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Effect of Oxygen on Development of Off-Flavors in Ultrahigh-Temperature Milk[1]

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ABSTRACT

The role of dissolved oxygen as a contributor to flavor deterioration in sterile milk during storage was investigated. Before processing, a concentrated aqueous solution of Tenox-2 was added to half of a batch of pasteurized-homogenized milk to give a final concentration of 400 ppm BHA on a fat basis in the milk. The other half was untreated. Half of each of those batches was treated to reduce oxygen concentrations by a combination of nitrogen sweep and sonication. The remaining two samples (Tenox-2 added and no-Tenox-2) did not receive the deoxygenation treatment. Oxygen levels in the preprocessed-deoxygenated milk were lower (4.6 ppm) than those in the untreated milk (6.9 ppm). All four lots were UHT-sterilized at 135°C for 5 s in an indirect UHT system constructed at Kansas State University. Sterilized milk was collected aseptically in a glove box in 250-ml amber glass bottles, which were closed with either Teflon-lined caps or sterile cotton plugs. Samples from each treatment were stored at 7° and 32°C for 4 months. Samples in capped bottles maintained relatively low (<4 ppm) dissolved oxygen concentrations, whereas those in cotton-plugged bottles had relatively high (7-7.5 ppm) dissolved oxygen concentrations. Dissolved oxygen affected the rate of stale flavor development. Sterile milk in bottles with cotton plugs, which had relatively high concentrations of dissolved oxygen during storage, developed a stale flavor sooner and with greater intensity than milks with lower levels of oxygen. However, acetaldehyde, propanal, n-pentanal, and n-hexanal, which are most likely products of lipid oxidation, did not appear to be principal contributors to staling in sterile milk during storage in this study. Furthermore, the stale flavor development did not parallel changes in thiobarbituric acid (TBA) values. Although antioxidant (40 ppm BHA on fat basis from Tenox-2) did retard oxidation slightly, it did not control staling. A decrease in the concentration of several volatile materials throughout the storage period probably was caused by dissipation of the volatile material through the cotton plug or by their interaction with other compounds in the milk. Acid degree values increased in sterile milk at 32°C during prolonged storage, but changes in ADVs did not parallel development of the stale flavor.

Chemical and flavor changes resulting from the dissolved oxygen concentration in ultrahigh-temperature (UHT) milk have been investigated by many researchers (*1,3,5,11,13*). Thomas et al. (*11*) found that flavor acceptability was independent of the oxygen concentration after the first few weeks of storage when UHT milk was stored at 20 ± 4°C. Milks with an initially high oxygen concentration were preferred up to 8-13 d, but thereafter, the dissolved oxygen concentration did not influence flavor. An increase in acceptability during the first few days was related to a decrease in cabbage-like flavors and occurred more rapidly in the presence of a high dissolved-oxygen content. This was followed by an increase in stale and oxidized flavors, which developed independently of the initial oxygen concentration.

Mehta and Bassette (*8*) found that sterile milk stored in cartons more permeable to oxygen (polyethylene lined) developed a stale flavor faster than that stored in cartons less permeable to oxygen (aluminum foil-lined). Also, an increase in compounds associated with oxidized flavors (propanal, n-pentanal, and n-hexanal) paralleled the stale flavor development.

Jeon et al. (*5*) also reported that, as the concentration of carbonyl compounds increased in stored UHT-milk, the stale intensity increased and flavor scores decreased. They proposed that aldehydes were the most important compounds contributing to the off-flavors and that an increase in aldehydes was dependent on oxygen concentration and storage temperature.

The objective of our research was to investigate the role of dissolved oxygen as a contributor to flavor deterioration in sterile milk during storage. Whole pasteurized-homogenized milk was sterilized and subjected to a 4-month, shelf life study. The effects of oxygen, antioxidants, and storage temperature were examined.

MATERIALS AND METHODS

Experimental design

Ten gallons of pasteurized-homogenized milk from the Kansas State University dairy processing plant were divided into two equal portions. A concentrated aqueous solution of the anti-

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oxidant Tenox-2 (20% butylated hyroxyanisol (BHA), 6% propylgallate, 4% citrate and 70% propylene glycol) was added to one portion to give a final concentration of 400 ppm BHA on a fat basis. Each of these lots was subdivided further into two equal portions, one of which was deoxygenated by subjecting the milk at 30°C to vacuum (20 mm of Hg) and sonication, while sweeping it with nitrogen. A schematic chart of the treatments of the experimental milk is shown in Fig. 1 and a diagram of the deoxygenation apparatus in Fig. 2.

