JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Impacts on Volatile Formation in High-Pressure-Processed Milk

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The effect of antioxidants on volatile formation in milk under high pressure was investigated. Raw milk samples with addition of either butylated hydroxyanisole (BHA), epicatechin, ascorbic acid, β-carotene, or L-cystine were pressurized under 655 MPa at 75 °C for 3, 5, and 10 min. Formation of selected volatile compounds including aldehydes, ketones, and sulfur compounds was studied using headspace solid-phase microextraction and gas chromatography. BHA and epicatechin effectively inhibited the aldehyde formation. Ascorbic acid and β-carotene also inhibited aldehyde formation but to a much lower extent. L-Cystine was capable of inhibiting aldehyde and hydrogen sulfide formation. In general, the inhibition of volatile formation was proportional to the concentration of the added antioxidants. Reducing oxygen contents in milk also decreased aldehyde formation. Results suggested that the inhibition of volatile formation under high pressure could be similar to that under normal-pressure condition.

KEYWORDS: High-pressure processing; volatile; antioxidant; SPME; milk; aldehyde; sulfur

INTRODUCTION

Thermal processing is the most commonly used technique to achieve milk safety and shelf life; however, it can change the flavor profile depending on the severity of the thermal treatment. Thermally pasteurized milk (common conditions being 72 °C for 15 s) has a reasonably good flavor, but the shelf life is only 20 days under refrigerated storage. Ultra high temperature (UHT) processing (135–150 °C for 3–5 s) produces a product that is shelf-stable for up to 6 months; however, this extreme heat treatment can induce strong "cooked" off-aroma notes (*1, 2*) and limits its acceptance in the United States and many other countries.

High hydrostatic pressure processing (HPP) has been proposed as an alternative technology to extend the shelf life of milk (*3, 4*) without compromising its "fresh" flavor. Pressure treatments of 400 MPa for 15 min or 500 MPa for 3 min at room temperature have been demonstrated to achieve microbiological reduction and shelf life similar to those of pasteurized milk (*5*). When processed at 55 °C and 586 MPa for 5 min, HPP can significantly extend the shelf life of milk (*6*).

It is known that high-pressure processing might induce chemical and physical changes in milk (*7–9*). At room temperature, HPP has a very limited impact on the volatile profile of milk. However, at higher temperature (60°), HPP generates a volatile profile different from that under normal atmospheric pressure (*10*). Heat treatment of milk under normal atmospheric conditions promotes mostly the formation of methyl ketones, nonanal, hydrogen sulfide, and methanethiol (*1, 10, 12*), whereas heat treatments under high pressure favor the formation of hexanal, heptanal, and hydrogen sulfide. In addition, high pressure seems to inhibit the formation of methanethiol and has no impact on the formation of methyl ketone (*10*).

Kinetic studies on the formation of off-flavor compounds in milk under high pressure demonstrated that the formation of straight-chain aldehydes follows a first-order reaction, with rate increases with the applied pressure and temperature (*11*). Hexanal formation has the lowest activation energy (E_a), which decreases with pressure. Activation volume change (ΔV^*) for straight-chain aldehydes is negative, suggesting that an increase in pressure would increase aldehyde formation. In addition, ΔV^* for straight-chain aldehydes decreases in absolute value with temperature, suggesting that the formation of aldehydes would be less sensitive to pressure at higher temperatures. The kinetic study also demonstrates that the formation of hydrogen sulfide follows a zero-order reaction and that its formation is affected by pressure, whereas the formation of methanethiol is inhibited by pressure and the formation of methyl ketone is not affected by pressure (*11*), although the formation of these compounds has been reported to increase with temperature under normal atmospheric pressure (*12–18*).

