	1.53	2.31	5.96	1.06	14.36	0.55
22	0.83	1.61	15.08	2.03	1.76	3.70	1.57	2.63	6.31	0.99	12.27	0.47
23	0.87	1.19	19.25	1.84	3.12	4.14	1.83	2.37	7.43	1.06	16.47	0.47
24	0.83	1.08	23.88	2.36	3.12	4.01	1.92	2.05	7.54	0.93	14.80	0.61
25	0.84	1.62	13.91	2.07	1.60	4.79	1.43	1.36	8.54	0.71	10.60	0.48
26	0.74	1.46	15.71	1.91	2.22	3.74	1.30	1.40	6.46	0.63	11.46	0.37
27	0.92	1.64	38.16	1.98	5.63	4.39	2.35	2.03	11.30	0.80	14.52	0.53
raw	0.75	0.93	13.43	1.05	1.05	4.11	1.44	0.88	3.80	0.53	8.05	0.34
past. A	0.21	0.05	8.83	1.11	0.06	6.35	0.13	0.51	1.18	0.21	5.91	2.73
past. B	0.22	0.15	7.72	0.76	0.14	3.02	0.14	0.62	0.42	0.20	1.67	0.91
UHT A	9.51	1.82	12.76	32.46	1.68	4.53	0.91	51.72	3.91	1.35	6.56	9.63
UHT B	9.68	1.93	13.52	35.62	1.73	4.65	1.02	54.28	4.06	1.38	6.85	10.10

(B) Volatile Sulfur and Other Compounds

treatment[b]	2-methyl-propanal	2,3-butane-dione	ethyl acetate	3-methyl-butanal	2-methyl-butanal	3-methyl-1-butanol	2-furalde-hyde	H₂S	MeSH	DMS	DMDS (ng/kg)	DMTS (ng/kg)	CS₂ (ng/kg)	DMSO	Me₂SO₂
1	0.33	1.34	0.28	1.14	5.06	0.39	0.98	1.83	3.41	5.27	61	42	36	620	1653
2	0.38	1.44	0.22	1.70	6.63	0.51	1.99	2.01	2.77	5.60	80	36	33	638	1480
3	0.38	1.50	0.23	1.69	6.37	0.46	2.07	1.95	3.24	5.51	76	42	33	606	1412
4	0.35	1.78	0.25	1.96	7.34	0.55	3.20	1.70	4.48	5.33	63	39	36	647	1328
5	0.42	1.65	0.30	1.83	5.50	0.45	1.34	7.15	9.18	5.64	66	46	42	702	1481
6	0.48	1.79	0.31	2.77	9.12	0.67	3.28	16.43	12.66	5.73	38	48	50	748	1776
7	0.59	1.95	0.40	2.87	8.21	0.71	3.77	21.46	22.37	7.99	89	49	44	872	1269
8	0.73	2.39	0.38	1.29	8.07	0.66	2.99	25.35	24.87	8.44	60	65	43	1074	1273
9	0.76	2.82	0.39	2.08	7.66	0.68	3.34	30.25	28.36	8.70	44	72	65	1241	2143
10	0.31	1.28	0.26	0.53	2.42	0.24	0.14	5.78	3.40	5.23	15	24	135	398	1166
11	0.45	1.32	0.33	0.83	3.43	0.30	0.45	8.09	7.60	7.07	20	30	108	592	1199
12	0.50	1.43	0.32	0.99	3.38	0.33	0.62	6.19	5.78	5.99	34	35	113	867	1725
13	0.54	1.41	0.31	0.96	3.55	0.31	0.76	1.71	5.02	6.61	95	36	104	757	1679
14	0.35	0.94	0.34	0.99	2.62	0.31	0.73	3.18	6.16	6.58	55	32	105	676	1171
15	0.23	0.69	0.18	0.50	2.14	0.21	0.71	7.33	3.41	5.96	19	19	129	520	1051
16	0.40	0.82	0.32	0.89	3.12	0.31	0.71	6.66	2.74	6.91	29	34	147	678	1528
17	0.43	1.19	0.32	0.85	3.17	0.31	0.59	6.48	3.63	6.52	41	35	104	759	1798
18	0.40	1.19	0.27	0.78	3.01	0.27	0.64	11.94	3.20	5.99	20	29	120	765	1719
19	0.44	2.15	0.39	1.86	5.13	0.46	1.74	12.37	7.08	6.13	27	27	49	967	1734
20	0.51	2.35	0.39	2.24	6.32	0.55	2.67	13.69	6.52	6.83	31	36	65	735	1518
21	0.44	2.11	0.30	1.88	6.31	0.55	1.80	8.01	5.68	7.65	23	38	39	559	1596
22	0.58	2.24	0.26	2.12	6.91	0.58	2.40	6.81	7.90	6.09	33	34	54	915	1507
23	0.45	1.65	0.27	1.93	6.47	0.54	1.82	9.02	8.50	6.36	31	33	47	831	1414
24	0.46	1.87	0.32	1.81	5.11	0.50	1.60	9.53	8.76	6.30	30	31	63	992	1920
25	0.47	2.05	0.47	2.21	6.16	0.59	2.27	14.47	4.25	5.93	14	34	64	711	1704
26	0.49	1.84	0.41	1.83	5.26	0.53	1.99	15.68	4.44	5.93	19	38	60	731	1552
27	0.47	1.98	0.32	2.09	6.68	0.54	2.78	17.95	3.26	6.83	32	41	69	683	1367
raw	0.36	1.42	0.25	1.51	6.02	0.45	1.68	1.93	3.14	5.46	72	40	34	621	1515
past. A	0.51	0.93	0.33	0.05	0.08	0.13	0.12	0.86	3.59	8.47	33	17	30	696	1346
past. B	0.28	2.09	0.43	0.09	0.12	0.13	0.11	1.02	4.28	16.08	17	19	33	790	2224
UHT A	2.51	7.37	2.17	1.14	0.91	0.14	0.37	12.25	23.92	21.12	33	47	69	1427	1268
UHT B	2.63	7.52	2.18	1.26	1.12	0.16	0.41	12.23	24.65	22.42	33	49	60	1478	1275