A hose was connected from the sonicating flask to the sample tank of a pilot-sized, indirect UHT system (*12*), and milk was forced under slight pressure from the flask into the reservoir tank of the UHT system. Each of the four lots of milk previously described was sterilized at 135°C for 5 s. After sterilization, milk was collected aseptically in a glove box in 250-ml amber colored bottles. Half of the bottles from each lot were closed with Teflon-lined caps and half with sterile cotton plugs. After each lot was processed, half of the capped and half of the cotton-plugged bottles were placed in a 32°C chamber. The remaining bottles were stored at refrigeration temperature (4°C). Figure 1 shows schematically the various sample treatments.

Samples of each of the four lots of preprocessed milk and the 16 samples of processed milk were analyzed for volatile materials, concentration of BHA, dissolved oxygen, thiobarbituric acid (TBA), acid degree values (ADV), and flavor 1 d after processing and at weeks 1, 2, 4, 8, 12, and 16. Total aerobic and anaerobic counts were determined for the milk the day after processing and at weeks 4 and 16.

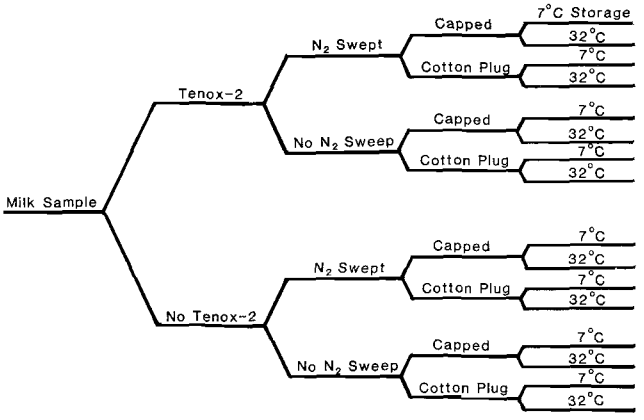

Figure 1. *Schematic diagram of treatments of experimental milk, including the preprocessing treatments of adding antioxidant (Tenox-2) and deoxygenation, as well as post sterilization bottle closure (capped or cotton plug) and storage temperatures.*

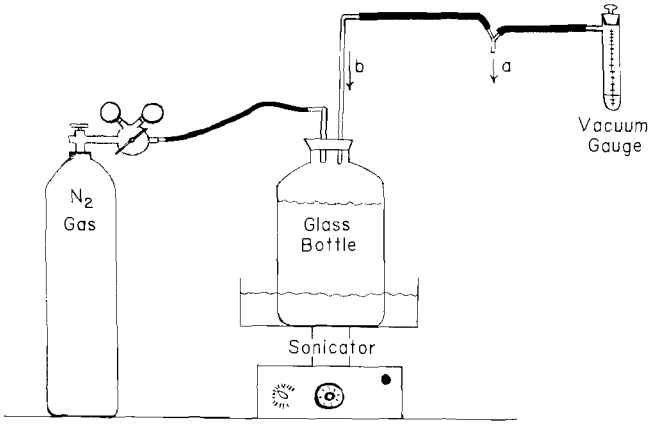

Figure 2. *Diagram of the deoxygenation apparatus.*

Analytical procedures

Microbial. All milk samples, except the preprocessed milks, were incubated 1 week at 32°C before plating to create sufficient growth so that any viable cells or cells from germinating spores present in the milk could be detected. Samples were plated in duplicate with dilutions of 10^0 and 10^{-1} for total aerobic counts on Plate Count Agar (Difco). Duplicate 10^0 dilutions were plated on anaerobic agar (Difco) and incubated in anaerobic jars for total anaerobic count. All plates were incubated at 37°C for 48 h and inspected for microbial growth.

Gas chromatographic analysis of sterile milk samples. Concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal in milk samples, as well as peak heights of other volatile materials were determined by the head space gas sampling and gas chromatographic methods of Bassette and Ward (*2*). Changes in these volatile materials were analyzed on an Aerograph Model 550-B gas chromatograph equipped with a Brown-Honeywell recorder. One milliliter of head space gas was drawn off the prepared sample and injected onto a 304.8 × 0.318-cm stainless steel column packed with 20% Carbowax 20M on 80/100 mesh Chromosorb P solid support. All analyses were duplicated and concentrations of the aldehydes were calculated from regression equations of standard curves of each aldehyde.