Inhibition of volatile off-flavor formation in heated milk under normal pressure has been extensively investigated. Antioxidants such as butylated hydoxyanisole (BHA) are widely used to prevent lipid oxidation. L-Cystine has been studied in UHT milk to minimize the formation of H_2S (*19*) and lower the "cooked" off-flavor note (*19, 20*). In addition, thiosulfonates and sulfocysteinates have been studied, and both have been demonstrated to have an inhibitory effect on "cooked" off-flavor formation (*21*). More recently, epicatechin has been used to inhibit the

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10.1021/jf0716174 CCC: $37.00
Published on Web 10/10/2007

Table 1. Effect of O_2 Content on Volatile Concentration (Micrograms per Kilogram) in Milk Treated under 655 MPa at 75 °C[a]

	raw milk (9.33 ± 0.06 mg/kg O_2)				degassed milk (3.23 ± 0.06 mg/kg O_2)			
compound	0 min	3 min	5 min	10 min	0 min	3 min	5 min	10 min
hexanal	1.1a	29c	74[e]	139g	1.2[a]	4.8b	57[d]	100f
heptanal	0.08a	4.4b	13d	30f	0.11a	0.15a	9.6c	24e
octanal	0.10a	2.5c	7.7e	15g	0.16a	1.0b	4.2d	11f
nonanal	1.7a	11c	27e	42g	1.9a	7.1b	22d	35f
decanal	4.1a	8.8b	11c	16d	3.9a	3.7a	8.9b	12c
2-heptanone	0.96a	3.9c	4.0c	4.3c	1.1a	3.2b	3.2b	4.0c
2-octanone	1.5a	1.3a	1.7b	1.8b	1.5a	1.8b	1.7b	1.4a
2-nonanone	0.21a	1.9c	1.8c	1.8c	0.18a	1.4b	1.5bc	1.1b
2-decanone	0.20a	1.8c	1.8bc	1.9c	0.17a	1.4b	2.0c	1.7bc
H_2S	2.2a	18c	21c	22c	2.4a	11b	10b	10b
MeSH	5.5a	6.9b	7.2b	7.8b	5.9a	5.9a	4.9a	5.8a
DMS	6.4c	5.0b	5.0b	5.7bc	5.8bc	3.8a	4.6b	4.1ab
DMDS[b]	28ab	29ab	32b	32b	25a	32b	33b	30ab
DMTS[b]	43a	77cd	72cd	87d	55b	73c	68c	69c

[a] Different letters for the same row denote significant difference (Tukey HSD 95%); reported values are the average of a triplicate. Relative standard deviation for the quantification < 10%. [b] Concentration units are ng/kg.

formation of Maillard reaction products in UHT milk, and a decrease in cooked off-flavor was observed (*1*, *22*). The objective of this study is to investigate the effect of antioxidants on volatile formation in milk under HPP conditions.

MATERIALS AND METHODS

Chemicals. L-Cystine was purchased from Alfa Aesar Co. (Ward Hill, MA); β-carotene was from TCI-EP Ltd. (Tokyo, Japan); BHA, epicatechin, hexanal, and ascorbic acid were from Sigma-Aldrich Inc. (St. Louis, MO). Heptanal, octanal, nonanal, decanal, *trans*-2-hexenal, 2-heptanone, 2-nonanone, 2-undecanone, 3-heptanone, 3-octanone, 4-decanone, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and isopropyl disulfide (IPDS) were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI); 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); dimethyl sulfide (DMS) and ethyl methyl sulfide (EtMeS) were from TCI America (Portland, OR).

Methanethiol (MeSH) stock solution (34.1 g/kg) was prepared by bubbling MeSH gas into frozen methanol. The stock solution was diluted with cold methanol to the desired concentration as described previously (*14*). The solution was kept at −17 °C and used within 4 days. All glassware used for the MeSH solution was deactivated by soaking it in a 5% dimethyldichlorosilane solution in toluene and then rinsed with toluene, methanol, and finally water.

Hydrogen sulfide (H_2S) solution was prepared by dissolving 0.077 g of sodium sulfide ($Na_2S \cdot 9H_2O$, Sigma Inc.) in 10 g of 50 mM phosphate buffer (pH 8.5) followed by acidification to convert the salt to hydrogen sulfide (*14*). The solution was kept at 4 °C before use (no more than 1 week).

