SOME CHEMICAL AND FLAVOR CHANGES OF STERILE CONCENTRATED MILK DURING STORAGE

by

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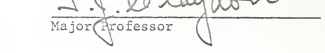

Major Professor

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INTRODUCTION

For years the production of foods with a high degree of palatability has been a challenge to the food scientist. An equal and constant challenge is the production of foods that will not undergo significant flavor deterioration during storage. In the area of dairy products the demand of the consumer in recent years for more "convenience foods" has intensified interest in the development of sterile milk products with improved flavor shelf-life. Ideally such milk could be stored at room temperatures for extended lengths of time in the market place or home, and remain suitable for use as a beverage milk or for general cooking use.

Other advantages await the successful commercialization of the process. Seasonal fluctuations in milk supply could be balanced somewhat with consumer demands in manufacturing the product. Also, wide usage by branches of the armed forces, where a fresh milk supply is often unavailable would seem reasonable. Other sterile dairy foods, currently experiencing similar flavor problems, might also be successfully processed if the sterile milk flavor problems were solved.

From the standpoint of container, warehouse and shipping cost it would be advantageous to the manufacturer if such improved sterile milk could be canned and distributed in a concentrated form, and later diluted with water to the normal solids content of milk by the consumer. Although sterilized evaporated milk has been marketed widely for many years for general home and institutional use, this product has lacked flavor acceptance as a beverage. Severe heat treatments required for physical stability and complete sterility, and failure to control staling during storage have created serious flavor quality problems.

The trend throughout the dairy industry, in improving flavor of sterile milk, has been toward using higher heat treatments and shorter holding times during processing. These ultra-high temperature (UHT) sterilization treatments range from about 138 C to 150 C with holding times of less than five seconds. Although recent advances in technology and equipment have overcome many of the flavor problems of fresh sterilized milk products, flavor deterioration during storage remains a serious problem.

The objective of the research reported herein was to determine organoleptically when and to what extent staling was progressing in sterile concentrated milks which were processed and stored under different conditions, and to evaluate these changes chemically by analytical techniques.

Viscosity, sediment and fat separation measurements also were made throughout this storage study to eliminate the chance of unknowingly having milk samples with abnormal properties.

REVIEW OF LITERATURE

Status of Sterile Milk Research

While ultra-high temperature (UHT) sterilization of milk has resulted in a product with less cooked or scorched flavor, it has presented physical stability problems of gelation and sedimentation during storage. The United States Steel Corporation, in conjunction with University of Wisconsin research workers (38), has described UHT processing conditions that gave a physically stable sterilized milk concentrate (SMC) during both processing and storage. Other recent publications (3, 4, 8, 34, 35) also describe equipment and processes used both in the United States and other countries, to make sterile

milk products. These procedures employ UHT to sterilize the milk, then it is aseptically filled into sterile containers which are either metal cans or paper "tetra-pak" units.

With processing conditions established, research work has now focused on problems related to the off-flavors encountered in sterilized milk during storage. In a recent study of consumer response to flavor and appeal of UHT processed SMC, McDivitt and Lowsma (18) reported that 34% of the consumers gave favorable comments in using diluted SMC as a fluid milk beverage, 34% gave unfavorable comments, and 32% reported little or no difference between SMC and fresh milk. A favorable response was received more often than not when this product was used on cereals or in coffee. This study, however, evaluated SMC only in the fresh condition or after only short storage at refrigerated temperatures.

Recently, the United States Steel Corporation (37) conducted a nationwide study of family acceptance of "Flavor Protected" sterilized milk concentrate (almost identical to most UHT processes) that had been subjected to 6 and 12 weeks of refrigerated storage, or after 4 weeks storage at room temperature, then 2 weeks at refrigeration temperature. Overall, 57% of the families rated the concentrated milk as good or excellent. The 12-week refrigerated milk was more desirable than the 6-week milk. This was attributed to the disappearance of some of the cooked flavor during storage. The milk stored at room temperature received the lowest rating. Three lots of "Flavor Protected" unconcentrated whole milk also were tested in this program. These samples were tested after storage at room temperature for 8 weeks, then with or without refrigerated storage in the home, or after 4 weeks at room temperature plus 4 weeks at refrigeration temperatures. These samples received good or excellent ratings from 53,

47 and 52% respectively of the families in the testing program. Overall, the unconcentrated whole milk received good or excellent ratings from 51% of the consumers.

Although the results of the above study indicated that from the consumer's standpoint, a good flavored heat sterilized milk can be manufactured, it was also evident that it must have an economical marketing advantage over regular fluid milk if it is to be used to supplement the latter product. To maintain low production and distribution costs, most manufacturers of this milk concentrate would probably have to substitute regular warehouse handling procedures for the more costly refrigerated storage and rapid distribution. For these less controlled warehousing procedures to be successful, the milk must be able to withstand extended storage periods at ambient temperatures without a significant loss in flavor stability.

In a flavor deterioration study, Sundararajan et al. (33) found that UHT, aseptically processed SMC had significantly better flavor scores initially than did milks made by longer heating processes. The flavor advantage, however, decreased throughout the storage period since the UHT milk deteriorated more rapidly during the first few weeks of storage. The initial flavor defect in these samples was described as cooked or caramel, while other defects - stale, storage, acidy, bitter, astringent and puckery - became apparent during the storage of the milk. These workers also reported more rapid flavor deterioration at 27 C than at 10 C storage regardless of processing conditions.

Flavor Compounds in Milk

Rapid development in the field of analytical instrumentation in the past decade has brought about the identification of many flavor producing

compounds in food products. Among the most prevalent compounds found in fresh and various types of off-flavored dairy products are carbonyls, sulfides, alcohols, lactones and free fatty acids.

In 1964 Bingham (7) summarized many of the compounds that have been isolated from some dairy products by various research workers. Those isolated from sterilized milks included acetone, pentanone, heptanone, ethanal, a pentyl acetate, dimethyl sulfide, delta-decalactone and delta-dodecalactone. In reporting the results of his research work, Bingham added methyl mercaptan, propanal, butanone, 2-hexanone and dimethyl disulfide to the above list.

In addition to many of the previously reported compounds, Muck et al. (21) reported that 2-nonanone, 2-undecanone, 2-tridecanone, gamma-dodecalactone and C-6 through C-16 even-numbered carbon chain saturated fatty acids were found in aged evaporated milk. A compound believed to be delta-tetradecalactone was also reported by these workers.

Arnold et al. (2) also isolated several flavor components from fresh and stale SMC. Those compounds found in the stale milk included 2-heptanone, 2-nonanone, 2-tridecanone, benzaldehyde, acetophenone, naphthalene, a dichlorabenzene, delta-decalactone, benzothiazole and O-aminoacetophenone. Only two of the compounds, dichlorabenzene and 2-heptanone, were extracted from the freshly prepared control SMC. The compounds isolated in this study were much less volatile than most of those previously reported. In another phase of the above study, volatile compounds were isolated from both the fresh and sterile SMC. Since these compounds were present in both milks at about the same concentrations, these workers concluded that the volatile compounds contributed little to the stale milk flavor.

To determine the role of phospholipids in the SMC aging process, Sprecher et al. (31) fractionated the phospholipids from fresh and stale milk, and studied their fatty acid composition. It was concluded from the study that the staling process did not involve oxidative attack on the unsaturated fatty acids of the phospholipids.