Analysis of BHA in milk. A modification of the steam distillation procedure of Bassette and Ward (*2*) was used to determine the concentration of BHA from Tenox-2 in milk. Fifty milliliters of milk and one drop of GE Antifoam 66 (100% active silicone defoamer) were placed in a digestion-distillation flask and steam-distilled with a Kemmerer-Hallet type micro-Kjeldahl nitrogen distillation apparatus. Twenty-five milliliters of distillate were collected in a marked tube in an ice-water bath. Approximately 10 min were required for the distillation. The system was cleaned before and after each trial with boiled pre-distilled water for 5 min. Two milliliters of carbon disulfide (Fisher Scientific) was added to the distillate to extract BHA. The mixture was shaken vigorously 100 times with the cap held on firmly and then was allowed to stand at room temperature until the BHA-containing carbon disulfide layer separated from the aqueous layer. The carbon disulfide layer at the bottom of the tube was drawn off with a 9-in. Pasteur pipette, placed in a closed vial and analyzed on a HP5880A GC using a flame ionization detector. A 1-μl sample was injected onto a 2 ft × 1/4 in. column of 0.2% OV-101 on 100/120 Chromosorb W/HP support. The GC signal was monitored by a HP Level 4 data system. Concentrations of BHA were derived from a regression equation of the standard curve of BHA in whole milk (3.3% fat) on a fat basis.

Measuring dissolved oxygen in milk. The dissolved oxygen concentration of each sample was measured by an oxygen-permeable membrane electrode with a YSI monitor Model 53 (Yellow Springs Instruments, Yellow Springs, OH) at 25°C. Four milliliters of milk was placed into the sample chamber and stirred at a constant rate by a magnetic stir bar. A chart recorder (Linear 1200) was used to measure oxygen levels (nmolar) and these levels were converted to ppm dissolved oxygen.

Thiobarbituric acid test (TBA). Lipid oxidation was measured by the method of King (*6*).

Acid degree values (ADV). The method of Thomas et al. (*10*)

was used to determine ADVs as a measure of rancidity.

Organoleptic analysis of milk flavor. All milk samples were evaluated organoleptically by a 4-member experienced taste panel on the day before and day after processing; then at 1, 2, 4, 8, 12, and 16 weeks of storage. A stale reference sample was available at each session for panelists to refresh their memory of the stale flavor. Samples were warmed to 24°C before taste evaluation. Each panelist was asked to evaluate the samples for any cooked, stale, and/or oxidized flavor on a scale of 1 (none) to 4 (pronounced). Also, panelists were asked to assign an overall flavor score to each sample using a hedonic scale ranging from 1 (dislike very much) to 7 (like very much) and encouraged to add any comments.

RESULTS AND DISCUSSION

Sterility and BHA

Total aerobic and anaerobic plate counts showed that milk remained sterile during the 4-month study. Within each treatment (at a specific week), samples with added antioxidant (Tenox-2) had approximately the concentration of BHA initially added and samples without added antioxidant were devoid of BHA. Antioxidant concentration did not change appreciably from week-to-week.

Dissolved oxygen

Although the samples receiving the deoxygenation treatment had lower dissolved oxygen concentrations than samples without deoxygenation before processing and on day 1, after 1 week of storage, the dissolved oxygen concentration in these samples subsequently was not significantly different ($P>0.05$) except at week 12. *Therefore, both deoxygenated and untreated samples were collectively grouped as "capped" samples.* A decrease in the dissolved oxygen concentration at 1 week may have resulted from oxidation of sulfhydryl groups formed during processing (*10,13*). By the 4th week of storage, dissolved oxygen concentrations had increased slightly and remained relatively constant until the 12th week, when a decrease occurred in both the cotton-plugged and the capped samples. Milk in bottles plugged with sterile cotton plugs had significantly ($P>0.05$) higher concentration of dissolved oxygen at both storage temperatures than did milk in the capped bottles. Changes in dissolved oxygen during storage are shown in Fig. 3.