Milk Samples. Samples of raw homogenized milk with 3.2% fat were donated by a local dairy (Lochmead Farms, Junction City, OR). Sodium azide (0.02%) (Mallinckrodt Baker Inc., Paris, KY) was added immediately to stop microbial growth. Milk from the same batch was used through the entire experiment to minimize compositional differences.

Milk with Reduced Oxygen. Five hundred milliliters of milk was placed into an Erlenmeyer flask and subjected to ultrasound at 50 Hz for 1 h using a model B32H Branson Ultrasonic Cleaner (Branson Cleaning Equipment Co., Shelton, CT). The flask was connected to a vacuum during ultrasound treatment. Dissolved oxygen content was measured in triplicate before and after ultrasound–vacuum treatments using a dissolved oxygen meter (model 95, YSI Inc., Yellow Springs, OH).

High-Pressure Treatments. Antioxidants were added individually to raw milk at two different concentrations: BHA was added at concentrations of 20 and 90 mg/kg and epicatechin at 0.1 and 1.0 g/kg; ascorbic acid was added at 0.5 and 1.0 g/kg, β-carotene at 0.1 and 1 g/kg, and L-cystine at 30 and 40 mg/kg (**Table 1**). Samples (20 g) containing the respective antioxidants were placed in sealed polyethylene bags and then subjected to high-pressure treatments. 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 apply the high pressure to milk. Samples were equilibrated at 25 °C before entering the high-pressure vessel. Loading time (1 min) and unloading time (1.5 min) were kept constant for all runs. The pressure ramp was 60 s on average. Samples were treated at 655 MPa and 75 °C for 3, 5, and 10 min. Each treatment was run in triplicate. Immediately after pressure treatment, samples were cooled in an ice bath and then stored at −38 °C until analyzed.

Volatile Analysis. Hexanal, heptanal, octanal, nonanal, decanal, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and dimethyl sulfide were analyzed using headspace solid-phase microextraction and gas chromatography with flame ionization detection (HS-GC/FID) as described previously (*13*). Twenty grams of sample was extracted with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm, 50/30 μm film, Supelco Co., Bellefonte, PA) at 35 °C for 1 h. Volatile analysis was carried out using an HP 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a FID and a HP-5 capillary column (50 m × 0.32 mm i.d., 0.52 μm film thickness, Hewlett-Packard). Calibration curves were constructed in raw milk using the standard addition technique by spiking the standards in the range from 0.1 to 150 μg/kg. Five internal standards, *trans*-2-hexenal, 3-heptanone, 3-octanone, *trans*-2-nonenal, and 4-decanone, were used to quantify the volatile compounds.

Hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide were analyzed using a headspace solid-phase microextraction and gas chromatography with pulsed-flame photometric detection (HS-SPME/GC-PFPD) technique previously developed (*14*). Ten grams of sample was extracted with a 1 cm, 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 pulsed-flame photometric detector (PFPD). Calibration for the selected sulfur-containing compounds was constructed in milk in the range from 2.6 to 60 μg/kg for hydrogen sulfide, methanethiol, and dimethyl sulfide and in the range from 2 to 80 ng/kg for dimethyl disulfide and dimethyl trisulfide by standard addition technique. Quantification was achieved using isopropyl disulfide and ethyl methyl sulfide as the internal standards.

Statistical Data Analysis. Statistical evaluations including linear regression and Tukey's Honest Significant Difference ($\alpha = 0.05\%$) were conducted using S-Plus 6.2 (Insightful Corp., Seattle, WA).

RESULTS AND DISCUSSION

Effect of Dissolved O_2 on Volatile Formation under High Pressure. Results showed that when high pressure (655 MPa)