[a] Each value represents the average of a triplicate treatment. Relative standard deviation was <8.9%. [b] For treatment descriptions, refer to **Table 1**.
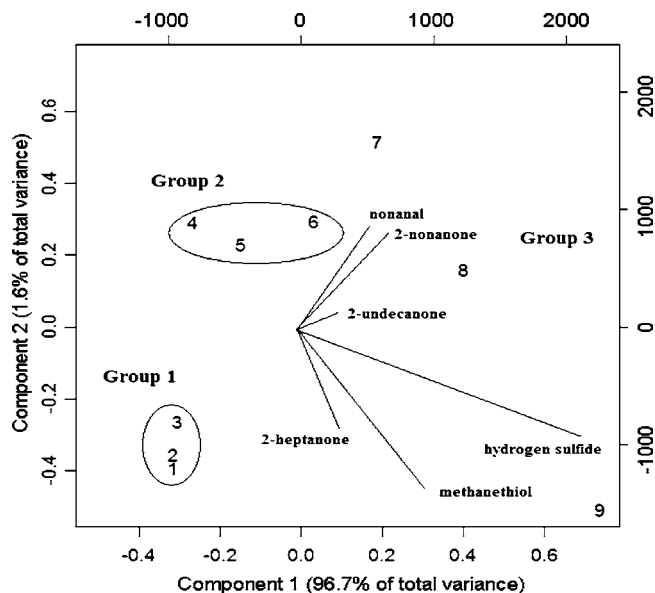
Figure 1. PCA plot for the volatile profile of milk subjected to different temperature treatments (treatments 1–9).

Volatile sulfur compounds can be generated from the thermal decomposition of sulfur-containing amino acids (*2*, *38*). H₂S is proposed to be mainly generated from cysteine through oxidation (*37*, *39*). MeSH is thought to be mainly liberated from methionine (*39*). Both H₂S and MeSH are highly reactive and can form other sulfur compounds such as DMS, DMDS, and DMTS (*40*).

MANOVA on normalized percent concentration changes showed that temperature and time, as well as their interaction, had significant effects on the volatile generation of milk ($P <$ 0.001). Temperature was the most important factor ($P <$ 0.0001). The PCA plot for temperature and time (**Figure 1**) demonstrated that temperature allowed for the differentiation of the treatments into three main groups. Group 1 included treatments 1, 2, and 3 (25 °C for 1, 3, and 5 min, respectively), group 2 included treatments 4, 5, and 6 (60 °C for 1, 3, and 5 min), and group 3 included treatments 7, 8, and 9 (80 °C for 1, 3, and 5 min). It was evident that the holding time did not scatter the samples at 25 °C (**Figure 1**, group 1), whereas it began to separate the samples at 60 °C (group 2), and the separation due to holding time became obvious at 80 °C (group 3). Although the holding time did not have a major impact at low temperature, it became critical at high temperature. These results demonstrated that the processing temperature was the determining factor for the volatile generation of milk.