TBA values

There was little change in least square means (LSM) of TBA values until the 2nd week when the LSM values in cotton-plugged samples were significantly ($P>0.05$) higher than the others (0.028-0.024). At the 16th week, storage temperature, antioxidant concentration, and dissolved oxygen concentration influenced TBA values. Samples stored for 16 weeks at 32°C, samples without antioxidant, and samples with high dissolved oxygen concentrations (cotton plugged) had significantly ($P>0.05$) higher TBA values than other samples. Although no attempt was made to determine significant difference from week-to-week, there was a measurable increase in the TBA values after the 4th week, with a larger increase in samples stored at 32°C than in those at 7°C (Fig. 4). Only at 16 weeks in the 32°C-stored samples did the TBA values reach 0.029, suggesting slight oxidation.

The LSM for ADVs were not significantly different up to and including the 4th week, but thereafter LSM were

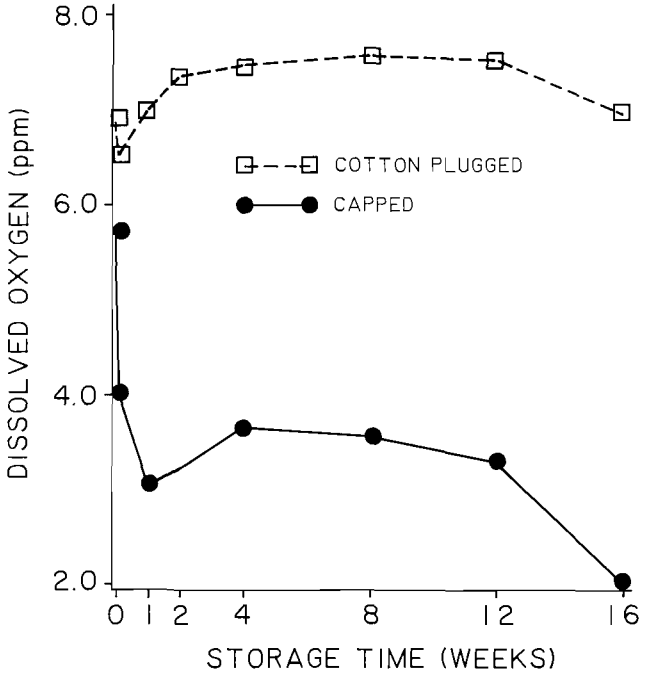

Figure 3. *Concentrations of dissolved oxygen in cotton-plugged and capped milk samples during storage.*

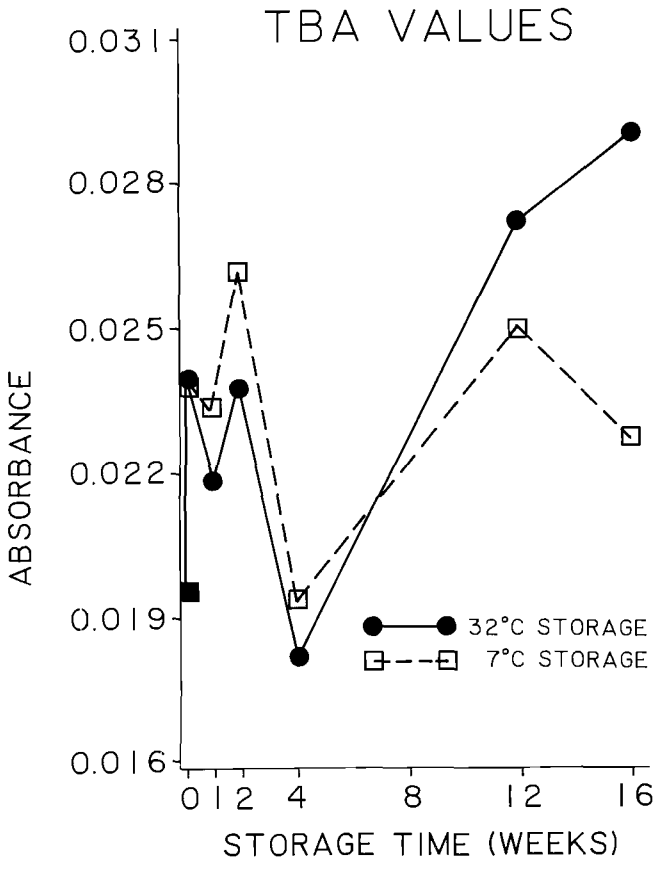

Figure 4. *TBA values of sterile milk samples stored at 32° and 7°C.*

significantly (P>0.05) lower in samples stored at 7°C than in those at 32°C. There was a greater increase in ADVs in samples stored at 32°C (Fig. 5). At the 12th week, the ADVs in samples stored at 7°C decreased, while the 32°C samples increased. Although samples stored at 32°C for 16 weeks reached ADVs of 1.4, they were not criticized for having a rancid flavor. Earley and Hansen (*18*) also found increases in ADVs with no detectable rancid flavor in the milk.