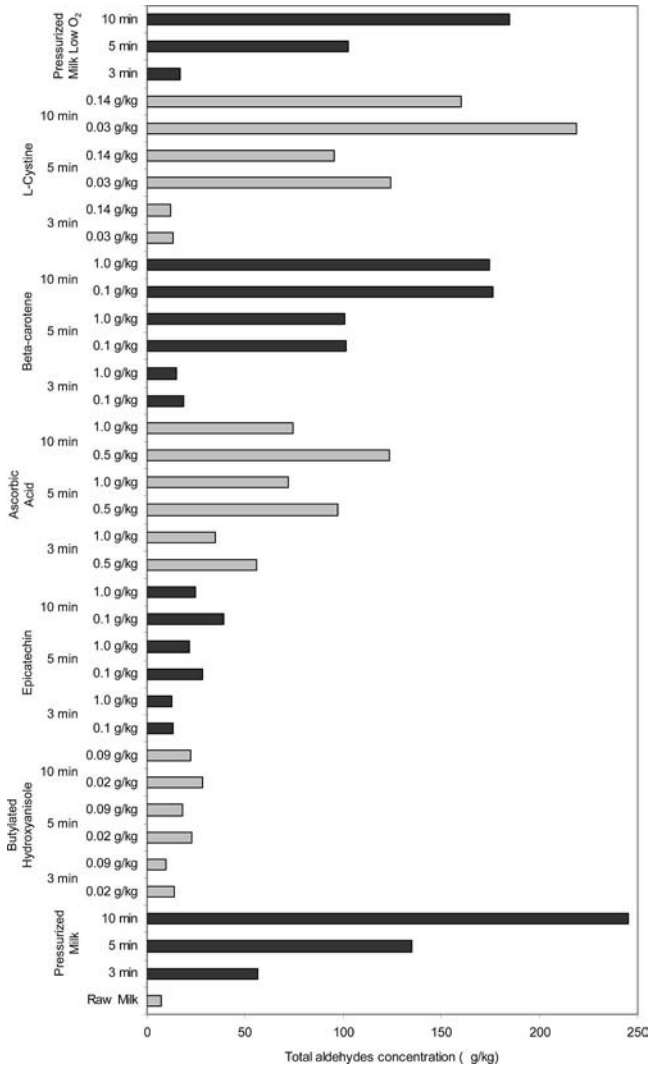

Figure 1. Effect of antioxidant on total concentration of aldehydes (hexanal, heptanal, octanal, nonanal, and decanal) (micrograms per kilogram) in milk treated under 655 MPa and 75 °C.

Table 2. Effect of Antioxidant on Aldehyde Concentration (Micrograms per Kilogram) in Raw Milk Treated under 655 MPa and 75 °C[a]

treatment			compound				
additive	concn (g/kg)	time (min)	hexanal	heptanal	octanal	nonanal	decanal
BHA	0.02	3	2.4b	0.09b	2.0d	4.1c	4.7b
BHA	0.09	3	1.5a	0.05a	0.66a	2.3a	4.8b
BHA	0.02	5	5.8e	0.55f	2.9f	6.8d	6.5cd
BHA	0.09	5	5.1e	0.25c	2.3de	4.3c	6.0c
BHA	0.02	10	6.6f	0.68g	3.2f	10f	7.8de
BHA	0.09	10	5.1e	0.28cd	2.1d	7.0de	7.7d
epicatechin	0.1	3	2.8c	0.18c	2.5e	3.3b	4.8b
epicatechin	1.0	3	2.2b	0.12b	2.0d	3.2b	4.8b
epicatechin	0.1	5	5.3e	0.64g	2.3de	11f	9.3ef
epicatechin	1.0	5	4.0d	0.32d	1.6c	7.5e	8.0de
epicatechin	0.1	10	6.7f	1.0i	4.3g	13g	14gh
epicatechin	1.0	10	5.7e	0.43e	0.87b	5.7d	13g
ascorbic acid	0.5	3	29i	4.5k	2.7ef	11f	8.9e
ascorbic acid	1.0	3	13h	2.3j	3.4f	8.0e	8.6de
ascorbic acid	0.5	5	54k	10no	4.0g	17h	11fg
ascorbic acid	1.0	5	38j	6.0l	3.0f	14g	11f
ascorbic acid	0.5	10	72l	11o	6.1h	19hi	15h
ascorbic acid	1.0	10	34ij	6.8lm	3.3f	15gh	14gh
β-carotene	0.1	3	6.5f	0.82h	1.1b	4.1c	6.0c
β-carotene	1.0	3	7.8g	1.0i	0.96b	2.1a	3.3a
β-carotene	0.1	5	52k	8.1m	6.8h	24ij	10f
β-carotene	1.0	5	54k	9.3mn	6.7h	21i	9.0e
β-carotene	0.1	10	91m	22p	12j	38kl	13gh
β-carotene	1.0	10	93m	24pq	12j	33k	12g
L-cystine	0.03	3	5.5e	0.54f	0.62a	3.1b	3.2a
L-cystine	0.14	3	4.4d	0.64g	0.56a	3.2b	3.5a
L-cystine	0.03	5	69l	11no	6.6h	26j	11f
L-cystine	0.14	5	50k	8.1m	5.8h	25ij	6.5cd
L-cystine	0.03	10	129n	25q	12j	42l	11f
L-cystine	0.14	10	84m	21p	9.5i	34k	10f

