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Organoleptic, Chemical and Microbiological Changes in Ultra-High-Temperature Sterilized Milk Stored at Room Temperature[1]

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ABSTRACT

The effects of carton materials on flavor of ultra-high-temperature sterilized milks stored 100 days at 22 ± 2 C was investigated. Flavor scores of stored milk decreased concurrent with an increase in stale flavor. At the same time propanal, pentanal, hexanal, and an unidentified compound increased; cooked flavor along with methyl sulfide and another unidentified compound decreased. Differences in browning were observed between ultra-high-temperature sterilized and reference (freshly pasteurized) milks and between 2- and 12-day-old ultra-high-temperature sterilized milks. Thiobarbituric acid values did not increase (indicating no lipid oxidation) until after the milk had been stored 22 days; however, those values were below that which would be detected organoleptically. Abnormally high acetaldehyde concentrations were related to the ethylene oxide sterilizing pretreatment of the carton board. Reference milk was superior in flavor to milk from all other treatments. Aluminum foil-lined cartons were less permeable to gases than were polyethylene-lined cartons. Milk in aluminum foil-lined cartons retained desirable flavor characteristics longer than did that stored in polyethylene-lined cartons. Wrapping cartons with Saran and aluminum foil was detrimental to flavor in all instances. Analysis of variance of microbiological data established that there were no differences in numbers of microorganisms in the different types of cartons during 42 days.

Milk sterilization has been practiced and studied for more than a century. Widespread acceptance of sterile milk in the United States, however, is limited because during storage it develops a stale flavor as cooked flavor disappears. Oxygen (*7, 11*) and light (*7*), thought to contribute to staling, and many other aspects of ultra-high-temperature sterilized (UHT) milk have been reviewed (*5*).

We stored aseptically packaged UHT milks and determined how well different carton materials preserved the milk flavor. Also, by using gas chromatography (GC), we attempted to relate changes in neutral volatile components to off-flavors produced by microbiological and chemical activity.

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MATERIALS AND METHODS

Part I. Milk stored 42 days

Processing and storage Between October 1975 and February 1976, nine batches of raw milk were sterilized at a commercial milk plant in Connecticut at 140 C for 3.5 sec by use of a De Laval Auto-Aseptic Processing System that included two-stage homogenization at 52 C. The milk was packaged aseptically with a Pure-Pak NLL machine in either aluminum foil (AL)-lined or plain polyethylene (PE)-lined pint cartons. The AL foil-lined carton board was laminated (from inside out): PE, AL, PE, paperboard, and PE. The PE carton board was laminated PE, paperboard, and PE. Within 2 days the filled cartons were shipped by air to Manhattan, Kansas. On arrival, half of each type (PE and AL foil-lined) cartons was wrapped in Saran[TM], AL and Saran[TM] to provide additional barriers to gases and light; cartons were stored at 22 ± 2 C exposed to 2150 lx (200 ft candles) of cool-white fluorescent light. Each of these three layers overlapped to provide as tight a seal as possible. They were examined at 10-day intervals for 42 days. The code letters used to identify these samples were: WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NPC = plain polyethylene-lined cartons and R = reference milk.

Test procedures. All UHT milks were evaluated by three microbiological tests: (*a*) The Standard Plate Count procedure recommended by the American Public Health Association (*1*) was modified so that 10 ml of milk was distributed among five petri-dishes. After Standard Methods Agar had been poured into the dishes with the milk, the milk-agar mixture was incubated at 32 C for 7 days; colonies then were counted. (*b*) Aerobic and anaerobic tests (*1*) for sterile products were modified in that 10 ml of milk was incubated at 32 C for 7 days; 0.8 ml of this milk, distributed equally among four plates, was mixed with Standard Methods Agar. Two of the plates were incubated aerobically and two anaerobically for 48 h at 32 C; colonies then were counted. (*c*) The litmus test (*6*) was modified in that 10 ml of the milk sample was added to 1 ml of 1% (w/w) sterile aqueous litmus solution. The tubes were incubated at 32 C for 7 days. If the litmus turned pink or white, the test was positive and indicated microbial growth.

Browning was measured by the hydroxymethylfurfural test (*8*) and lipid oxidation by the thiobarbituric acid test (*9*). Neutral volatile compounds were quantified by using the gas chromatographic procedure of Bassette and Ward (*4*).