Volatile materials in the stored milk

Gas chromatographic analysis was used to analyze concentrations of some volatile materials in all experimental samples. Figure 6 illustrates changes in acetone throughout the storage period. Acetone in capped samples stored at 32°C gradually increased, whereas it remained relatively constant in samples stored at 7°C. Scanlan (*9*) and Kirk et al. (*7*) found acetone to be heat-induced. Capped samples had significantly (P>0.05) higher acetone LSM than the cotton-plugged samples, and those stored at 32°C had significantly (P>0.05) more than the 7°C capped samples only after the 12th week. Figure 6 illustrates a gradual decrease in acetone in the cotton-plugged samples. Samples stored at 32°C had significantly (P>0.05) less acetone than those stored at 7°C only after the 12th week, but they were consistently lower. The decrease of acetone in the cotton-plugged samples at both storage temperatures may have resulted from losses through the cotton plug or from being consumed in a reaction faster than it was being formed, thus showing greater losses at the higher storage temperatures.

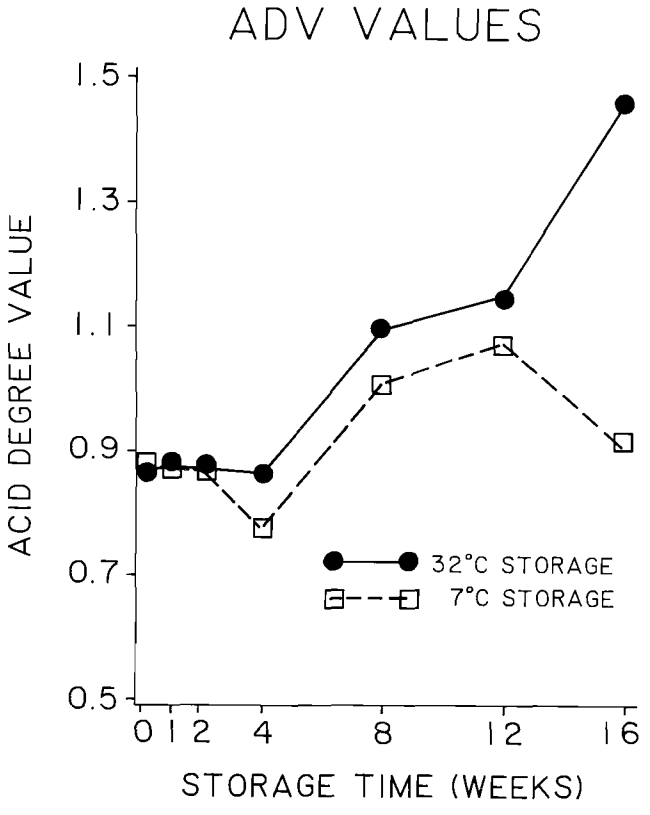

Figure 5. *Acid degree values (ADV) of sterile milk samples stored at 32° and 7°C.*

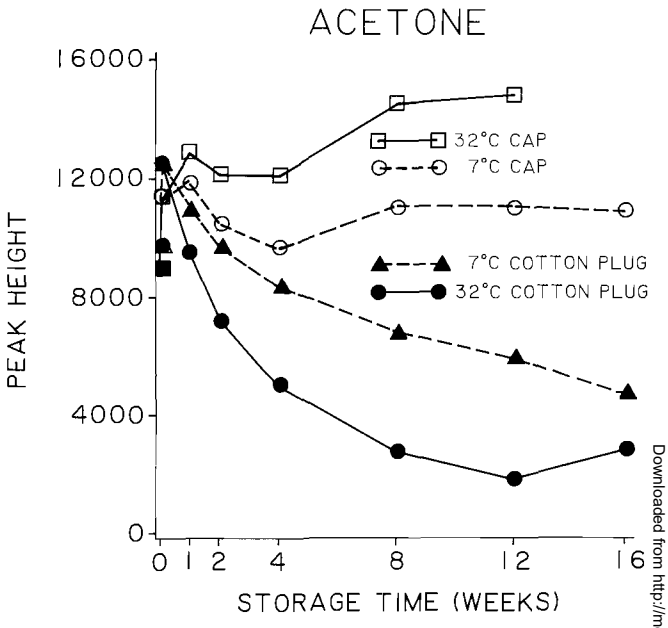

Figure 6. *Changes in concentration of acetone in sterile milk stored in capped or cotton-plugged bottles at either 32° or 7°C.*