[a] Different letters for the same column denote significant difference (Tukey HSD 95%); reported values are the average of a triplicate. Relative standard deviation for the quantification < 10%.

was applied to raw milk samples at 75 °C, the concentrations of aldehydes increased dramatically (**Table 1** and **Figure 1**). Hexanal, heptanal, octanal, and nonanal were preferentially formed, reaching concentrations of 140, 31, 16, and 42 μg/kg, respectively, at 10 min treatment. This was consistent with our previous studies that aldehyde formation was dramatically promoted by high pressure (*10, 11*). The formation of aldehydes follows first-order kinetics with rate constants increasing with pressure and temperature (*11*).

The concentrations of methyl ketones in pressure-treated milk experienced some increases over the untreated raw milk, but generally the increases were much less than the aldehydes and were not affected by holding time (**Table 1**). This result does not contradict our previous studies that high pressure does not affect methyl ketone formation (*10, 11*) because the concentration increase observed in this study is probably just caused by temperature effect.

The effect of high pressure on volatile sulfur compounds is complex. Previous studies (*10, 11*) have demonstrated that high pressure enhances the formation of H_2S, inhibits the formation of MeSH, and has little effect on other sulfur compounds. The enhancing effect of high pressure on H_2S formation was further demonstrated in this study, with a 10-fold concentration increase after 10 min of treatment at 655 MPa at 75 °C. DMTS, and to lesser extents MeSH and DMDS, showed a slight increase in concentration after high-pressure treatment (**Table 1**). Similarly, this slight increase may be attributed to heat treatment of the samples before and after each HPP run, and not to the pressure treatment itself.

It was postulated that the enhanced formation of aldehyde under high pressure could be related to the content of dissolved oxygen. Thus, degassed milk samples were prepared with ultrasound and vacuum. Degassing noticeably reduced the formation of aldehydes under HPP, and the degree of reduction was dependent upon the compound and the pressurization time (**Table 1**). These results suggested that aldehyde formation under HPP was dependent on oxygen availability. Although the exact mechanism for the accelerated aldehyde formation is not known, it has been previously proposed that oxygen will be distributed in a micelle/water system and that the partition coefficient in the aqueous phase and in the micelle phase at equilibrium will be affected by pressure (*23*). Under high hydrostatic pressure, dissolved oxygen could migrate from the aqueous solution to the inside of the fat globule, which will increase the availability of oxygen in the fat phase, leading to an increase in lipid oxidation.

Oxygen reduction had no impact on the concentrations of methyl ketones under high pressure. Both aldehydes and ketones

Table 3. Effect of Antioxidant on Methyl Ketone Concentration (Micrograms per Kilogram) in Milk Treated under 655 MPa and 75 °C[a]

