

## Effects of Added Copper on Concentrations of Volatile Materials Produced in Milk

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### ABSTRACT

Analyzing the steam distillate from milk enabled measurement of increases in concentrations of n-hexanal, n-pentanal, propanal, acetaldehyde in raw, laboratory-pasteurized, and plant-pasteurized-homogenized milk exposed to 0 and 5 ppm added copper. Greatest changes were in the copper-treated laboratory-pasteurized milk and the least in Vacu-Therm pasteurized-homogenized milk. The 2-thiobarbituric acid values (TBA) in the exposed milk, laboratory pasteurized, .090; raw, .078; plant pasteurized, .056, reflected changes in volatile materials. Acetaldehyde concentrations increased about the same in whole pasteurized-homogenized milk as in skimmilk exposed to 1 or 2 ppm copper. The other carbonyl compounds increased consistently in whole milk, but little or none in skimmilk. Increases in concentration of the carbonyl compounds (other than acetaldehyde, acetone, and butanone) produced in pasteurized-homogenized milk exposed to 4 ppm copper for 44 h were reduced by adding 150 mg of ascorbic acid per liter of milk. However, the concentrations were greater in ascorbic acid-copper-treated milk than in milk not treated with copper. Adding the steam distillate from copper-oxidized milk produced the typical copper-oxidized flavor in good-flavored milk.

Oxidized flavors in milk and other dairy products have been studied extensively (5, 12). Though many carbonyl compounds have been isolated from oxidized dairy products (12), there is no clear agreement on which compound (or compounds) is responsible for the early off-flavor in oxidized fluid milk. Forss et al. (6) isolated and identified acetone, acetaldehyde, n-hexanal, crotonaldehyde, and series of C<sub>4</sub>-C<sub>11</sub> 2-enals and C<sub>6</sub>-C<sub>11</sub> 2,4-dienal from skimmilk oxidized with 5 ppm copper and 0.05 ml of 30% H<sub>2</sub>O<sub>2</sub>/ml and held 3 days. They found that 2-octenal, 2-nonenal, 2,4-heptadienal, and 2,4-nonadienal resembled the cardboard flavor in skimmilk at 10<sup>-7</sup>—10<sup>-9</sup> dilutions. Day and Lillard (3), working with oxidized milk fat, concluded that a wide spectrum of n-alkanals, alk-2-enals, alk-2,4-dienals, and alkan-2-ones was necessary to impart the oxidized flavor. Stark and Forss (11), who isolated and identified oct-1-en-3-one in oxidized milk fat, reported that it was responsible for the metallic flavor in dairy products. They also reported that when the compound was added to skimmilk, graders criticized the milk as "cardboard and oxidized." Day et al. (4) stated that although that compound produced a metallic flavor, it was not the oxidized flavor. Hammond and Seals (7) attempted to simulate the oxidized-flavor defect in milk by adding 1 or 10 ppb of oct-1-en-3-one and/or octanal to homogenized

milk. They revealed that frequent criticisms among 53 judges of homogenized milk (in nine laboratories) to which the compounds had been added were: "typical oxidized," "metallic," and "cardboard flavor." Those same chemical compounds, however, also educed criticisms of "atypical" and of flavors other than oxidized, metallic, or cardboard much more frequently than did copper-induced-oxidized milk samples.

Interpreting early research on off-flavor compounds in oxidized products is difficult in part because then there were inadequate quantitative measurements. Much of the early research dealt with extensive oxidation or long storage periods of dried products, changes that may or may not apply directly to the "typical" flavor that occurs in oxidized fluid milk. The development of a simple method to measure minute (ppb) changes in volatile materials in milk (2) enabled me to study early changes in copper-induced oxidized flavor.

### EXPERIMENTAL PROCEDURE

#### *Measuring volatile materials in milk*

Steam distillation and GLC analysis of the distillate were used to measure quantitatively the volatile materials in milk. Standard deviations of 10% of the mean value or less were obtained when four series of replicates of normal and 50 ppb spiked milk samples were analyzed for some volatile materials by this method (2). GLC peak components were identified by subtractive techniques (1). Aldehydes were distinguished from ketones by treating distillates with one drop of saturated potassium permanganate before the GLC procedure to oxidize and eliminate the aldehyde peaks. This is a modification of a procedure reported by Hoff and Feit (8).