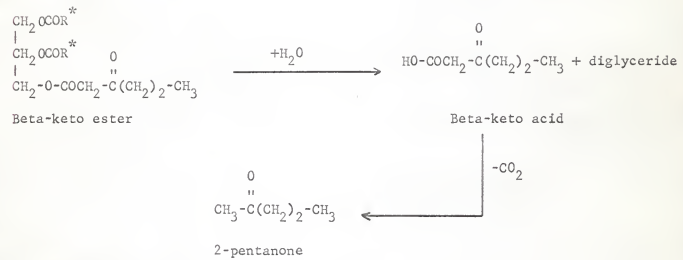
Most of the compounds isolated from stale SMC also have been reported in stale whole milk powder (7,29). However, in addition to the compounds found in sterile milk, aldehydes of C-2 through C-14 carbon chain length, were present in the stale dry milk. This fact would indicate that oxidative deterioration in dry milk is more important than in sterile milk.

The flavor effect of many of the chemical compounds which have been isolated from milk is somewhat questionable at the concentrations in which they are present in the milk. Langler and Day (16) determined the average flavor threshold (AFT) for some ketones by adding known concentrations of the compounds to homogenized milk and submitting the milk to a trained taste panel. The AFT for acetone, which is found in almost all dairy foods, was reported to be 79.5 parts per million (ppm). As the chain length of the ketones increased, the AFT dropped rapidly until a minimum value was reached at 0.7 ppm for 2-heptanone, than increases again as the chain length of the ketone increased. The AFT for 2-tridecanone was 18.43 ppm.

Wong et al. (40) determined the AFT of 2-pentanone and 2-heptanone in water and their results were in close agreement with the milk AFT determination previously described. Toan et al. (36) reported the AFT of methyl sulfide in homogenized milk to be 115 parts per billion. While most of these compounds in SCM are present at concentrations below the threshold values, they may still impart off-flavors to the milk by an additive effect (40).

Sources of Chemical Compounds

In 1958 Wong et al. (40) demonstrated that ketones were formed when whole milk or cream were heated, but not when skimmilk was heated. These workers suggested that the ketones might orginate from beta-keto acids in the lipid phase. Several workers (16, 22, 30, 39) have since studied the heat formation of ketones from butter oil. In three of these studies (16, 30, 39) it was found that water must be present before the ketones could be formed. Van der Ven (39) concluded that beta-keto esters are the precursors of methyl ketones in butter. Thus the 2-pentanone formation according to this theory would proceed as follows:



* R - alkyl chain

Parks et al. (22) reported that triglycerides containing one beta-keto acid and two fatty acid moieties accounted for 0.045% of butterfat samples they studied.

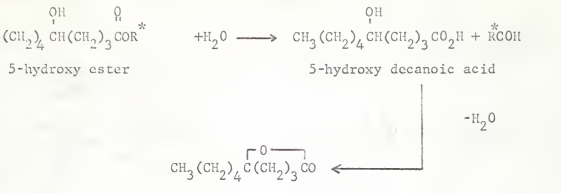
According to Langler and Day (16), all of the odd-numbered, straight carbon chained n-ketones from C-3 through C-15, and some butanone were formed when milk fat was heated in the presence of water. Although the concentration

of the individual ketones produced varied somewhat depending on the condition of the fat and the heat treatment, substantially more of the acetone, heptanone and pentadecanone were consistently formed. As pointed out by the above workers (16), it appeared that little correlation existed between the concentration of the specific beta-keto ester chain length found in the butter oil and the concentration of the respective fatty acid chain length normally present in milk fat.

In the study by Langler and Day (16) and a similar publication by Schwartz et al. (30) a maximum level of the total ketones was produced after certain levels of heat treatments, in the presence of ample water. Further heating beyond this point actually resulted in a slight decline in the total amount of ketones present. The former workers (16) found a maximum of 1.754 millimoles of ketones/kg of fat, while the latter investigators found a maximum of 0.65 millimoles/kg of fat. The results of the above studies would indicate that a wide variation in ketone formation might be expected, depending on the history of the particular butter oil.

Hawke (10) has recently published a review of the methyl ketone formation and metabolism in dairy products. The review includes evidence for the mechanism for biosynthesis of the beta-keto acids in tryglycerides.

The precursor of delta-lactones is believed to be either a simple ester of 5-hydroxy acid (17) or a 5-hydroxy ester bound to a glyceride (25). In both cases it has been suggested that the machanism proceeds as follows for delta-decalactone:



* R = Alkyl or diglyceride

Parliment et al. (15) has presented additional evidence that the delta-lactone precursor in milk fat is the monohydroxy-acyl-triglyceride. This evidence was based on molecular weight considerations by gel filtrations of milk fat. Jurriens and Oele (11) increased the concentration of lactones 2- to 3-fold in milk fat by heating the fat at 140 C. Keeney and Patton (13) determined that 9-decenoic acid was not a precursor of delta-decalactone as had been earlier suggested.

Parks et al. (23) suggested that potential precursors of 0-aminoaceto-phenone, which has been isolated from both stale dry milk and stale SMC, were tryptophane, indican and kynurenine. While tryptophane and indican were known constituents of milk, only recently has kynurenine been shown to be present in milk (24). Parks et al. (24) used an alkaline degradation procedure to convert a fraction of milk, believed to be kynurenine, to 0-amino-acetophenone. A yield of one micromole/liter of raw milk serum was obtained. Thin-layer chromatography was used to verify further that the isolated fraction contained kynurenine. These workers also observed a slight increase in 0-aminoacetophenone when milk was heated above 76.7 C, and a continuous decrease in the compound when a temperature of 93.3 C for 15 sec was exceeded.

By thin-layer chromatography methods a similar pattern for kynurenine concentration was observed as milk was heated.

The Maillard browning process is known to be detrimental to the flavor of food products. The browning reaction in dairy products has been reviewed by Patton (27). In the review furfuryl alcohol, 5-hydroxymethylfurfural, maltol, acetol, methyl glyoxal; butyric, propionic, acetic, formic, lactic and pyruvic acids; hydrogen sulfide and carbon disulfide are listed among the suspected browning degradation products in dairy products. No doubt some of the flavor compounds detected in aged sterilized milk are by-products of the browning reaction since high heat treatments during sterilization and prolonged storage periods are both conducive to the Maillard browning reaction.

Bell et al. (6) reported that milk processed by high-temperature short-time methods was essentially free of browning. Adams et al. (1) recently reported that evaporated milk stored 4 years at refrigeration temperature did not undergo any significant color changes by visual detection methods, but did show some browning by reflectance measurement methods.

Some carboxylic acid formation, by hydrolysis of triglycerides during heat sterilization of milk, might be expected in evaporated milk. Kern et al. (14) observed an increase in butyric, propionic, acetic and pyruvic acids during autoclave sterilization of whole milk. Later Morr et al. (20) observed these same acids plus formic and lactic acid, and three unidentical compounds during prolonged heat treatment of skimmilk. These data would probably indicate compound formation primarily by lactose degradation rather than simple fat hydrolysis, since similar observations were made with skimmilk and whole milk.

Oxidation during storage is not considered to be a problem during storage of sterilized milk (29). The reason for the control of oxidation is believed to be related to the reducing groups present in the milk and the lack of available oxygen in the container.

It is often difficult to determine which changes in milk products are caused by a particular reaction as many of these mechanisms proceed under the same conditions.

Methods of Isolating and Identifying Flavor Compounds

In the study of flavor components of foods, gas chromatography has been successfully employed almost universally by flavor chemists. Often other analytical equipment and techniques, such as mass and infrared spectrometry, and various functional group reaction methods are used in conjunction with gas chromatography to establish more evidence for compound identification.

Even with the sensitive detectors used with gas chromatographs, it is not possible to detect many of the compounds which are responsible for the good or bad flavor of a food at their low concentrations. Many enrichments procedures have been used to increase the concentration of flavor compounds to a detectable level.