treatment			compound			
additive	concn (g/kg)	time (min)	2-heptanone	2-octanone	2-nonanone	2-decanone
BHA	0.02	3	4.3c	1.5ab	2.3cd	1.6b
BHA	0.09	3	3.7ab	1.1a	2.1c	1.4ab
BHA	0.02	5	3.6ab	1.8bc	2.6d	2.2c
BHA	0.09	5	3.3a	1.8bc	2.5cd	1.8bc
BHA	0.02	10	4.2c	2.1c	2.0c	1.7bc
BHA	0.09	10	4.2c	2.2c	2.4cd	1.9c
epicatechin	0.1	3	3.0a	1.8bc	1.7bc	1.1a
epicatechin	1.0	3	3.9bc	1.3ab	1.7bc	1.1a
epicatechin	0.1	5	3.0a	1.9bc	1.9cd	2.2c
epicatechin	1.0	5	3.2a	1.6ab	1.5bc	1.6b
epicatechin	0.1	10	3.9bc	2.1c	2.4cd	1.9c
epicatechin	1.0	10	4.8c	2.1c	2.0c	2.1c
ascorbic acid	0.5	3	3.3a	1.2a	1.6bc	1.7bc
ascorbic acid	1.0	3	3.3a	1.6ab	1.4ab	2.0c
ascorbic acid	0.5	5	3.9bc	2.0bc	1.7bc	1.7bc
ascorbic acid	1.0	5	4.1bc	2.1c	2.1c	1.4ab
ascorbic acid	0.5	10	3.5ab	1.7b	2.3cd	1.8bc
ascorbic acid	1.0	10	4.5c	1.9b	2.1cd	2.2c
β-carotene	0.1	3	3.8b	1.6b	1.8c	1.0a
β-carotene	1.0	3	3.3ab	1.4ab	1.3ab	1.4ab
β-carotene	0.1	5	3.9b	1.3ab	1.4bc	1.7bc
β-carotene	1.0	5	3.2a	1.5ab	1.7bc	1.8bc
β-carotene	0.1	10	4.5c	2.0bc	1.4bc	2.1c
β-carotene	1.0	10	4.4c	1.4ab	1.8c	1.9c
L-cystine	0.03	3	2.8a	1.8bc	0.9a	1.4ab
L-cystine	0.14	3	3.6b	1.4ab	1.3ab	1.5b
L-cystine	0.03	5	3.1a	1.9bc	1.7bc	2.0c
L-cystine	0.14	5	3.4ab	1.1a	1.7bc	2.2c
L-cystine	0.03	10	4.9c	1.8bc	1.1a	2.1c
L-cystine	0.14	10	4.1b	2.0c	2.0c	1.6b

[a] Different letters for the same column denote significant difference (Tukey HSD 95%); reported values are the average of a triplicate. Relative standard deviation for the quantification < 10%.

Table 4. Effect of Antioxidant on Sulfur Compound Concentration in Milk Treated under 655 MPa and 75 °C[a]

treatment			compound				
additive	concn (g/kg)	time (min)	H_2S (μg/kg)	MeSH (μg/kg)	DMS (μg/kg)	DMDA (ng/kg)	DMTS (ng/kg)
BHA	0.02	3	26c	6.0ab	5.1ab	24a	73ab
BHA	0.09	3	19c	6.6b	4.5a	24a	77ab
BHA	0.02	5	23c	7.1b	5.2ab	36b	75ab
BHA	0.09	5	25c	7.1b	5.0ab	26a	77ab
BHA	0.02	10	22c	6.5b	5.5ab	30ab	81b
BHA	0.09	10	22c	6.8b	4.9ab	28ab	78ab
epicatechin	0.1	3	20c	6.7b	5.2ab	27ab	79ab
epicatechin	1.0	3	21c	7.6b	5.7ab	22a	78ab
epicatechin	0.1	5	19c	6.9b	6.0b	28ab	75ab
epicatechin	1.0	5	20c	7.2b	5.5ab	25a	81b
epicatechin	0.1	10	22c	6.7n	5.5ab	29ab	78ab
epicatechin	1.0	10	23c	6.9b	5.0ab	27ab	76ab
ascorbic acid	0.5	3	23c	5.9ab	5.0ab	30ab	65a
ascorbic acid	1.0	3	21c	6.5b	5.3ab	29ab	74ab
ascorbic acid	0.5	5	20c	7.0b	5.3ab	31b	80ab
ascorbic acid	1.0	5	23c	6.9b	4.9ab	34b	84b
ascorbic acid	0.5	10	23c	7.1b	5.0ab	30ab	79ab
ascorbic acid	1.0	10	21c	7.5b	5.4ab	34b	78ab
β-carotene	0.1	3	22c	6.1ab	5.3ab	30ab	71ab
β-carotene	1.0	3	23c	7.5b	5.2ab	28ab	73ab
β-carotene	0.1	5	20c	6.0ab	6.1b	31ab	77ab
β-carotene	1.0	5	23c	6.4b	6.0b	33b	69ab
β-carotene	0.1	10	21c	6.8b	5.2ab	28ab	81b
β-carotene	1.0	10	23c	7.1b	5.2ab	32b	77ab
L-cystine	0.03	3	5.0a	6.0ab	5.5ab	25a	65ab
L-cystine	0.14	3	4.8a	5.4a	5.3ab	29ab	62a
L-cystine	0.03	5	6.7b	5.2a	6.1b	29ab	75ab
L-cystine	0.14	5	5.6a	6.2b	5.5ab	30ab	67ab
L-cystine	0.03	10	6.1ab	5.9ab	6.0b	33b	63a
L-cystine	0.14	10	5.2a	5.9ab	5.4ab	29ab	67ab