#### *Changes in concentrations of volatile materials in raw, laboratory-pasteurized, and commercial Vacu-Therm pasteurized-homogenized milk exposed to copper*

A portion of raw milk collected at the surge tank of a DeLaval Vacu-Therm pasteurizer was laboratory pasteurized at 63 C for 30 min with gentle agitation in an Erlenmeyer flask. Shortly after the raw milk was collected, a sample of the Vacu-Therm pasteurized-homogenized milk (76.6 C/15 sec) was collected at the outlet pipe from the pasteurizer. Copper sulfate was added to aliquots of each of these milks to give a concentration of 5 ppm copper. A portion of each milk was untreated; it served as a control. All milk samples were stored in a refrigerator at 5 C for 23 h, then analyzed for volatile materials as described. The 2-thiobarbituric acid reaction values (TBA) were determined on the samples by King's method (9).

#### *Volatile materials in commercial skimmilk and pasteurized-homogenized whole milk were compared after exposure to added copper*

Samples of packaged skimmilk and whole, homogenized-pasteurized milk from the same milk supply (processed on the same day) were taken

from the University Dairy milk cooler. Aliquots from each carton were spiked with 0, 1, and 2 ppm copper as copper sulfate and stored at 5 C. These samples were analyzed for volatile materials, as described, at 16 and 45 h.

*Effects of added ascorbic acid on volatile materials produced when copper was added to pasteurized-homogenized milk*

Samples were from freshly pasteurized-homogenized, packaged milk from the University Dairy plant: control milk (no treatment); milk with 150 mg of ascorbic acid per liter; milk with 4 ppm added copper; and milk with 150 mg of ascorbic acid and 4 ppm added copper. These milks were analyzed for volatile materials at 20 and 44 h.

*Organoleptic study of milk prepared by adding distillates from oxidized and control milk to good-quality milk*

Pasteurized-homogenized milk exposed to 5 ppm copper for 45 h was distilled as described. The 5-ml distillates thus obtained were reconstituted into 45-ml aliquots of untreated homogenized milk and compared with the copper-oxidized milk and with the control milk diluted with distilled water (5 ml to 45 ml milk). Distillates from control milks also were blended with untreated milk (5 ml to 45 ml) and compared with all of the other samples. These milk samples were analyzed organoleptically by two experienced milk judges.

## RESULTS AND DISCUSSION

*Effects of some processing conditions on copper-induced production of volatile materials*

Table 1 shows the concentration of volatile materials

TABLE 1. Changes in concentrations of some constituents of raw, laboratory-pasteurized, and plant pasteurized-homogenized milk exposed to 0 and 5 ppm copper for 23 h

Treatment	Raw milk	Lab past. <sup>a</sup>	Plant past. <sup>b</sup>
	<i>Acetaldehyde (ppb)</i>		
Control	8.0	4.8	4.8
5 ppm Cu <sup>c</sup>	15.2	17.0	11.5
	<i>Methyl sulfide (ppb)</i>		
Control	10.2	7.1	7.8
5 ppm Cu	8.3	7.9	6.2
	<i>Propanal (ppb)</i>		
Control	2.7	2.4	2.4
5 ppm Cu	17.0	19.8	7.1
	<i>Acetone (ppb)</i>		
Control	385.5	306.5	295.5
5 ppm Cu	344.5	365.5	301.8
	<i>Butanone (ppb)</i>		
Control	60.0	47.8	47.1
5 ppm Cu	53.2	53.6	50.1
	<i>n-Pentanal (ppb)</i>		
Control	4.9	3.9	4.1
5 ppm Cu	96.0	114.7	48.5
	<i>n-Hexanal (ppb)</i>		
Control	4.9	3.9	4.1
5 ppm Cu	452.3	541.7	165.2
	<i>TBA values</i>		
Control	.019	.019	.013
5 ppm Cu	.078	.090	.056

<sup>a</sup>63 C for 30 min.

<sup>b</sup>Vacu-Therm, HTST pasteurized-homogenized.