In 1958 Wong et al. (40) used reduced-pressure distillation, paper and liquid chromatography, and 2,4-dinitrophenylhydrazine derivatives to concentrate, separate and identify flavor components from commercial evaporated milk. Patel et al. (26) also used a reduced-pressure milk distillation procedure, but also distilled and fractionally condensed the distillate. The second distillation separated most of the water from the more volative compounds, that were studied by gas chromatography.

Using a solvent extraction technique, Patton (28) recovered flavor components from aged evaporated milk and subjected them to gas chromatographic analysis. Apparently this method was successful in extracting the off-flavored components from the stale milk since the solvent had a stale-like odor after the extraction and the orginal stale flavor was removed from the milk. Muck et al. (21) used an extraction procedure similar to Patton's, in conjunction with paper and gas chromatography to separate and identify flavor components from aged sterilized milk.

A nitrogen distillation system, similar to the procedure developed by Morgan et al. (19), was used by Bingham (7) in studying the flavor compounds in sterile milk. In this method, anhydrous sodium sulfate is added to milk, nitrogen is bubbled through the milk and vapors are condensed in a 2 foot section of a chromatographic column immersed in a liquid nitrogen bath. At the end of the distillation period the short column is connected to the front end of a longer gas chromatographic column and the analysis carried out by gas chromatography temperature programming.

Bingham (7) also attempted to use the head space gas sampling method developed by Bassette et al. (5), but reported little chromatographic response to the injected gas sample. This method has the advantage of direct vapor sampling with little chance for the formation of artifacts.

In 1966 Arnold et al. (2) used a procedure which involved first lyophilizing large quantities of sterilized milk, extracting components from the dehydrated powder and subjecting the concentrated extract to gas chromatographic analysis. Many of the separated compounds were collected from several sample injections in a cold trap after being eluted from the chromatographic unit. The trapped fraction was re-chromatographed, and subjected to mass spectral analysis for compound identification.

While all of the reviewed methods have been successfully used to separate minute amounts of chemical compounds from milk, care must be taken to be sure these compounds are not artifacts produced during their isolation due to heating, or contamination from equipment or solvents used.

MATERIALS AND EXPERIMENTAL PROCEDURE

Primarily nine lots of sterilized concentrated whole milk were studied in this experiment. Three procedures, ultra-high temperature (UHT), high-temperature short-time (HTST) and conventional, were used to process the milk. Three temperatures of storage, 4, 20 and 37 C, were employed. In addition, some HTST sterilized skimmilk concentrate was manufactured and stored at each of the three temperatures.

The term UHT in this report applies to concentrated milk which was sterilized at UHT for a few sec in small diameter tubing and filled into previously sterilized cans under aseptic conditions. HTST milk refers to milk which received a heat treatment which required more holding time than the UHT but less than the conventional milk. The term conventional as used throughout this report refers to concentrate milk processed in the same manner as the evaporated milk commonly found in the food market today. The conventional process requires long holding times during forewarming and sterilization which often result in severe cooked flavor and some browning of the milk. All types of milk were concentrated 2:1 on a total solids basis.

Each lot of concentrated whole milk was examined fresh, and then monthly for a period of 8 months. Only a limited amount of the sterilized skimmilk was available for storage at each temperature. Therefore, it was not analyzed each month. Monthly analyses consisted of organoleptic, gas

chromatographic, rancidity and browning measurements. Changes in the physical properties of the milks were also determined throughout the storage period.

Concentrated milk samples were diluted to approximately the normal solids concentration of whole milk by mixing the milk 1:1 (w/w) with distilled water before the organoleptic, browning, and rancidity measurements were made. Gas chromatographic and physical property measurements were made of the concentrated milk before dilution.

Processing and Storage of Milk

The UHT, HTST and conventional milks were processed from the same tank of raw milk. This milk was standardized for the correct fat to solids-not-fat ratio before treatment. Carrageenan fat stabilizer was also added at this point. The skimmilk, while not from the same tank of milk, was from the same general milk supply.

The UHT milk was preheated to 84 C, forewarmed to 116.7 C in a Roswell heater and held at that temperature for 2.5 min before being passed into a falling film vacuum pan for condensing at 41 C. The milk was pumped continuously from the pan at about 31% total solids to a surge tank feeding the concentrate heat treatment system. In this process the milk was heated in a plate preheater to 84 C, then further heated by live steam injection to 113 C. After a 4 min holding period the milk was cooled to 60 C with a tubular cooler and prehomogenized at 70 kg/cm^2 pressure, then cooled to 4 C for standardization of total solids. The milk was sterilized at 144 C for 4 sec, cooled to 64 C and homogenized at 220 + 35 kg/cm^2 pressure, then further cooled to 37 C for aseptic filling into sterile cans and sealing with a Dole aseptic canner.

The HTST evaporated milk and the concentrated skimmilk were forewarmed in the same manner as the aseptic milk through the condensing step. After concentration, these milks were homogenized at 210 kg/cm^2 pressure at 41 C, then cooled to 4 C for standardization. Following standardization, the milk was canned at a product temperature of 4 C, then sterilized at 112.8 C for 4.9 min plus 125.6 C for 2.7 min in a Food Machinery Corporation (FMC) sterilizer.

The conventional milk was preheated to 85 C, forewarmed in a continuous flow vat to 90.6 C and held for 20 min, then heated further to 96 C in a hotwell and given an additional 10 min hold at this temperature. The hot milk was introduced into the vacuum pan for concentration, then homogenized, cooled and canned under the same conditions as the HTST milk. This milk was also sterilized with a FMC sterilizer, but at 117.2 C for 12.3 minutes. Epon coated, sanitary-type cans were used for all samples.

Storage samples were coded to indicate processing treatments and storage temperatures as follows:

Code	Process	Storage Temperature (C)
U-4	UHT	4
U-20	UHT	20
U-37	UHT	37
H-4	HTST	4
H-20	HTST	20
H-37	HTST	37
C-4	Conventional	4
C-20	Conventional	20
C-37	Conventional	37
S-4	HTST - Skimmilk	4
S-20	HTST - Skimmilk	20
S-37	HTST - SKimmilk	37

The concentrated whole milks ranges from 7.87 to 7.96% in milk fat and from 25.55 to 25.94% in total solids. The concentrated skimmilk contained 0.16% milk fat and 18.0% total solids.

Organoleptic Analyses

Each month the concentrated whole milks, after dilution, were evaluated for flavor qualities by a five member trained taste panel. Duplicate sets of the nine milk samples were randomly presented to the panel members in foil wrapped glass stoppered Erlenmeyer flasks. Since no more than nine single samples were evaluated at one tasting session, two testing periods were required. The two sessions were separated by at least a three hour time interval.

A one to nine hedonic flavor scoring system was used to evaluate the samples. A score of one indicated the milk was objectionable and a score of nine indicated the milk had an excellent flavor. Also included on this score card were flavor criticisms with the intensity of each defect indicated by a scale from one to five. A rating of one was considered a slight defect and five was considered intense. The score card used for the flavor evaluation is shown in Table 1.