[a] Different letters for the same column denote significant difference (Tukey HSD 95%); reported values are the average of a triplicate. Relative standard deviation for the quantification < 10%.

are generated from lipid oxidation (*24*); however, the reaction pathways that lead to ketones are different from those leading to aldehydes. Methyl ketones are generated from the β-oxidation of saturated fatty acids, followed by decarboxylation (*25*). They can also be formed by decarboxylation of β-ketoacids naturally present in milk fat mediated by heat (*24*, *26*), whereas aldehydes are formed from the spontaneous decomposition of hydroperoxides of unsaturated fatty acids that yields an aldehyde and another radical (*24*). The spontaneous self-catalytic nature of the aldehyde formation pathway could be favored under high pressure by making more oxygen available for hydroperoxide formation.

Reducing the concentrations of oxygen prior to HPP treatment also decreased the formation of H_2S and barely reduced the formation of MeSH and DMS, whereas it had no significant impact on the formation of DMDS and DMTS. H_2S was proposed to be mainly generated from cysteine oxidation (*27*, *28*). MeSH and DMS are thought to be mainly liberated from methionine through thermal breakdown of the sulfur-bearing side chain (*21*, *28*). It has been proposed that DMDS and DMTS are probably formed from the oxidation of MeSH (*14*, *21*).

Effect of Antioxidants on Lipid-Derived Volatile Formation under High Pressure. Antioxidants can be added to foods to prevent the autoxidation of lipids and therefore minimize off-flavor formation. Several commonly used antioxidants were investigated in this study, and they showed different degrees of inhibition for aldehyde formation under high pressure (**Table 2** and **Figure 1**). BHA showed the highest inhibition on the formation of aldehydes, and the inhibition effect increased with the concentration of BHA. However, the inhibition effect generally decreased with pressurization time, even at the highest level of antioxidant concentration (**Table 2**). This observation suggested that increasing pressurization time could quench the antioxidant capability of BHA. BHA is a synthetic phenolic that works as a primary antioxidant. It accepts a free radical, converting it into a more stable, nonradical product during initiation and propagation steps. The fact that BHA can effectively inhibit the formation of aldehydes in pressurized milk indicated that the formation of aldehydes under high pressure followed free radical reaction pathways.

Epicatechin had the second most effective inhibitory effect on aldehyde formation (**Table 2** and **Figure 1**). Similar to BHA, hexanal inhibition was achieved for epicatechin, but the concentration needed was much higher (0.1 and 1 g/kg). Even at those high concentrations, epicatechin was much less efficient in inhibiting the formation of heptanal, octanal, nonanal, and decanal than BHA, especially at longer pressurization time. Epicatechin works as a primary antioxidant to prevent lipid

oxidation. It has been proved that epicatechin can effectively reduce the development of stale off-flavor in skim milk powder during drying and storage (*22*). Addition of epicatechin to milk prior to ultrahigh-temperature processing also significantly reduces the production of Maillard off-flavor compounds and lowers the cooked flavor intensity (*29*). It has been proposed that epicatechin inhibits off-flavor development in heated milk by quenching reactive compounds and forming a more stable dimer (*30*).