The milk was judged organoleptically according to the procedure used in the National Collegiate Student Contest. The recommended flavor-score ranges for the presence of slight, definite, and pronounced for the three predominant criticisms were 36, 34, and 31 for oxidized; 37, 34, and 31 for stale; and 39, 37, and 35 for cooked. As a modification of the contest procedures, we asked panelists also to assign an intensity score (on a scale of 0 = none to 9 = very pronounced) to the specific off-flavors observed.

Protein (by Kjeldahl), fat (Babcock procedure), and total solids were determined as described in AOAC (2).

Statistical analysis. The experimental design for the analysis of variance (ANOVA) was a randomized complete block design with a 2-factor cross-classified treatment structure; there were five levels for each of the two factors, days, and treatments (types of cartons). This model was used:

$Y_{bdt} = \mu + \beta_b + \gamma_t + (\beta\gamma)_{dt} + E_{bdt}$

Y_{bdt} = response (e.g., stale-flavor intensity, concentration of hexanal).

μ = mean

α_b = block effects (blocks 1-9)

β_d = day effects (2, 12, 22, 32, and 42 days)

γ_t = treatments effects (WAC = Wrapped AL; NAC = non wrapped AL; WPC = wrapped PE; NPC = nonwrapped PE; and R = reference milk, freshly pasteurized at 72 C for 15 sec)

$(\beta\gamma)_{dt}$ = interaction between days and treatments

E_{bdt} = random experimental error, as a deviation from the mean for the dt^{th} treatment combination of the b^{th} block. $E \sim N(0, \sigma^2)$ where σ^2 is estimated by error mean square.

From the 225 samples analyzed, 15 to 17 observations for most variables were lost because the cartons were not sterile; all results from non-sterile cartons of milk were discarded.

The model was used as presented when we analyzed data for all effects except the day effects (β_d); to analyze for β_d effects, we removed data for the R milks, which were freshly pasteurized and had not been stored 42 days.

After the data had been tested for homogeneity, we obtained regression equation relating the flavor criticisms (scores) to the components observed chromatographically. A program for multiple deletion regression (with a 0.10 alpha level) was used to select the most prominent associated factors.

Part II. Milks stored 100 days

Of the nine batches (blocks) used in Part I, the milks of the last six were evaluated again at 100 days. Microbiological, chemical, organoleptic and gas chromatographic analyses were conducted as described previously on milks, except that WPC samples were not included.

The experimental design for the ANOVA of the extended 100-day study was the same as for the 42-day study except for the following changes:

α_b = block effects (blocks 4-9)

β_d = day effects (2, 12, 22, 32, 42, and 100 days)

γ_t = treatment effects (when analyzing data at 100 days, we did not include WPC cartons because there were insufficient samples).

RESULTS

Part I. Milk stored 42 days

ANOVA of the microbiological data (Table 1) established that there were no differences in the number of microorganisms among the various treatments (four types of cartons) and among the days of storage (2-42). Results from litmus test of milk, not presented here, also agreed with results from other microbiological tests. The similarities were expected because obviously spoiled samples had been discarded and were not included in the statistical analysis. Even though statistically there was no difference among the selected cartons, the wrapped cartons (WAC and WPC) had higher average mean bacteria counts than did the nonwrapped.

Flavors (Table 2) deteriorated with time. Reference (R) milk flavor was best, followed by milk in AL and PE cartons. Milk in nonwrapped cartons had higher flavor scores than did that in the wrapped counterpart cartons. Flavor-score trends were similar to those for stale-intensity scores (Table 2), indicating that "stale" was the principal flavor criticism of the panel. Generally, as the stale flavor increased, the cooked flavor decreased (Table 2 and Fig. 3) for the UHT milks. We might have observed, however, a masking effect of the cooked flavor on the stale flavor and vice versa.

TABLE 1. *Probabilities and means for the microbiological analyses of UHT milk stored 42 days at room temperature.*

Item	Standard plate count	Aerobic count	Anaerobic count
		Probabilities	
Days	.2365	.7964	.3409
Treatments	.1566	.2182	.7319
Days		*Means*[1,2]	
2	-0.055	1.271	0.111
12	-0.061	0.928	0.481
22	0.238	1.315	0.584
32	-0.020	1.225	0.524
42	0.121	0.886	0.579
Treatments[3]		Means[1,4]	
WAC	0.296	1.208	0.692
NAC	-0.007	0.718	0.485
WPC	0.359	1.540	0.831
NPC	0.199	1.033	0.615

[1]Logarithmic values (base 10) of microbiological counts (colonies/ml) are used.