Chemical Analyses

Gas Chromatography. The instruments used for gas chromatographic separations were: Aerograph models 600-B (Instrument A) in conjunction with a 1.05 mv Brown-Honeywell recorder and a no. 500-C (Instrument B) with a 1 mv Brown-Honeywell recorder. Both instruments were equipped with hydrogen flame ionization detectors. A 3.05 m x 0.318 cm stainless steel

Table 1. Flavor score card used for evaluating milk samples

FLAVOR SCORE CARD FOR CONCENTRATED MILK FOR BEVERAGE PURPOSES

Name _____ Date _____

Please check proper score		Sample Number 1	2	3	4	5	6	7	8	9	10
EXCELLENT	9										
	8										
DESIRABLE	7										
	6										
No strong like or dislike	5										
UNDESIRABLE	4										
	3										
OBJECTIONABLE	2										
	1										

Please Indicate Intensity of Off-flavor by Scoring Proper Criticism
(+1) Slight (+2) Weak (+3) Moderate (+4) Strong (+5) Intense

	1	2	3	4	5	6	7	8	9	10
ASTRINGENT										
CHALKY										
COOKED										
SCORCHED										
STALE										

column, packed with 20% carbowax 20M on 60/80 mesh, HMDS treated chromosorb P, was installed in each instrument. The operating conditions of the two instruments were:

	Instrument A	Instrument B
Column temperature	100 C	100 C
Injector temperature	192 C	192 C
Nitrogen flow	14.1 ml/min	16.3 ml/min
Hydrogen flow	24.4 ml/min	110 ml/min
Oxygen flow	120 ml/min	110 ml/min
Chart speed	0.85 cm/min	0.85 cm/min

Other materials used were:

Sampling bottles - serum vials, 15 x 52 mm, of 5 ml capacity with self-sealing rubber caps.

Syringe - 1 ml gas tight, Hamilton no. 1001, with a no. 25 needle 5.08 cm long.

Mechanical shaker - Fisher clinical shaker operated at 275 to 285 oscillations per minute.

Reagents - sodium sulfate, anhydrous, ACS grade; mercuric chloride, anhydrous, ACS grade; acidic and basic hydroxylamine solutions prepared according to the methods of Bassette at al. (5).

The head space sampling method as described by Bassette et al. (5), and modified by Toan et al. (36) was used in the analysis of milk samples. In this method 2 ml of concentrated milk was saturated with sodium sulfate in a serum vial, heated in 60 C water bath for 2 min and mixed on a shaker for 5 min. After mixing, a clean cap was placed on the vial and it was again heated in the 60 C bath for 8 min. The syringe needle was inserted through the vial cap and 1 ml of head space gas was withdrawn from above the milk and injected into the chromatograph.

Chromatographic peak times were recorded in minutes from time of sample injection, with acetone as a standard having a retention time of 4.0 min in all samples.

All total peak heights were corrected for daily instrument sensitivity changes. This was accomplished by determining the total peak height (% of full scale recorder deflection x attenuation) for 1 ppm acetone each day and dividing this value into the maximum peak height found for 1 ppm acetone, 1600, to get an adjustment factor. The total peak height of each compound for that day was then multiplied by this factor to get an adjusted total peak height.

Tentative identification of chromatographic peaks was made by a comparison of their retention times with those of known compounds analyzed under the same conditions. Additional identification was supplied by prechromatographic reaction methods as described by Bassette at al. (5) for esters, carbonyls and sulfides. By this method, ester and carbonyl peaks were eliminated from the chromatograph after the sample was reacted with basic hydroxylamine. If the sample was mixed with acidic hydroxylamine, only the peaks representing carbonyl compounds were removed. In the same manner sulfides were eliminated by treating the sample with mercuric chloride before removing the head space sample for analysis. The boric acid on-column-reaction technique described by Ikeda (10) was employed for the removal of alcohol peaks from the vapors from the milk.

Rancidity Measurements. The acid degree value (ADV) determination described in Standard Methods for the Examination of Dairy Products (32) was used to determine the degree of rancidity in the diluted milk samples. The fat was recovered with a nonionic surface-active agent and titrated

with 0.02 N KOH. ADV by this definition is the ml of 1 N alkali required to neutralize 100 g of fat. An ADV of 1.5 or greater is considered to indicate definite rancidity in fresh milk.

Browning Measurements. An estimation of the amount of browning in the milk was made by the method B procedure of Keeney and Bassette (12). This spectrophotometric method measures the concentration of 5-hydroxymethylfurfural (HMF), an intermediate compound formed in the Maillard-type browning reaction. The HMF reacts with 2-thiobarbituric acid to give a colored product which was measured with a Beckman DU spectrophotometer at 443 mμ. A reaction time of 35 min at 40 C was used.

Physical Property Analyses

Concentrated milk used for the evaluation of physical properties was discarded after the measurement was made rather than using it for the organoleptic or chemical analyses.

Fat Separation. The fat complex layer, which has risen to the top of the milk during quiescent storage, was dipped from the milk immediately after opening each can of milk and weighed on a triple-beam balance. These measurements were made only at 0, 6 and 8 months storage.

It should be pointed out here that the HTST and conventional milk containers were of 10 fl oz capacity, while the UHT containers were 8 fl oz.

Viscosity. A Brookfield multi-speed LVF viscometer, equipped with a no. one spindle, and operated at 60 rpm was used to determine the viscosity of the milk. The sample was poured into a 600 ml beaker with minimum agitation, then 180 ml were carefully transferred to a 5 x 10 cm, wide-mouth bottle, and the viscosity determination made at 24-25 C.

Sediment. The thickness of the layer of solid material remaining in the can after pouring the milk out was measured and recorded as mm of sediment. The sample was not agitated before this measurement was made.

RESULTS AND DISCUSSION

Organoleptic Analyses

The average monthly hedonic flavor score and total defect intensity ratings of each of the concentrated whole milks are summarized in Tables 2, 3 and 4. These average flavor scores include 10 judgments per analysis since five panel members tasted duplicate samples of each milk. No attempts were made to organoleptically evaluate the flavor of the concentrated skimmilk.

A cooked flavor was essentially the only defect in all of the three types of milks at the beginning of the storage study. The UHT milk (Table 2) was less severely cooked than the HTST milk (Table 3), and the conventional milk (Table 4) was cooked and received two judgments indicating a scorched flavor. Initial average flavor scores also were in this order as the UHT was scored 7.6, HTST 6.8 and conventional received the lowest score, 6.2 based on the one to nine hedonic scale.

During storage a slightly oxidized flavor defect became apparent in both the U-4 and U-20 milk. Altogether 14 oxidized flavor judgments with a combined intensity of 27 were assigned to these two groups of samples throughout the entire storage period. Only one other sample, H-20 (Table 3) at the sixth month examination, was marked as oxidized. This criticism did not appear on the printed score card. Therefore, it was necessary for the judges to write this defect on the card when it was found. It was possible

that U-37 samples were slightly oxidized, but undetected due to the covering effect of the stale flavor that was present. Also, an oxidized flavor could probably be detected easier in the U-4 milk, since it had fewer other defects to mask this flavor.

An astringent flavor was noticed in several of the milk samples. This defect did not appear to be characteristic of any particular type of milk or storage condition. A chalky flavor defect was observed which had about the same intensity characteristics as the astringent flavor, but was less apparent in the samples stored at 37 C than in the samples stored at 20 and 4 C.

Stale and scorched flavor criticisms were used by the judges to describe the flavor properties of the aged milk. These defects were seldom marked in the early stages of the flavor study, but the frequency and intensity of both defects increased in the 20 and 37 C milk samples as storage time was increased (Tables 2, 3 and 4). As the stale and scorched defects became more intense, the cooked flavor defect which became less noticeable, was apparently covered somewhat.

One of the judges indicated that the milks stored at 37 C occasionally had a high acid-like flavor. The titratable acidity was found to be within the normal range for evaporated milk when it was determined for some of these samples. The relationship between this high acid defect and the high acid degree values described later in this report is not known.

Linear regression equations for the rate of deterioration during storage were calculated for each type of milk at each storage temperature. These linear regressions and the actual monthly flavor scores of the samples are shown in Figure 1. In all groups of samples the greatest deviation

Table 2. Effect of storage on flavor of UHT processed milk.