The PCA loadings (**Table 3**) showed that increases in temperature and time mostly promoted the formation of H₂S, MeSH, 2-nonanone, nonanal, 2-undecanone, and 2-heptanone. These compounds were the most important contributors to separate the treatments in the PCA plot (**Figure 1**). The dramatic increase of these compounds from 60 to 80 °C supports previous reports that heat treatments above 74 °C start to change the volatile composition and the sensory quality of milk (*2*, *38*). Of special interest is the high percent of total variance (96.7%) explained by principal component 1 in the PCA (**Table 3**). This allowed for a discrimination of the volatile profile of the different treatments using the linear direction of a single component, in this case, component 1, whereby component 2 could be considered as irrelevant.

Effect of High-Pressure−Moderate Heating on the Generation of Volatile Compounds in Milk. The effect of high

hydrostatic pressure and mild heating on volatile generation in milk was investigated. Methyl ketone formation was affected by temperature (**Table 1**, treatments 10−27). At 25 °C, the concentrations of 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and 2-undecanone in all treatments were close to those of the raw milk, demonstrating that these methyl ketones are not generated at pressures below 620 MPa. This is consistent with the results from heat-only treatment that volatile compounds were not formed at 25 °C. It was observed that the concentrations for 2-pentanone and 2-hexanone in high-pressure-treated samples were even slightly lower than that in the raw milk. At 586 and 620 MPa, further subtle decreases of 2-pentanone and 2-hexanone with increasing holding time were noted. Yet, these observations need to be proved by further experimentation. At 60 °C, the concentrations of methyl ketones in all high-pressure-treated samples increased. However, the increasing trends and magnitudes were similar to those of heat-only samples. The results suggested that high pressure had no major effect on the formation of methyl ketones (**Table 2A**).

Similar to methyl ketones, there was no aldehyde formation at 25 °C at any of the pressure−holding time treatments. With the exception of heptanal and 2-methylpropanal, the concentrations of all aldehydes in the high-pressure-treated samples were even slightly lower than those in the raw milk sample, which is consistent with previous results for some methyl ketones. If this observation is confirmed by further experimentation, it would suggest that HPP could affect flavor perception even at low temperature. At 60 °C, a general increasing trend of formation was observed for straight-chain aldehydes. Under high pressure, hexanal had much higher increases than the samples heated under normal atmospheric pressure, whereas nonanal had a much lower increase. Both pressure and holding time enhanced the formation of aldehydes. This trend was magnified when the pressure was increased to 620 MPa. Among the straight-chain aldehydes, hexanal and heptanal had the most dramatic increase in concentration, and up to 284 and 436% increases, respectively, at the highest pressure−time treatment. It is worthwhile to note that their concentrations were much higher than for the corresponding homologous heat treatments (treatments 4, 5, and 6). The formation of branched-chain aldehydes seemed not to be affected by pressure.

The mechanisms of aldehyde and ketone formation under normal pressure have been well studied. Both autoxidation and thermal oxidation can generate aldehydes and ketones. Unlike heat-induced reactions, very few literature studies have published the mechanisms of volatile formation under high hydrostatic pressure (*24*). The fact that the concentration of aldehydes (hexanal and octanal) increased dramatically under high pressure, while the concentration of methyl ketones was similar to that of samples subjected to heat-only treatments, suggested that the volatile formation under high pressure could be different from that under normal pressure. Although the actual formation mechanisms under high pressure are not known, oxygen becomes more soluble under high pressure, therefore potentially increasing the formation of hydroperoxides and leading to more aldehyde generation. It is also possible that high pressure affects the kinetics of volatile formation. According to Le Chatelier's principle (*41*), if the formation of hydroperoxides from oxygen and fatty acids involves equilibrium reactions with a volume reduction, high pressure will favor this reaction and thus lead to more aldehyde generation. Another highly likely possibility is that the hydrostatic pressure affects the rate of formation according to its reaction activation volume (ΔV^*) defined as the difference between the partial molar volume of the transition