<sup>c</sup>Held 23 h at 5 C.

in raw, laboratory-pasteurized, and plant-pasteurized-homogenized milk exposed to 5 ppm copper for 23 h. The concentrations of each of the chemical compounds in the control milks did not differ appreciably (see raw, laboratory-pasteurized, or plant-pasteurized control milks, Table 1). n-Hexanal increased in concentration more than any other component in milk exposed to copper. Relatively large increases also were observed for n-pentanal, propanal, and acetaldehyde in milk exposed

to copper. Concentrations of chemical compounds differed little in either copper-treated raw or laboratory-pasteurized milk. However, concentrations of all carbonyl compounds were lower in the copper-treated, plant-pasteurized sample, perhaps because the Vacu-Therm treatment of the milk, reduced the dissolved oxygen, before experimental treatment. Methyl sulfide did not change appreciably from exposure to copper. Several additional small peaks were not identified but these did not change with the treatments.

TBA values of milks exposed to different processing conditions and held 23 h without added copper were low, indicating no oxidation. The relatively lower TBA value for the plant-pasteurized sample than for the other samples exposed to copper generally paralleled the lower concentrations of volatile materials.

*Volatile materials in skimmilk and whole-homogenized milk exposed to added copper*

Table 2 shows the changes in concentration of the

TABLE 2. Changes in concentrations of some compounds in commercial skim and whole pasteurized-homogenized milk with 1 and 2 ppm copper added

Exposure time (h)	Whole milk <sup>a</sup> added copper			Skimmilk <sup>b</sup> added copper		
	0	1 ppm	2 ppm	0	1 ppm	2 ppm
	<i>Acetaldehyde (ppb)</i>					
16	5.3	6.1	7.1	5.3	5.6	6.7
45	7.7	8.2	10.3	5.1	6.7	8.0
	<i>Methyl sulfide (ppb)</i>					
16	6.1	6.4	6.8	6.1	5.8	6.6
45	7.6	7.8	6.9	6.4	6.1	6.3
	<i>Propanal (ppb)</i>					
16	2.7	3.2	3.1	2.4	2.5	2.5
45	2.9	3.7	4.3	2.7	2.7	2.7
	<i>Acetone (ppb)</i>					
16	327.1	322.1	324.1	333.2	298.0	289.1
45	343.7	354.1	361.8	331.8	305.2	304.0
	<i>Butanone (ppb)</i>					
16	49.2	63.0	46.7	48.0	52.6	48.8
45	50.9	50.9	48.8	48.4	49.0	51.6
	<i>n-Pentanal (ppb)</i>					
16	6.0	9.6	8.9	-0-	-0-	-0-
45	11.0	19.7	25.4	-0-	-0-	-0-
	<i>n-Hexanal (ppb)</i>					
16	16.0	31.0	31.6	8.7	11.3	12.9
45	19.6	38.9	54.0	11.3	11.3	13.4

<sup>a</sup>Vacu-Therm, HTST pasteurized-homogenized.

<sup>b</sup>Commercial skimmilk with 0.14% fat and fortified with 1% NFDM solids.

volatile materials in commercial pasteurized skimmilk and pasteurized, whole, homogenized milk with 0, 1, and 2 ppm copper added. The consistency with which acetaldehyde increased with added copper in either skim or whole milk strongly indicated that the nonfat component of milk was the precursor of this compound. Increases in concentrations of the other carbonyl compounds were definite and consistent in the whole milk but slight or nonexistent in the skimmilk. From these data, I concur with the generally accepted view that lipids are precursors of these carbonyl compounds in copper-induced, oxidized milk. No precise pattern could be discerned for methyl sulfide in this study.

TABLE 3. Changes in concentrations of some volatile materials in pasteurized-homogenized milk with and without added copper and ascorbic acid

Identified component <sup>a</sup>	Control		Ascorbic acid <sup>b</sup>		Added Cu <sup>c</sup>		Cu + ascorbic acid <sup>d</sup>	
	20 h	44 h	20 h	44 h	20 h	44 h	20 h	44 h
	(ppb)							
Acetaldehyde	7.5	7.7	7.4	9.0	10.3	15.4	8.0	15.1
Methyl sulfide	7.2	9.4	7.2	9.6	7.2	10.0	7.2	8.8
Propanal	2.9	3.1	2.8	3.1	3.8	5.4	3.0	3.6
Acetone	267.6	279.8	259.4	315.0	288.6	317.4	270.8	313.4
Butanone	65.8	65.6	62.5	74.4	70.0	74.7	64.4	73.4
n-Pentanal	21.1	21.1	17.5	21.8	29.0	44.9	25.4	31.9
n-Hexanal	32.1	31.6	32.6	35.8	61.2	104.4	54.5	68.5

<sup>a</sup>Identification based upon subtractive techniques and agreement of retention times.