Sample[a]	Storage Time (mo)	Intensity and number of flavor defect judgments														Avg hedonic flavor score
		Astringent		Chalky		Cooked		Scorched		Stale		Oxidized		Acid		
		(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	
U-4	0			1	1	26	10									7.6
"	1	1	1			26	10									6.9
"	2					24	10					1	1			6.6
"	3	1	1	1	1	15	9	5	2	2	1	3	1			6.6
"	4	3	2			21	10									6.7
"	5	2	2	8	3	25	10					2	1			6.1
"	6	2	2			25	10									6.4
"	7	5	3	4	2	21	10			2	1					6.4
"	8	3	1	2	1	18	8			4	4	7	3			5.7
U-20	0			1	1	26	10									7.6
"	1					25	10									7.0
"	2					21	9	1	1			2	2			6.3
"	3	4	3	1	1	25	9			1	1	3	1			6.0
"	4	3	1	4	2	21	9					5	3			5.9
"	5	2	1	4	1	30	9			6	5					5.9
"	6	3	2			27	10	1	1	5	4	4	2			5.1
"	7	1	1	3	2	25	10	1	1	3	3					5.9
"	8	1	1	3	3	19	8	1	1	7	5					5.6
U-37	0			1	1	26	10									7.6
"	1					26	8	4	1	4	3					5.7
"	2	7	3			26	9			7	5					4.5
"	3					18	5	13	5	10	6					4.5
"	4	9	4			9	4	19	6	11	6					3.7
"	5	2	1	4	1	21	6	13	4	14	7					3.5
"	6	7	4			12	4	19	5	17	6					3.3
"	7	2	1			3	1	26	6	16	6					2.2
"	8	6	2			8	2	20	6	34	9			2	1	2.3

[a] U-4 = 4 C storage, U-20 = 20 C storage and U 37 = 37 C storage.

[b] Intensity obtained for each duplicate set of samples by totaling all judges' defect intensity ratings for each defect.

[c] Total number of times the defect was marked by the judges for each duplicate set of samples.

Table 3. Effect of storage on flavor of HTST processed milk.

Sample[a]	Storage Time (mo)	Intensity and number of flavor defect judgments														Avg hedonic flavor score
		Astringent		Chalky		Cooked		Scorched		Stale		Oxidized		Acid		
		(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	
H-4	0					31	10			1	1					6.8
"	1					21	8	3	1	5	2					6.2
"	2					24	9	3	1							6.3
"	3	2	2			20	8	3	2	1	1					6.4
"	4					26	9	3	1	1	1					6.3
"	5	6	3			29	10									5.9
"	6	1	1			22	10			2	2					6.0
"	7	3	2	2	1	24	9			6	4					5.7
"	8	3	2	2	2	18	8			7	4					5.8
H-20	0					31	10			1	1					6.8
"	1					23	8			5	3					5.8
"	2	3	1	3	1	26	9			2	2					5.6
"	3	1	1			24	8	6	2	2	2					5.7
"	4					21	7	9	4	6	3					5.2
"	5	4	2	3	1	35	10	1	1	2	2					5.1
"	6	2	2	1	1	27	9	5	3	4	3	2	1			5.1
"	7					25	9	5	3	10	7					5.1
"	8	2	1	3	1	21	8	1	1	7	4					4.9
H-37	0					31	10			1	1					6.8
"	1	2	1			25	7	8	2	3	2					5.2
"	2					21	6	10	4	8	4					4.2
"	3			1	1	7	2	12	4	14	7					3.6
"	4					15	5	18	5	12	6					3.8
"	5					14	4	21	6	19	8					3.0
"	6	5	3			10	3	22	6	22	7					2.6
"	7	1	1					32	8	33	10					2.1
"	8	6	2			9	2	19	5	32	8			4	2	2.0

[a] H-4 = 4 C Storage, H-20 = 20 C storage, H-37 = 37 C storage.

[b] Intensity obtained for each duplicate set of samples by totaling all judges' defect intensity ratings for each defect.

[c] Total number of times the defect was marked by the judges for each duplicate set of samples.

Table 4. Effect of storage on flavor of conventionally processed milks.

Sample[a]	Storage Time (mo)	Intensity and number of flavor defect judgments														Avg hedonic flavor score
		Astringent		Chalky		Cooked		Scorched		Stale		Oxidized		Acid		
		(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	(b)	(c)	
C-4	0					23	8	3	2							6.2
"	1	1	1	1	1	12	5	10	4	4	2					5.3
"	2	2	1			22	7	4	2	2	2					5.2
"	3	2	2			22	7	8	4	3	3					5.3
"	4	3	2			23	9	3	1							6.4
"	5					32	10	3	2	2	2					5.3
"	6	3	2	1	1	25	10			5	4					5.7
"	7	1	1	2	1	28	10	4	3	4	3					5.4
"	8	1	1	3	2	20	8	3	3	4	2					5.8
C-20	0					23	8	3	2							6.2
"	1	2	2			16	6	8	3	4	2					5.5
"	2			2	1	24	8	6	2							5.2
"	3	1		2		25	8	6	3							5.4
"	4	6	2			26	8	9	4	3	3					4.8
"	5	1	1	6	2	31	9	5	2	5	3					4.4
"	6	5	3	1	1	17	7	5	2	15	7					4.5
"	7					30	10	6	4	9	5					4.6
"	8			5	2	19	7	4	3	8	4					5.0
C-37	0					23	8	3	2							6.2
"	1	2	1			10	2	14	4	12	5					4.1
"	2					21	6	12	4	8	4					4.0
"	3					5	1	25	7	19	7			2	1	3.0
"	4	3		1		22	6	1	4	17	7					3.6
"	5	2	1	3	1	18	5	17	4	18	8					3.0
"	6	2	1			9	3	21	6	19	7					2.6
"	7	1	1			3	1	31	8	27	8					2.2
"	8	6	2			8	2	21	5	34	8			4	2	2.0

[a] C-4 = 4 C storage, C-20 = 20 C storage and C-37 = 37 C storage.

[b] Intensity obtained for each duplicate set of samples by totaling all judges' defect intensity ratings for each defect.

[c] Total number of times the defect was marked by the judges for each duplicate set of samples.

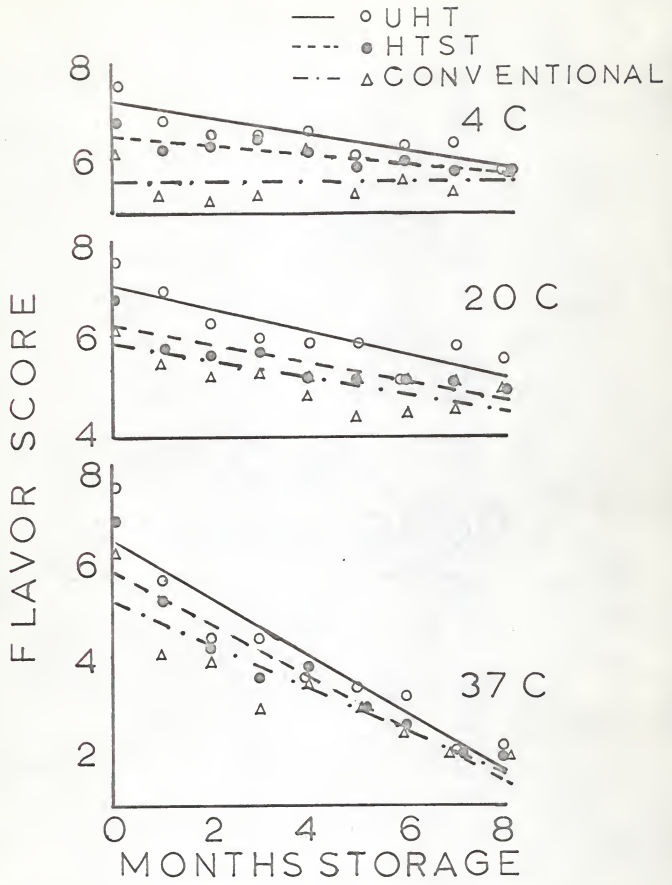


Figure 1. Linear regression (lines) and average monthly flavor scores (symbols) of UHT, HTST and conventionally processed milk stored at 4, 20 and 37 C using a 1-9 hedonic scoring system.

of flavor scores from regression lines was in the early months of storage where the rate of deterioration was greater than it was near the end of the study.