Table 3. PCA Loadings for the Volatile Compounds of Milk Subjected to Different Treatments[a]

compound	temperature + time, **Figure 1** (treatments 1–9)		temperature + pressure + time, **Figure 2** (treatments 10–27)		temperature + time and temperature + pressure + time, **Figure 3** (treatments 1–27)		treatments 1–27 and pasteurized and UHT milk, **Figure 4**	
	component 1 (96.7%)[b]	component 2 (1.6%)	component 1 (85.5%)	component 2 (9.4%)	component 1 (81.8%)	component 2 (11.4%)	component 1 (87.0%)	component 2 (10.6%)
2-methylbutanal	0.043	0.015	−0.010	−0.061	0.033	−0.041	0.080	0.006
2,3-butanedione	0.032	0.001	−0.035	−0.136	0.036	−0.039	0.056	0.017
ethyl acetate	0.024	0.056	−0.025	−0.019	0.023	0.015	**0.098**	−0.011
3-methylbutanal	0.009	0.163	−0.049	−0.161	0.030	−0.046	−0.001	0.039
2-methylbutanal	0.012	0.078	−0.030	−0.113	0.025	−0.059	−0.007	0.035
2-pentanone	0.057	0.213	−0.027	−0.120	0.057	−0.125	**0.158**	0.009
3-methyl-1-butanol	0.020	0.084	−0.035	−0.114	0.031	−0.047	−0.006	0.039
2-hexanone	0.029	0.170	−0.071	−0.158	0.051	−0.046	0.015	0.055
hexanal	0.010	−0.064	**−0.072**	−0.125	0.033	0.038	0.002	0.043
2-furaldehyde	0.035	0.202	−0.064	−0.167	0.057	−0.090	−0.006	0.066
2-heptanone	**0.127**	−0.257	−0.054	−0.195	**0.105**	−0.084	**0.426**	−0.032
heptanal	0.048	0.070	**−0.142**	**−0.228**	0.082	0.063	0.005	**0.096**
2-octanone	0.007	−0.016	0.001	0.002	0.004	−0.004	0.001	0.002
octanal	0.083	0.056	−0.032	−0.110	0.077	−0.147	−0.001	**0.083**
2-nonanone	**0.288**	0.275	**−0.074**	**−0.446**	**0.247**	−0.501	**0.791**	−0.016
nonanal	**0.203**	0.315	−0.098	−0.191	0.191	−0.294	0.001	**0.196**
2-decanone	0.041	0.111	−0.027	**−0.252**	0.040	−0.082	0.019	0.040
decanal	0.066	**0.280**	−0.054	**−0.258**	0.073	−0.147	−0.002	**0.079**
2-undecanone	**0.137**	0.063	−0.025	−0.144	**0.109**	0.192	**0.368**	−0.061
hydrogen sulfide	**0.829**	−0.286	**−0.841**	0.225	**0.882**	0.444	0.032	**0.932**
methanethiol	**0.312**	**0.429**	0.015	**−0.331**	0.221	−0.511	0.084	0.187
carbon disulfide	0.025	−0.014	**0.096**	**0.422**	−0.041	**0.165**	−0.007	−0.019
dimethyl disulfide	−0.009	0.023	0.018	0.014	−0.005	−0.067	−0.001	−0.004
dimethyl trisulfide	0.028	−0.020	−0.010	−0.020	0.027	−0.055	0.005	0.028
dimethyl sulfoxide	0.035	−0.018	−0.004	−0.060	0.025	−0.039	0.016	0.018
dimethyl sulfone	0.008	−0.080	−0.005	−0.001	0.007	0.001	−0.002	0.005
dimethyl sulfide	0.024	0.011	0.001	−0.026	0.015	0.020	0.037	−0.009

[a] Boldfaced numbers represent the loadings with the six highest values. For treatment descriptions, refer to **Table 1**. [b] Proportion of total variance.

or activated state and of the reactant at the same temperature and pressure (*42*). When pressure is applied, $\Delta V^* < 0$ leads to an increase in reaction rate, whereas $\Delta V^* > 0$ has the opposite effect. The sensitivity of a chemical reaction to pressure will increase with the absolute value of ΔV^* (*43*). The determination of ΔV^* values is not possible with the experimental data available in this study and will require more comprehensive kinetic studies.