Methyl sulfide increased in concentration in all samples the day after processing and then decreased with time. It was lost rapidly from the cotton-plugged samples with a more gradual loss from the capped samples. The cotton-plugged samples had a significantly (P>0.05) lower concentration of methyl sulfide than the capped samples after only 1 week of storage. Capped samples maintained a relatively high concentration even after 16 weeks of storage.

Similar patterns were observed for changes in concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal during storage. In general, less of each compound was formed at 7°C than at 32°C. Also, samples containing antioxidants produced slightly less of these volatile materials at both storage temperatures than did the counterpart samples without antioxidant. As was shown by the acetone (Fig. 6), volatile compounds decreased to lower concentrations in the cotton-plugged samples. Figure 7 shows similar increases in concentrations of these aldehydes in the cotton-plugged samples, and on the day after processing in samples with high dissolved oxygen contents. The high heat treatment may have contributed to formation of these reactive compounds, which in turn may be precursors of other compounds.

Concentrations of acetaldehyde were significantly (P>0.05) higher from 4-16 weeks in samples held at 32°C than in those at 7°C. Also, although not significant, LSM of acetaldehyde in samples containing the antioxidant was lower than samples without antioxidant. The cotton-plugged samples had significantly (P>0.05) lower concentrations of acetaldehyde at 4, 12, and 16 weeks. This was probably due to escape of the very volatile compound through the cotton plug. Propanal concentrations in the 32°C capped samples (no BHA) were consistently higher than in the other samples. Also, samples containing the antioxidant formed less propanal at each

storage temperature than did samples without antioxidant (Fig. 7).

Samples held at 32°C had significantly ($P>0.05$) more n-pentanal from the 4th-16th week than those stored at 7°C. Cotton-plugged samples had significantly lower concentrations than capped samples over the same period, with samples at 32°C lower than those at 7°C. Samples with added antioxidant again had lower concentrations of n-pentanal at both storage temperatures.

At 4 and 12 weeks, LSM of the n-hexanal concentration in samples without antioxidant were significantly ($P>0.05$) higher than those containing antioxidant and were consistently higher throughout the study. The 7°C cotton-plugged samples increased in hexanal throughout storage after a slight decline at weeks 1 and 2, while in the 32°C cotton-plugged samples, it decreased after an