At all temperatures, the flavor scores were usually in the descending order of UHT, HTST and conventional milks, but the rate of deterioration was in the reverse order (Fig. 1). By the end of the fourth month of storage, all types of processed samples at each storage temperature were approaching equality and at the end of the study the samples had essentially the same flavor scores at each temperature (see Tables 2, 3 and 4).

As might be expected the rate of flavor deterioration was greater as the temperature of storage was increased (Fig. 1). The conventionally processed milk, which has a lower initial flavor score than the other two types of milk, had a rate of flavor deterioration of only 0.005 points per month (one-nine hedonic range) at 4 C storage. The rate of flavor deterioration for the same lot stored at 37 C was 0.4417 points per month as determined by the regression equation.

An analysis of variance was performed on the flavor scores to determine the importance of treatments of milk during processing and storage. These results are presented in Table 5. The F-test shows that time of storage, process, and storage temperature are all significant sources of variation in the flavor scores at the 1% level. Also, an interaction of storage time on temperature was significant at the 1% level, while an interaction of storage time on processes was significant only at the 5% level.

Table 5. Analysis of variance of flavor scores of milk among processing conditions, months storage and storage temperatures.

Source of variation	D/F	Sum of squares	Mean square	F value	Significance
Months	8	463	57.88	68.27	**
Processes	2	107	53.50	63.11	**
Temp.	2	818	409.00	482.46	**
Month x Proc.	16	23	1.44	1.77	*
Proc. x Temp.	4	2	0.50	0.59	ns
Month x Temp.	16	203	12.69	14.99	**
Prox. x Temp. x Mo.	32	20	0.63	0.74	ns
Remainder	729	617	.84		

* Significant at 1% level.

** Significant at 5% level.

ns Not significant.

Least significant differences (LSD) of the above differences are shown in Table 6. The LSD of the mean flavor scores for each month of storage indicated that the 1 through 8 month ratings were significantly lower at the 1% level than the average initial flavor scores. Similarly all flavor scores from the 2 through 8 month evaluations were significantly lower than the 1 month flavor scores, and the 5 through 8 month scores were significantly lower than the 2 through 4 month flavor scores at the 1% level. The scores of flavor evaluations for the 2 through 4 month samples were not significantly different at the 5% level. Also, the 5 through 8 month flavor scores were not significantly different at the same confidence level.

Table 6. The least significant difference (LSD) of the mean flavor scores among months, processes and storage temperatures.

A.	Mean monthly flavor scores (LSD = 0.39)								
	0	1	2	3	4	5	6	7	8
	6.87*	5.74*	5.32ns	5.17ns	5.16*	4.61ns	4.55ns	4.40ns	4.35

B.	Mean flavor scores for processes (LSD = 0.22)				
	UHT		HTST		conventional
	5.59	*	5.11	*	4.70

C.	Mean flavor scores for storage temperatures (LSD = 0.22)				
	4 C		20 C		37 C
	6.11	*	5.54	*	3.75

* Indicates significant differences at the 1% level.

ns Indicates not significantly different at the 1% level.

The LSD test for processes indicate (Table 6) that all treatments were significant (1% level) sources of flavor score variation when averaged for all storage temperatures and months. The average UHT milk scores were higher than HTST, and the conventionally processed milk received the lowest rating. All temperatures of storage are shown to be significant sources (1% level) of variation in flavor scores by the LSD test. The average flavor score of the 4 C samples was higher than the 20 C samples, and the 37 C storage samples received the lowest average flavor scores.

These organoleptic data indicate that the best flavored milk was obtained when it was sterilized by UHT methods and stored at 4 C for relatively

short periods. With an increase in heat during processing, storage temperature, and storage time, the flavor quality was lowered. This change was shown by both decreases in hedonic scores and increases in criticism intensities (Table 2, 3 and 4).

Gas Chromatographic Analyses

Volatile Compound Identification and Changes during Storage. Representative gas chromatographic patterns of volatile compounds found in the milk samples are shown in Fig. 2. The patterns shown are from vapors of conventionally processed milk 2 days after processing and again after 8 months storage at 37 C. All compounds encountered by the head space technique used in this experiment are shown in this illustration, except a small peak with a retention time of 12.5 min that was found only in the skimmilk. Not all of these components were necessarily present in all milk samples.

The retention time of each peak, the suspected compound, and evidence for its identification are presented in Table 7. Three peaks that were observed in all analyses are not shown in Fig. 2 and Table 7. The first such peak had a retention time of 1 min and was related to the changes in column pressure when the vapor sample was injected. The other two peaks, with retention times of 1.2 and 1.8 min, were encountered not only in all milk analyses, but also when room air or vapors from distilled and recently boiled water were injected into the instrument. Therefore, these three peaks were not considered to be of any significance in the milk analyses.

Fig. 3-11 show peak height changes in some of the volatile components during the storage of milk samples. Also included in each of the figures is an estimate of the concentration of the compound. The estimate was made by comparing measured peak heights with a standard curve of each compound.

The retention time of peak A, 2.4 min, agreed with that of acetaldehyde. The reaction characteristics of this compound with acid hydroxylamine offered additional evidence that it was acetaldehyde. Peak A appeared in all milk samples and was the only aldehyde isolated from the milk. As indicated in Fig. 3, acetaldehyde had similar peak heights overall in most of the 3 types of milk except higher levels of this compound were observed in the fresh conventionally processed milk and in most of the milk samples near the end of the 37 C storage. The acetaldehyde peak heights were erratic throughout the monthly examinations.

Peak B had a retention time of 3.1 min, which was identical to that of authentic dimethyl sulfide. Since reaction of the milk with mercuric chloride removed this peak, it was concluded that this peak did represent dimethyl sulfide. The monthly peak heights of dimethyl sulfide are shown in Fig. 4. This compound was considerably higher in concentration in the skimmilk than in the other three milks, which had essentially the same concentrations. The dimethylsulfide peak heights did not change much at any of the storage conditions.

A compound with a retention time of 3.5 min was represented by Peak C. Propanal, which has been reported in sterilized milk (7), and furan have retention times of 3.5 min. Since this peak was not affected by reacting the milk with acid hydroxylamine, it was believed that this compound was not propanal. It is also known that this compound was not an ester or

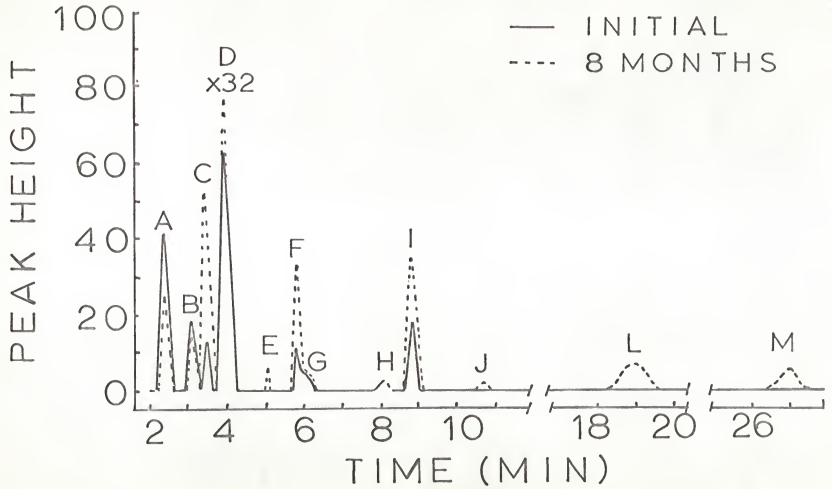


Figure 2. Gas chromatographic peak heights of volatile components from conventionally sterilized milk initially and after 8 months storage at 37 C (all peaks have an attenuation factor of X 4 unless otherwise indicated above peak).