[2]Each mean represents the average of the four UHT milks over 9 replication.

[3]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, and NPC = plain polyethylene-lined cartons.

[4]Each mean represent the average of the milk over 9 replications during the 42-day trail period.

Statistical differences in the oxidized flavor intensities probably were artifacts because the mean intensities were less than 1 on a 0-to-9 scale. TBA values for UHT milk increased slightly when the milk was stored; however there was no significant difference between TBA values of UHT milks and reference milk.

Browning of milk increased slightly but significantly when stored between 2 and 12 days (Free HMF, Table 2) but none beyond 12 days. That, compared with low HMF levels in the R milk, indicated that some heat damage had occurred in the UHT milks. Total HMF, which measures potential as well as actual browning (*8*), did not change significantly during the 42-day period. Though HMF values of milk stored in AL foil and PE cartons differed, differences were not great enough to be significant. The R milks had significantly lower concentrations of these compounds than did the UHT milks.

Table 3 presents components isolated from steam distillates by use of GC. When concentrations or peak heights were compared with intensity scores of specific criticisms, certain relationships were observed. These are shown in the last column. Peaks designated with "x" were unrelated to either chemical or organoleptic tests studied. Statistical analyses of peaks found to be related to other factors are presented in Table 4.

The abnormally high initial concentrations of acetaldehyde were associated with an ethylene oxide-sterilizing treatment of empty milk cartons. That was confirmed by comparing acetaldehyde levels in milk from aseptically filled cartons that had been treated with ethylene oxide

TABLE 2. *Probabilities and separation of means for the chemical tests and organoleptic analyses of UHT milk stored 42 days at room temperature.*

Item	Organoleptic analyses [1]				Chemical tests		
	Flavor score	Stale flavor	Cooked flavor	Oxidized flavor	Free HMF	Total HMF	TBA
				Probabilities			
Days	.0000	.0000	.0000	.0000	.0000	.0730	.0021
Treatments	.0000	.0000	.0520	.0000	.0000	.0000	.7192
				Means[2,3]	(Micromoles HMF/liter)		(Absorbance 443 m)
Days							
2	35.62	1.26	4.73	0.58 a	0.60	4.31 a	.014 ab
12	35.00	2.69	3.49 a	0.37 ab	0.88 a	4.46 a	.016 abc
22	34.15 a	3.68	3.17 ab	0.14 b	1.00 a	4.75 a	.013 ab
32	33.92 ab	4.66 a	3.12 ab	0.17 b	0.97 a	4.73 a	.023 c
42	33.49 b	4.77 a	2.72 b	0.17 b	0.99 a	4.09 a	.021 bc
Treatments[4]				*Means*[2,5]			
WAC	34.90 a	2.97 a	3.93 b	0.30 b	0.86 a	4.26 a	.016 a
NAC	35.14 a	2.57 a	3.94 b	0.25 ab	0.82 a	4.30 a	.016 a
WPC	33.23	4.71	2.64 a	0.32 b	0.95 a	4.70 a	.018 a
NPC	34.50	3.47	3.28	0.27 ab	0.92 a	4.62 a	.019 a
R	38.04	0.03	2.37 a	0.11 a	0.57	2.09	.016 a

[1]Intensity scores 0-9; 0 = none to 9 = very pronounced.
[2]Means not significantly different at 1% level are joined with a common letter of the alphabet.
[3]Each mean represents the average of the four UHT milks over 9 replications.
[4]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NPC = plain polyethylene-lined cartons, and R = reference milk.
[5]Each mean represent the average of the milk over 9 replications during the 42-day trial period.

TABLE 3. *Components isolated by steam distillation of UHT milk and analyzed by gas liquid chromatography.*

	Retention[1] time	Name of compound	Associated with
	(min)		
X	1.7	not identified	—
	2.4	acetaldehyde	ethylene oxide
	3.1	methyl sulfide	cooked
	3.6	propanal	stale
X	4.0	acetone	—
X	4.8	acrolein	—
X	5.4	not identified	—
	9.5	pentanal	stale
X	11.0	not identified	—
	13.8	not identified	stale
	16.6	hexanal	stale
X	25.0	not identified	—
	28.0	not identified	stale

[1]An "X" by the peak retention time indicates that after statistical analysis peak could not be related to chemical or organoleptic changes.