Ascorbic acid also inhibited aldehyde formation under high pressure (**Table 2**) but it was much less effective than BHA and epicatechin. Increasing the concentration of ascorbic acid improved its effectiveness in inhibiting the formation of hexanal, heptanal, and octanal. However, the effect of increased concentration was much smaller for nonanal and decanal. Similar to BHA and epicatechin, pressurization time lowered the antioxidant effect of ascorbic acid. Ascorbic acid prevents oxidation by scavenging oxygen, therefore slowing hydroperoxide formation (*31*). It can also scavenge free radicals and chelate free metal ions to prevent oxidation.

β-Carotene had a much lower inhibitory effect on aldehyde formation (**Table 2**). Increasing 10 times the concentration of β-carotene had very limited impact on aldehyde concentration, and the inhibitory effect of β-carotene was lowered by increasing the pressurization holding time. β-Carotene is a fat-soluble compound that can quench singlet oxygen in the system (*31*). β-Carotene absorbs energy from singlet oxygen to prevent the initiation of autoxidation of lipids. When singlet oxygen is not present in the system, β-carotene can scavenge free radicals. Carotenoids are unstable, and their antioxidant properties can be destroyed by heat (*31*).

L-Cystine was added to inhibit volatile sulfur formation. It was interesting to observe that L-cystine caused a reduction in aldehyde concentration (**Table 2** and **Figure 1**), which was proportional to the concentration of L-cystine, especially for the 5 and 10 min pressure treatments. It is possible that heat will decompose L-cystine to L-cysteine residues (*28*), which in turn have antioxidant activity mediated by the sulfhydryl group (*31*).

Compared with control samples treated under the same pressure and time (**Table 1**), addition of antioxidants had little effect on methyl ketone formation (**Table 3**). Previous studies have demonstrated that the formation of methyl ketone is not promoted by high pressure (*10*, *11*). Because methyl ketones can be formed from decomposition of β-ketoacids naturally present in milk by heat (*24*, *26*) and this formation pathway does not involve the formation of hydroperoxides or oxygen attack, antioxidants will not affect their formation under high pressure. Overall, antioxidants used in this study had no effect on the concentration of methyl ketones under high-pressure treatments, and although a slight reduction was noted in a few cases, they were not found to be meaningful and probably are within the natural variability of the assay.

Inhibition of Volatile Sulfur Off-Flavor Compounds under High Pressure. As expected, the addition of antioxidants had no impact on the formation of volatile sulfur compounds under high pressure. The formation of H_2S under high pressure was dramatically inhibited by L-cystine (**Table 4**), and the inhibition was related to the concentration of L-cystine. It is interesting to observe that the inhibition was effective even at prolonged pressurization holding time. Formation of MeSH and DMTS was slightly reduced with L-cystine addition; however, this reduction was not obvious and probably not significant. L-Cystine had no effect on the formation of DMS and DMDS under high pressure. Methanethiol, hydrogen sulfide, and dimethyl sulfide are the most important sulfur compounds contributing to the cooked off-flavor in heated milk (*13*, *14*). Under high-pressure treatment, the formation of methanethiol is greatly inhibited, whereas H_2S formation appears to remain similar to that found with heating under atmospheric pressure conditions (*10*). Addition of cystine can further inhibit the formation of H_2S under high pressure.

In summary, antioxidants can inhibit the formation of some important volatile off-flavor compounds in milk under high pressure. BHA and epicatechin were particularly effective in inhibiting the formation of aldehydes, whereas L-cystine was effective against the formation of H_2S. With the addition of antioxidants, the levels of volatile off-flavor compounds analyzed in pressurized milk were similar to those in untreated raw milk, even after severe high-pressure treatment under 655 MPa at 75 °C for 10 min. The combination of high pressure, heat, and antioxidants could be used to develop a commercial product that is much more shelf-stable while possibly reducing or completely eliminating cooked off-flavor.

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Received for review June 1, 2007. Revised manuscript received August 25, 2007. Accepted September 1, 2007.

JF0716174