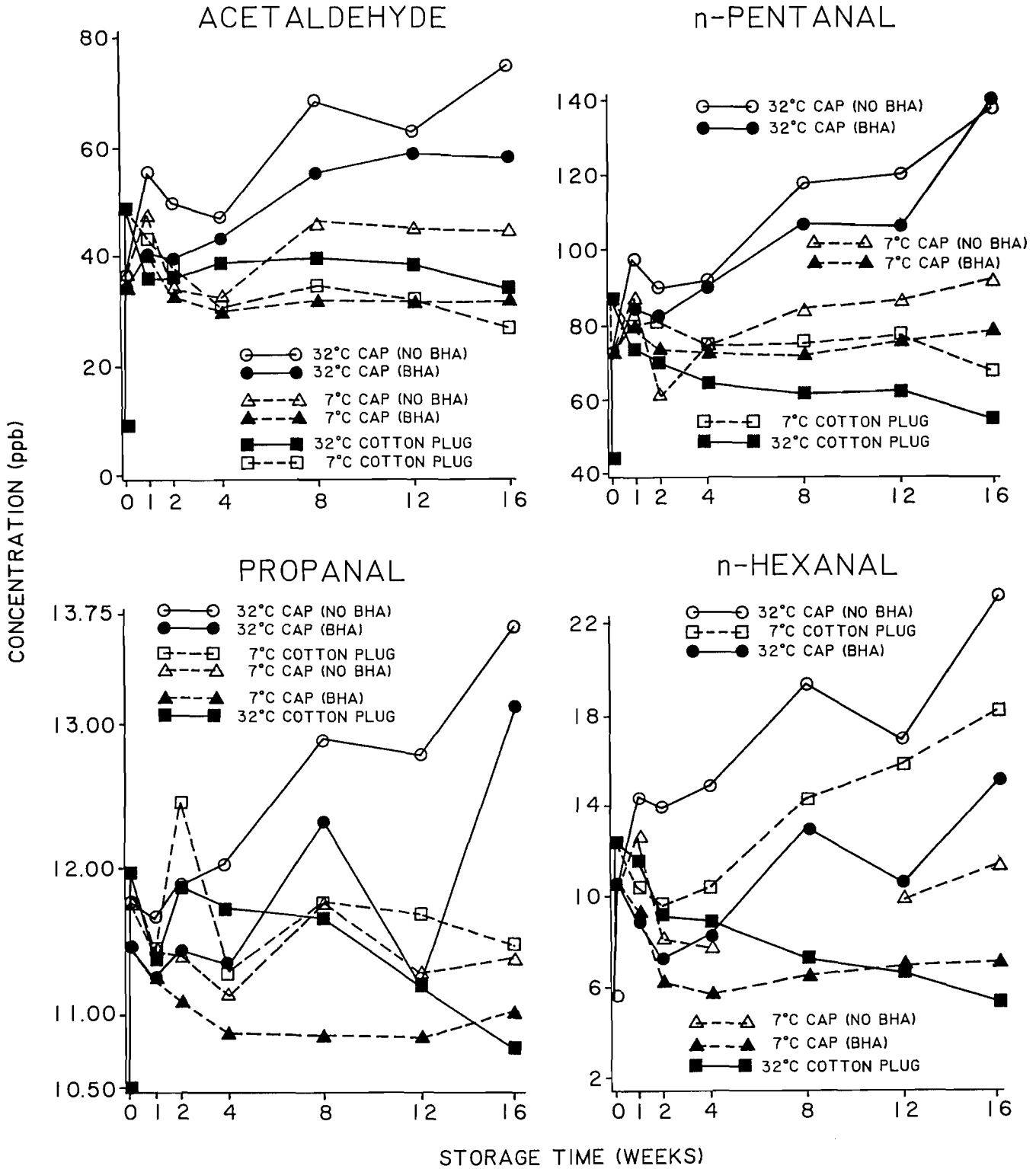

Figure 7. *Effect of BHA, temperature, and dissolved oxygen on concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal in sterile milk during storage.*

increase on day 1. This might be because the longer chain aldehydes are less volatile than shorter chain aldehydes, and at the lower storage temperature hexanal was retained in the milk while its formation continued; hence the concentration increased. However, at the higher storage temperature, hexanal may still be volatile enough to be lost through the cotton plug or consumed in other reactions.

Flavor changes during storage

The cooked flavor in whole milk increased after processing and then decreased upon storage. As expected, a similar pattern occurred in the concentration of methyl sulfide because it is one of the compounds that contributes to the cooked flavor (*4*). Initially, the cooked flavor decreased rapidly, but after 2 weeks it remained relatively constant. Samples stored at 32°C with cotton plugs received lower cooked-flavor scores and had lower methyl sulfide concentrations.

Storage temperature appears to be a major contributor to development of the stale flavor. Samples stored at 32°C were significantly ($P>0.05$) staler at 2-16 weeks than samples stored at 7°C. Figure 8 illustrates that samples stored at the higher temperature developed a more pronounced stale flavor as early as 4 weeks (cotton-plugged), whereas samples stored at 7°C were only slightly stale after 16 weeks of storage. A strong stale flavor developed early in the cotton-plugged samples stored at 32°C. Therefore, one would surmise that the concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal probably are not directly responsible for the stale flavor, because the 32°C cotton-plugged samples usually had low concentrations of these compounds in all treatments and yet quickly developed a stale flavor. However, the 32°C capped samples without antioxidant had the highest concentration of these compounds and developed approximately the same stale intensity 8 weeks later.

Flavor scores appear to be inversely related to overall stale flavor (Fig. 9) development. A decrease in flavor acceptability paralleled an increase in stale intensity (Fig. 8 and 9). At 32°C storage, flavor scores decreased proportionally to the increase in stale intensity. In samples stored at 7°C, the flavor decreased slightly but scores were still relatively high after 16 weeks of storage. The 7°C samples scored higher in acceptability and lower in stale intensity. At 4, 12 and 16 weeks, samples stored at 7°C had significantly higher scores than those stored at 32°C.

TBA values increased after the 4th week of storage, but stale flavor development did not parallel changes in TBA values. This suggests that products of lipid oxidation are not major contributors to the stale flavor. Although the antioxidant did retard lipid oxidation slightly, it did not control staling.