either 24 h or one week before filling. For milk in cartons treated 24 h before filling, the acetaldehyde peak was extremely high; for that in one treated week before, the acetaldehyde peak was insignificant. Milk used to fill both types of cartons was from the same source. Methyl sulfide has been associated with cooked flavor (*10*) and so we observed. Since it decreased as the stale flavor increased, methyl sulfide could be a precursor of some other component that causes staling. Conceivably, however, methyl sulfide has a masking effect. Concentrations of propanal, pentanal, and hexanal increased with time and paralleled increased in intensities of stale flavors; the compounds also have been associated with light-induced and oxidized flavor (*3*). Because the 13.8-min GC peak decreased as the flavor intensity increased, this compound could be a precursor of staling. The compound responsible for the 28-min peak, like pentanal and hexanal, also might be associated with staling.

The mean protein, fat, and total solids contents of the milks in this study were 3.47, 3.50, and 11.80,[2] respectively, and their respective standard deviation were .13, .06, and .12.[2] These values show that the composition in the different blocks varied considerably, however, those differences should not have affected our results.

Part II. Milk stored 100 days

Trends we obseved for milk stored 42 days continued for microbiological, organoleptic, chemical, and GC evaluations of milks stored 100 days. Thus details of the statistical analyses are not presented; however, we plotted organoleptic changes to illustrate these trends (Fig. 1-3). Differences in flavor and stale-flavor intensity scores observed among cartons through 42 days were clearer at 100 days; the flavor indices changed almost linearly with time (Fig. 1, 2). Cooked intensity scores leveled off in the UHT milk stored 100 days.

DISCUSSION

This research indicates that AL foil lining is slightly permeable to gases, probably at the seams but also probably through microscopic pores in the AL foil lining as evidenced by the fact that wrapping AL-lined cartons reduced flavor scores.

However, plain PE-lined cartons were more permeable to gases than the aluminum foil-lined cartons. This is apparent from the more rapid loss of methyl sulfide, and cooked flavor as well as a greater increase in carbonyl compounds and stale flavor in the PE lined than foil lined cartons. These results concur with those of Flückiger (*7*) who compared changes in milk packaged in liter PE-lined cartons with and without foil lining. His aluminum foil-lined cartons did not lose weight during 6

[2]*The total solids means encompass values for only blocks 1-6.*

TABLE 4. *Probabilities and separation of means for the gas-liquid-chromatographic components of UHT milk associated with defects when the milk is stored 42 days at room temperature.*

Item	Component and/or retention time shown on the chromatogram: 2.4 min[1] acetaldehyde	3.1 min[1] methyl sulfide	3.6 min[1] propanal	9.5 min[1] pentanal	13.8 min[2]	16.6 min[1] hexanal	28.0 min[2]
				Probabilities			
Days	.0000	.0000	.0000	.0000	.1852	.0000	.0002
Treatments	.0143	.0000	.0000	.0000	.0000	.0000	.0000
Days					*Means*[3,4]		
2	5237	36.11	14.2 a	212 a	160 a	68.91	38 a
12	2088 a	29.10 a	15.6 ab	326 ab	151 a	71.80 a	52 ab
22	785 a	27.40 a	17.7 bc	464 bc	155 a	73.46 a	98 bc
32	914 a	27.30 a	19.6 c	427 bc	105 a	73.95 a	96 bc
42	933 a	29.11 a	19.0 c	517 c	113 a	74.03 a	109 c
Treatments[5]					*Means*[3,6]		
WAC	1384 ab	31.07 b	16.2 a	322 a	130 a	70.94 ab	92 a
NAC	1270 ab	31.30 b	15.2 a	328 a	104 a	69.99 a	77 ab
WPC	2756 b	28.42 a	20.6	473 b	211	76.74	93 a
NPC	2529 b	28.53 a	16.7 a	439 ab	102 a	72.06 b	53 b
R	676 a	27.58 a	11.2	180	104 a	69.48 a	6

[1]Concentrations in ppb calculated from the regression equation of the standard curve.
[2]Peak heights relative to the peak height of 1 ppm acetone = 1000 units (% full-scale deflection peak height × attenuation).
[3]Means not significantly different at 1% level are joined with a common letter of the alphabet.
[4]Each mean represents the average of the four UHT milks over 9 replications.
[5]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NFC = plain polyethylene-lined cartons, and R = reference milk.
[6]Each mean represent the average of the milk over 9 replication during the 42-day trial period.