Table 7. Characterization of volatile compounds found in sterile milk.

Peak	Retention time (min)	Reaction characteristics with: Mercuric chloride	Acid hydroxyl-amine	Basic hydroxyl-amine	Boric acid column	Suspected compound
A	2.4	*	++	++	*	Acetaldehyde
B	3.1	++	*	*	*	Dimethyl Sulfide
C	3.5	+	*	*	*	Furan
C	4.0	*	++	++	*	Acetone
E	5.1	++	*	*	*	2-Methyl furan
F	5.9	*	++	++	*	Butanone
G	6.2	*	*	*	++	Ethyl alcohol
H	8.1	x	x	x	x	Unknown
I	8.8	*	++	++	*	2-Pentanone
J	10.5	x	x	x	x	Unknown
K	12.5	*	*	*	*	Unknown
L	18.8	*	*	*	++	n-Butyl alcohol
M	26.0	*	++	++	*	2-Heptanone

* No change in peak height.

\+ Peak height reduced.

++ Peak completely removed.

x Initial peak too small to determine.

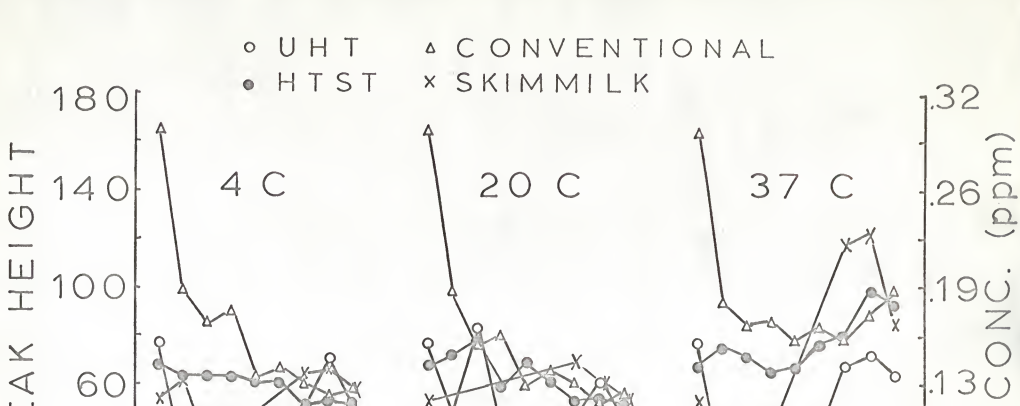


Figure 3. Monthly changes in acetaldehyde gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

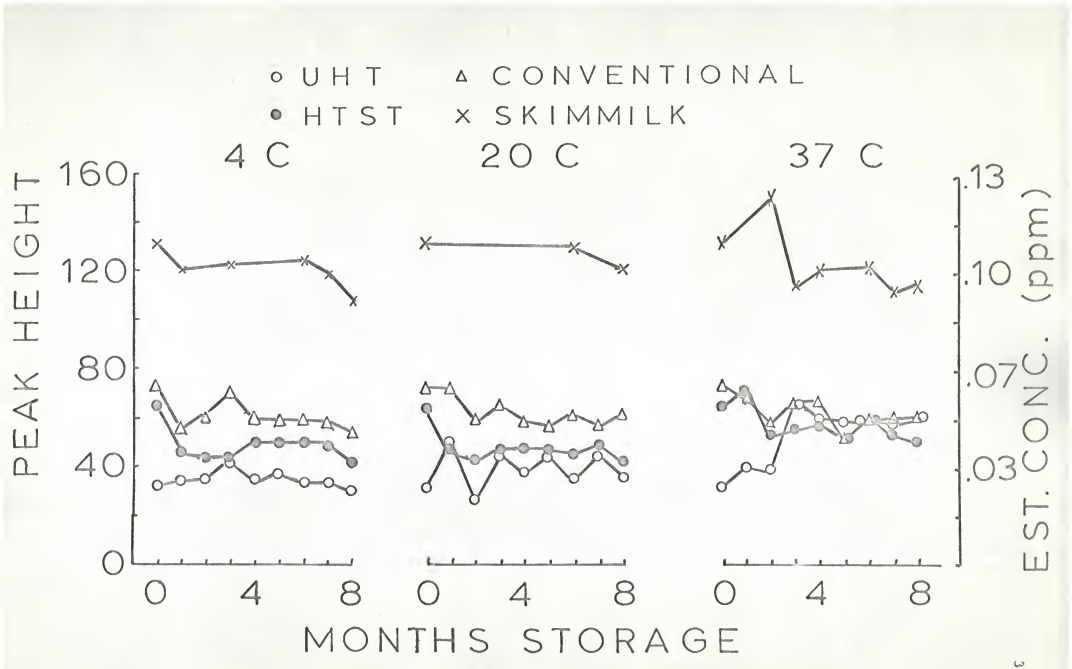


Figure 4. Monthly changes in dimethyl sulfide gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

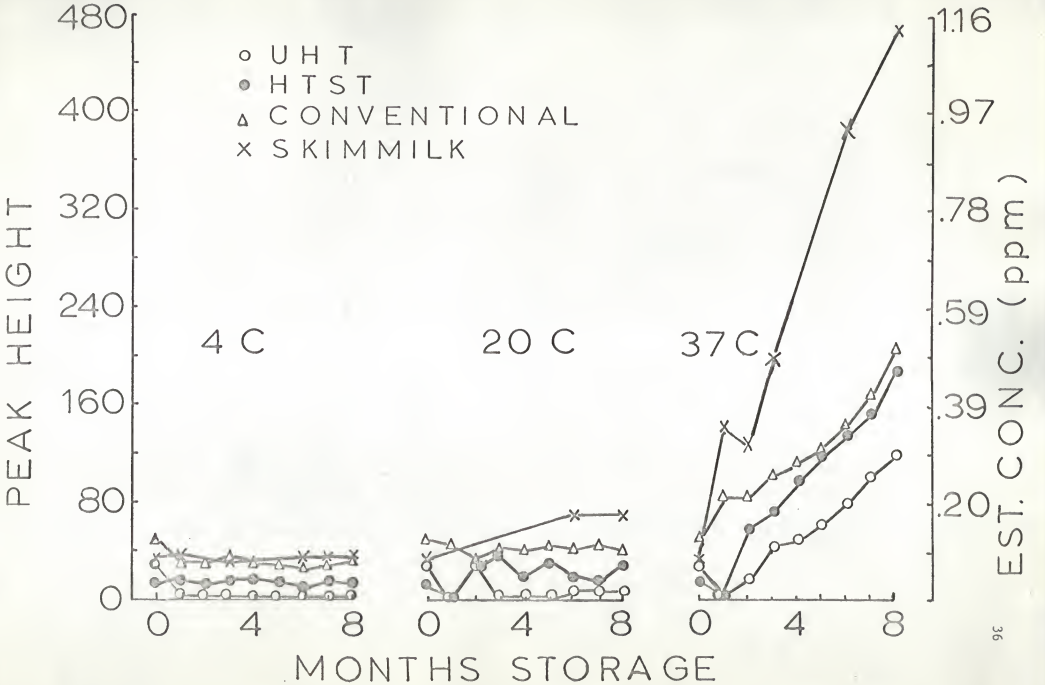


Figure 5. Monthly changes in furan gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