Acid Degree Values did increase in sterile milk stored at 32°C during the later stages of storage, but changes in ADVs did not parallel development of the stale flavor.

These data suggest that relatively high concentrations of dissolved oxygen and high storage temperature accelerate

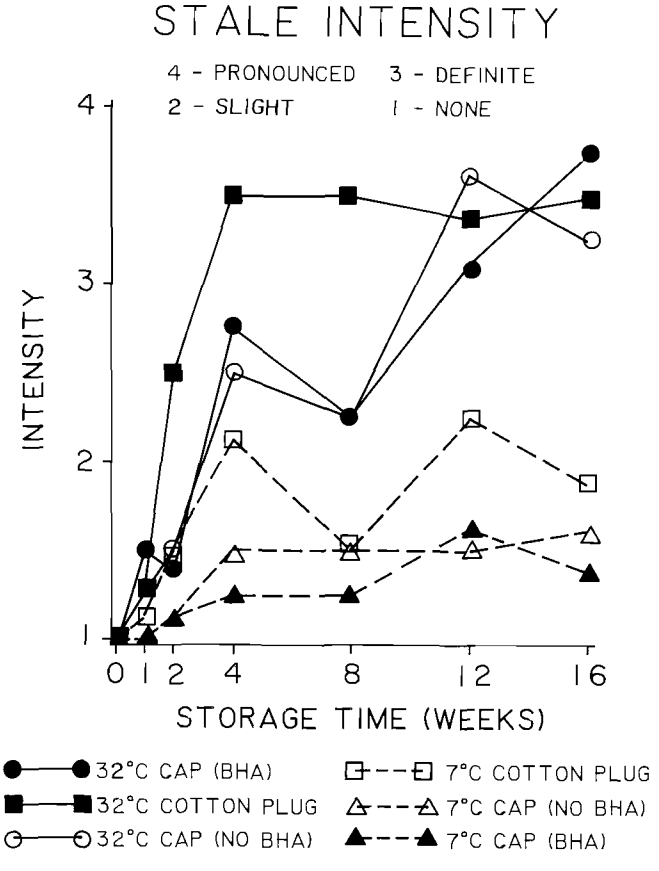

Figure 8. Effect of BHA, temperature, and dissolved oxygen on the development of staling in sterile milk during storage.

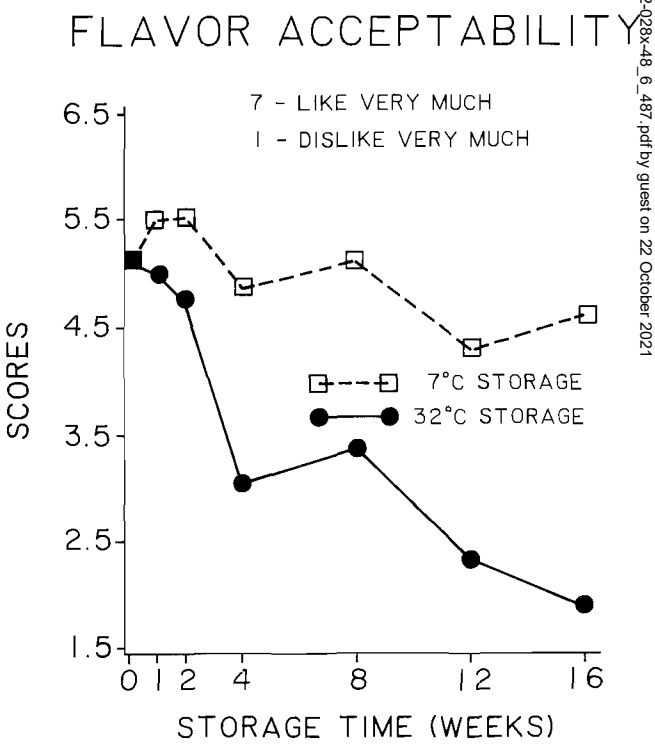

Figure 9. Effect of storage temperature and time on flavor scores of sterile milk.

ate staling in milk. Neither TBA values nor ADV correlated with stale intensity. Furthermore, changes in the concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal did not parallel either increases in stale intensity or changes in flavor scores. The antioxidant, Tenox-2, had only a minor effect on reducing the concentrations of carbonyl compounds or on influencing the flavor.

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