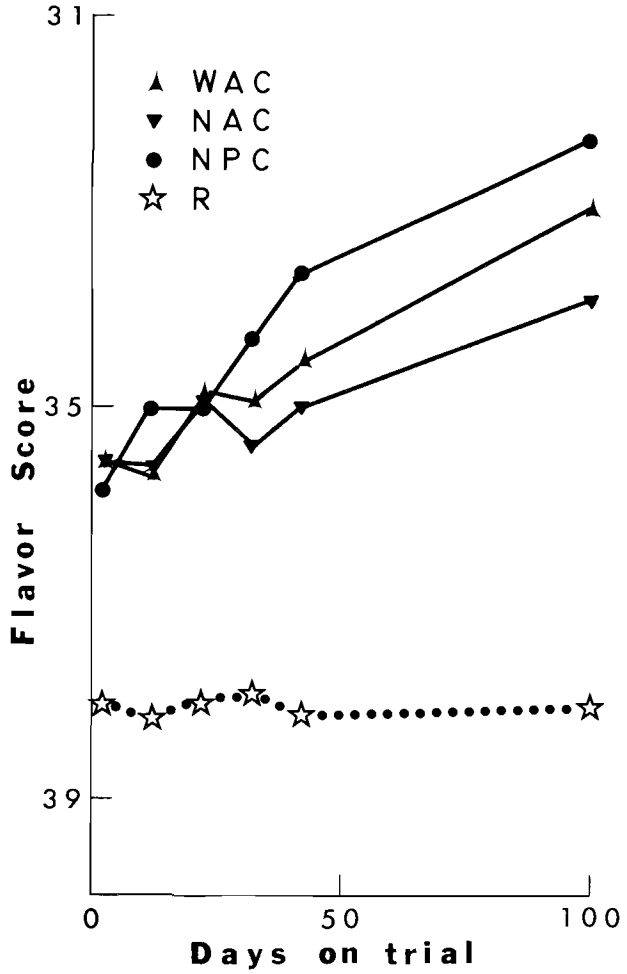

Figure 1. *Changes in flavor scores of UHT milks from cartons exposed to fluorescent light for 100 days. WAC + wrapped AL foil-lined, NAC + AL foil-lined, NPC = PE-lined, and R = reference milk.*

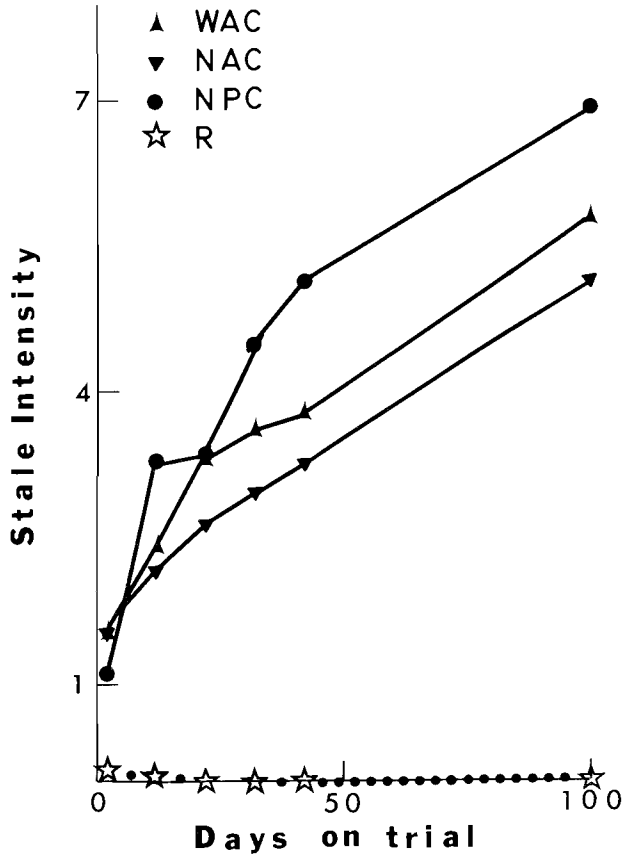

Figure 2. *Changes in stale intensities of UHT milks from cartons exposed to fluorescent light for 100 days. WAC + wrapped AL foil-lined, NAC + Al foil-lined, NPC + PE-lined, and R + reference milk.*