The formation of H_2S seemed to be affected by both pressure and holding time. A dramatic increase of H_2S was observed under high-pressure treatments even at 25 °C (**Table 2B**). The increases seemed to be holding time dependent. As expected, H_2S increased even more at 60 °C, and its concentrations in high-pressure-treated samples were generally higher than in the corresponding heat-only samples. Although the formation of H_2S could be pressure dependent, it was not obvious, however, because the trend was not consistent for all treatments. The concentrations of MeSH also increased at 25 °C under high pressure. However, when the pressure increased to 620 MPa, the concentration of MeSH decreased. The same behavior was observed at 60 °C, with the lowest concentration occurring at 620 MPa and 5 min of holding. Although methanethiol formation appeared to be inhibited under pressure, it is also possible that it was converted to other compounds. In addition, the formation and conversion of methanethiol could be pH dependent, and the effect of pH on volatile formation was not taken into consideration in this study, because pressure-induced pH shifts cannot be measured in the experimental HPP vessel as there are no pressure-resistant probes currently available. There was only a slight increase for DMS under high pressure, and this increase was undistinguishable from 25 to 60 °C. The concentrations of other sulfur compounds were similar to that
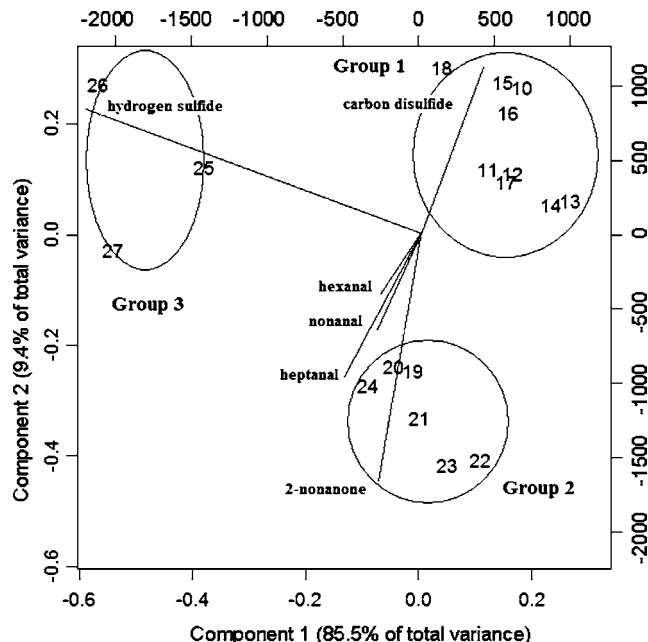

Figure 2. PCA plot for the volatile profile of milk subjected to different high hydrostatic pressure and temperature treatments (treatments 10–27).

of the homologous heat treatment. Many sulfur compounds are extremely reactive, and more studies are needed to understand their formation behavior under high pressure.

The impact of HPP on volatile formation can be better illustrated by MANOVA on normalized percentage change. From the results of MANOVA, it can be concluded that
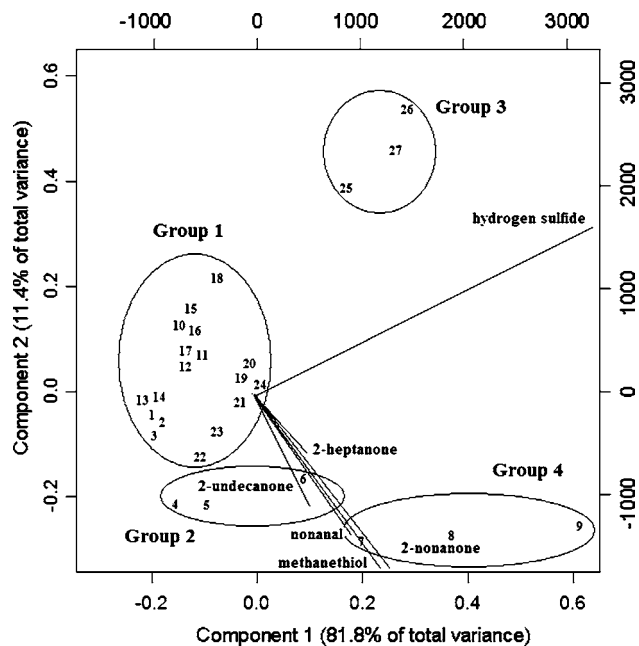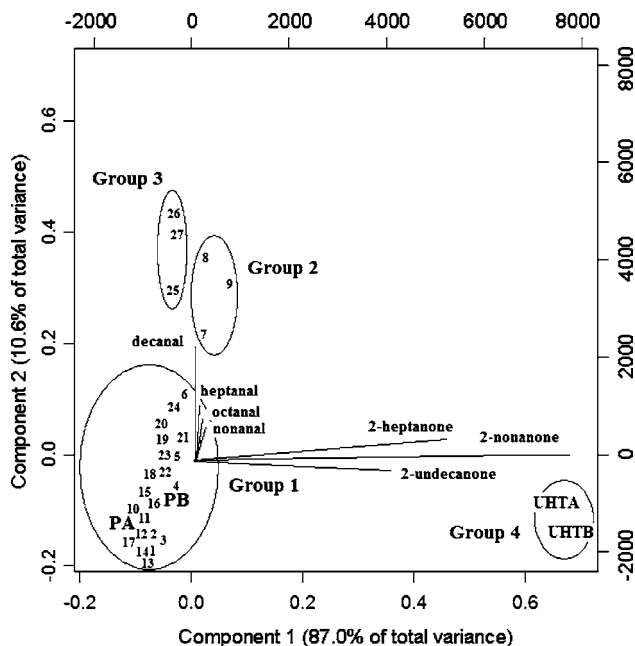
Figure 3. Spatial distribution of volatile profile of milk subjected to temperature and high hydrostatic pressure–temperature treatments.