<sup>b</sup>Added 150 mg of ascorbic acid per liter of milk.

<sup>c</sup>Added 4 ppm Cu as CuSO<sub>4</sub>.

<sup>d</sup>Added 4 ppm Cu and 150 mg per liter of ascorbic acid.

#### Ascorbic acid and added copper as related to volatile materials produced in milk

The changes in concentration of the volatile materials in milk exposed to 4 ppm copper for 20 and 44 h with and without 150 mg ascorbic acid per liter are shown in Table 3. Obviously, ascorbic acid affected oxidative changes; the antioxidant effect, however, was relatively small. Though its effect was apparent at 20 h, ascorbic acid after 44 h seemingly inhibited the production of the lipid-related volatile compounds more than did the nonlipid-related acetaldehyde.

The carbonyl compounds that I measured have been identified in other studies of oxidized dairy products. Comparable increases in acetaldehyde in copper-exposed skim milk and whole milk indicate that a nonfat component is its precursor. Propanal is the classical dismutation product of one of the linolenic acid hydroperoxides. The mode of n-pentanal formation in homogenized milk is not readily apparent, however, it appears to originate from the milk lipids. Skim milk did not contain n-pentanal and none developed when it was exposed to copper. Hexanal is one of the dismutation products of the linoleic acid hydroperoxides and has been recovered consistently from oxidized fats. The quantitative measurements made in this study may be useful to those who may wish to further investigate organoleptic properties of oxidized milkfat. Similar measurements might be used to evaluate methods of controlling lipid oxidation.

#### Organoleptic properties of milk distillate-treated milks

Table 4 gives flavor scores and criticisms of coded milks prepared by mixing distillates from normal and copper-oxidized milk with untreated milk as compared with scores and criticisms of oxidized and control milks. Obviously, the distillates contain the oxidized flavor characteristics. The one judgment of "cooked" and "oxidized" for the control milk with distillate from the control milk may have reflected a carry-over judgment of a previous sample. Holding the refrigerated distillates 2 days eliminated the cooked flavor character.

A subsequent study in which the distillates of control and oxidized milks were added to good quality milk and compared with the control or oxidized milk confirmed

TABLE 4. Organoleptic properties of milks prepared by adding the distillates of control and oxidized milks (2) to good-quality milk

Milk samples <sup>a,b</sup>	Judge 1		Judge 2	
	Score	Criticism	Score	Criticism
Control milk <sup>c</sup>	38.0	Sl. cooked	39.0	Cooked
Control milk <sup>c</sup>	38.0	Sl. cooked	38.5	Cooked
Distillate from Control milk <sup>d</sup>	37.5	Cooked	38.5	Cooked
Distillate from control milk <sup>d</sup>	37.0	Cooked	35.0	Cooked/oxid.
Cu oxidized milk <sup>e</sup>	33.0	Oxid.	35.0	Oxid.
Cu oxidized milk <sup>e</sup>	32.0	Oxid.	34.0	Oxid.
Distillate from Cu-oxidized milk <sup>f</sup>	33.5	Oxid.	33.0	Oxid.
Distillate from Cu-oxidized milk <sup>f</sup>	33.0	Oxid.	34.0	Oxid.

<sup>a</sup>Coded milk samples presented to judges in random order.

<sup>b</sup>When distillates were added to control milks, each sample was prepared from a new distillation.

<sup>c</sup>45 ml of pasteurized-homogenized milk + 5 ml of distilled water.

<sup>d</sup>45 ml of pasteurized-homogenized milk + 5 ml of distillate from the control milk.

<sup>e</sup>45 ml of Cu-oxidized pasteurized-homogenized milk + 5 ml of distilled water.

<sup>f</sup>45 ml of pasteurized-homogenized milk + 5 ml of distillate from Cu-oxidized milk.

findings shown in Table 4 (10). In that study, 3 to 5 panelists with 80 observations criticized all milks as oxidized when distillates from 1 or 2 ppm copper oxidized milk was added to good milk. When only 0.1 ppm copper-oxidized milk distillates was added to good quality milk, 79% of the samples (28 observations) were criticized as being oxidized.

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