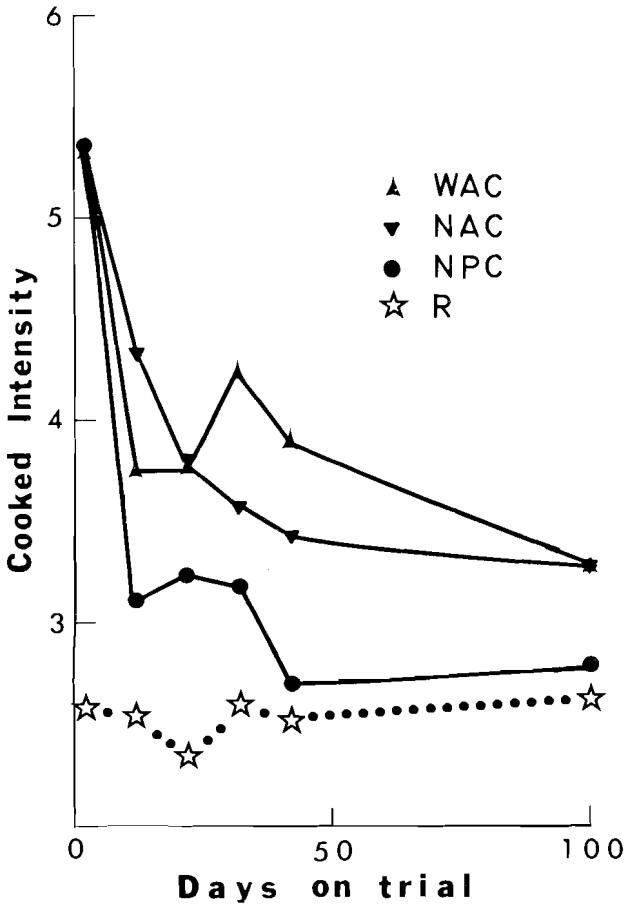

Figure 3. *Changes in cooked intensities of UHT milks from cartons exposed to fluorescent light for 100 days. WAC = wrapped AL foil-lined, NAC = AL foil-lined, NPC = PE-lined, and R = reference milk.*

weeks of storage; oxygen level in the milk remained constant at 1 ppm and milk was organoleptically acceptable for 2 months at room temperature. On the other hand, during the same period the plain PE cartons lost about 0.2% of their original weight at 20 C and 1% at 38 C; the milk was saturated with oxygen (8-9 ppm) after a few days and the milk was organoleptically acceptable only up to 3 weeks at 15 C. Flückiger felt most oxidative changes in the plain PE cartons took place in the first 2-3 days after processing and the reducing substances in the milk offered little protection in cartons without foil lining. The Saran-AL foil-Saran wrapping quite likely not only excluded outside air from the milk but also served to contain volatile compounds exuding from either growth of microorganisms on the surface of the cartons or from the wrapping material itself. Mean bacterial counts were higher in milk from wrapped cartons than from nonwrapped ones, even though they were not found to be statistically significant. One would suspect that the area under the wrapping could be loci for bacteria and perhaps odor. There was no evidence that light contributed to the changes that were observed.

We propose that the UHT milks exhibited two types of off-flavors: (*a*) cooked from volatile sulfhydryl compounds induced by heat, such as methyl sulfide; and (*b*) stale caused by volatile carbonyl compounds produced from the milk, from one or more of the carton layers, from the outer surface of the carton by microorganisms, or from the wrapping materials themselves. Heat damage from the UHT treatment, chemical changes that occur in the milk, and effect of the package all could account for changes in concentration of the off-flavors mentioned. Decreases in these components (as measured by GC) could be caused either by their escape to the atmosphere or by their interaction with other components. A decrease in the oxidation-reduction potential with heat treatment and in the liberation of sulfhydryls are known to produce stability against oxidative deterioration. Whether the mechanism for staling depends on the oxidation-reduction potential has not been established, though development of staling as cooked flavor disappears might suggest that it does. These assumptions, and the assumption that the AL foil carton is slightly permeable (although less so to exchange of gases than is the PE carton), account for our conclusion that AL foil lining helped the milk retain its desirable flavor characteristics.

Wrapping the cartons 2 days after they had been filled with milk proved detrimental to milk flavor. We speculate that that might have been caused by an odor-laden environment at the surface of the carton, confined by the wrappings. Odor could have been emitted by growing microorganisms on the surface of the cartons, by the Saran wrap, or by the carton material itself. Wrapping either the PE-lined or AL foil lined cartons had an adverse effect upon the flavor of the packaged milk during storage. This wrapping effect was less pronounced with AL foil lined cartons than with PE-lined cartons.

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