PA: Pasterized sample A
PB: Pasterized sample B

Figure 4. Comparison of volatile profile in commercially processed milk with samples subjected to temperature and high-pressure treatments.

temperature, pressure, and time, as well as their double and triple interactions, were important factors influencing the formation of volatile compounds in milk ($P < 0.001$). The PCA plot for the pressurized samples (**Figure 2**) confirmed that temperature had an important effect, separating the samples treated at 25 °C (group 1) from those treated at 60 °C (groups 2 and 3). At 25 °C, neither pressure nor holding time appeared to affect the volatile profile of milk, as all treatments at 25 °C were grouped closely together (group 1). However, when temperature was increased to 60 °C, pressure became an important factor separating samples treated at 620 MPa (group 3) from those treated at 586 and 482 MPa (group 2) at the same temperature. The results demonstrated that pressure had an impact on the volatile generation of milk but only at moderately high temperature (60 °C). Samples treated at 60 °C and 620 MPa were the only ones clearly scattered due to holding time (group 3), therefore suggesting that time has an important effect only at high levels of pressure and temperature.

To understand how the pressure and temperature will affect the volatile formation in milk, the spatial distribution of all HPP-treated and non-HPP-treated milk samples was plotted in **Figure 3**. Milk treated under milder conditions, either with pressure below 620 MPa or with temperature below 60 °C, formed one cluster (group 1), indicating that the milk volatile profile did not change significantly under these conditions. Heat-treated samples at 60 °C (group 2) and 80 °C (group 4) formed clusters clearly separated from group 1. On the other hand, those treated at 60 °C and 620 MPa formed a different cluster (group 3). It was interesting to note that the samples in group 3 were clustered in the opposite side of their heat-treated homologues of group 2 in the PCA plot. The PCA loadings for the compounds (**Table 3**) showed that the major changes in group 3 (620 MPa, 60 °C) were mostly defined by an increase in concentrations of H_2S, hexanal, heptanal, and nonanal, which were different from their corresponding heat-only treatments (group 2), where the greatest increases were for H_2S, MeSH, 2-nonanone, nonanal, 2-undecanone, and 2-heptanone.

Figure 4 compares the volatile profiles of milk samples under all pressure, temperature, and time treatments with two com-

mercial pasteurized and two commercial UHT samples. Although these commercially processed samples were not obtained from the same source of raw milk used for the experiments, they could provide a relative comparison between the high-pressure treatments and commercial thermal processing. Pasteurized samples were located inside a major cluster (**Figure 4**, group 1) that included the milder treatments of heating (60 °C and lower) and all pressurization runs except those at the highest pressure and temperature combinations (620 MPa and 60 °C). From this PCA plot (**Figure 4**), in addition to previous studies on off-flavor compounds in heated milk (*8, 9*), it could be inferred that all of the samples in group 1 have aroma profiles similar to that of pasteurized milk. Samples located in groups 2 and 3 were those submitted to the highest levels of heat and pressure treatments and could present aroma profiles different from those of samples in group 1. UHT milk samples (group 4) were located far from any other cluster in the PCA chart, because they had the highest concentration of volatile compounds and could impart a "cooked" off-flavor note, yet further sensory analysis is needed to prove this prediction.

In summary, high-pressure processing at low temperature causes minimum change of the volatile composition of milk. However, under extreme pressure and temperature conditions, volatile compound formation is different from that under atmospheric pressure conditions. Heat treatment at high temperature promotes the formation of both aldehydes and methyl ketones, whereas high pressure at high temperature favors the formation of aldehydes. The formation of sulfur compounds was also different under high pressure. Further determinations of the kinetic behavior of these compounds may help us to understand their formation under high hydrostatic pressure.

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