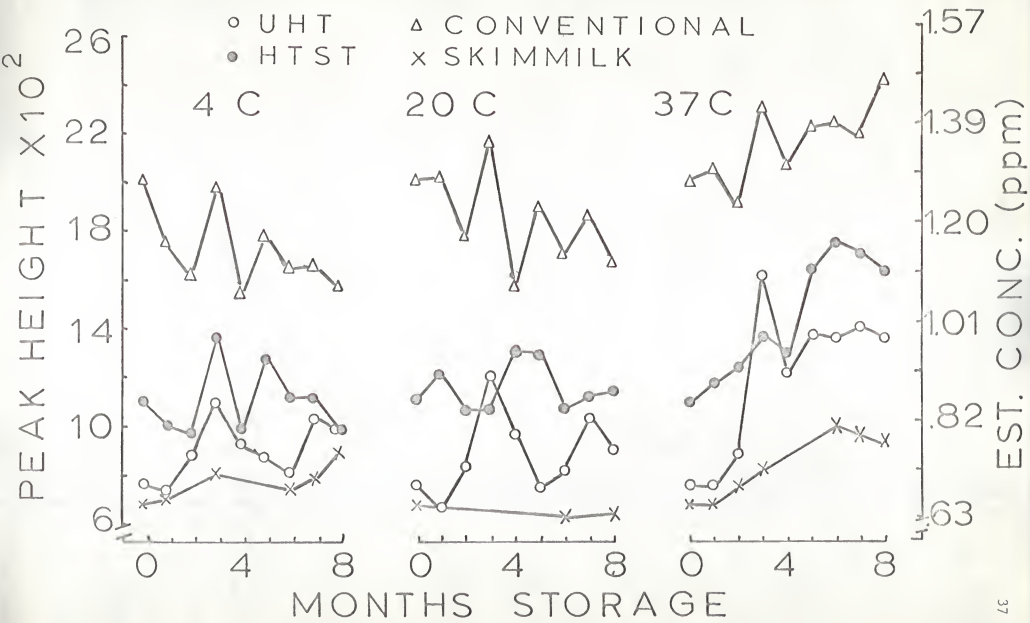


Figure 6. Monthly changes in acetone gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

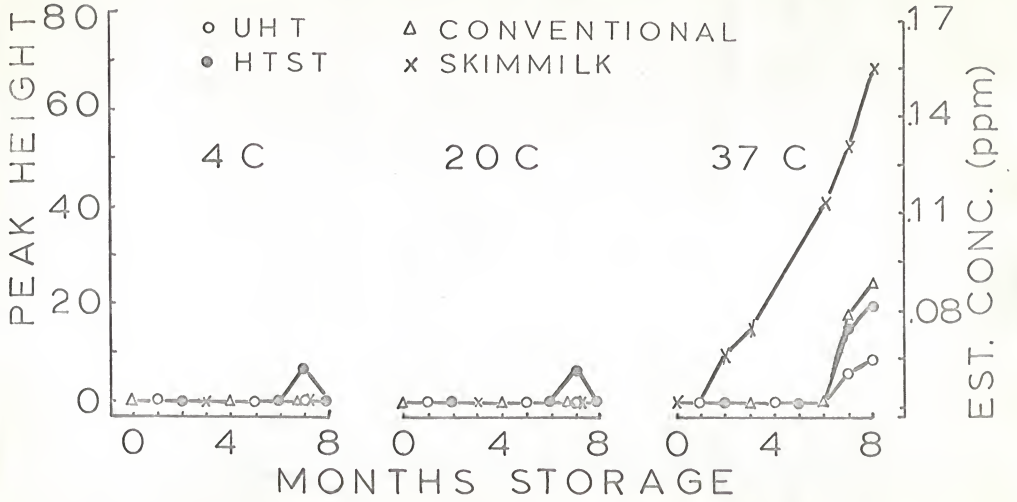


Figure 7. Monthly changes in 2-methyl furan gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

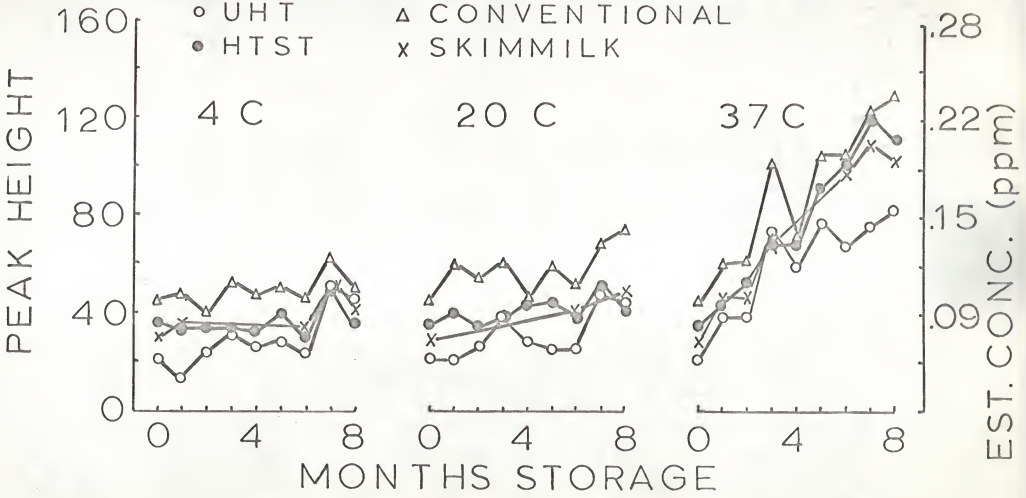


Figure 8. Monthly changes in butanone gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

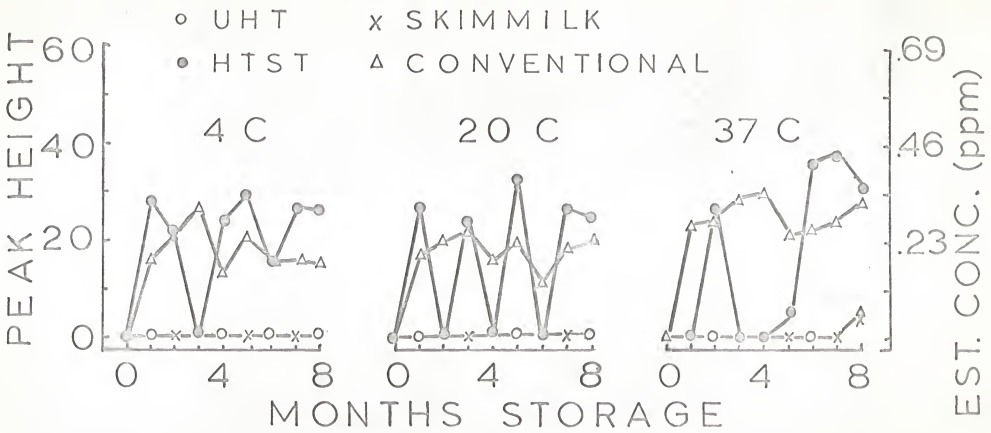


Figure 9. Monthly changes in n-butyl alcohol gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

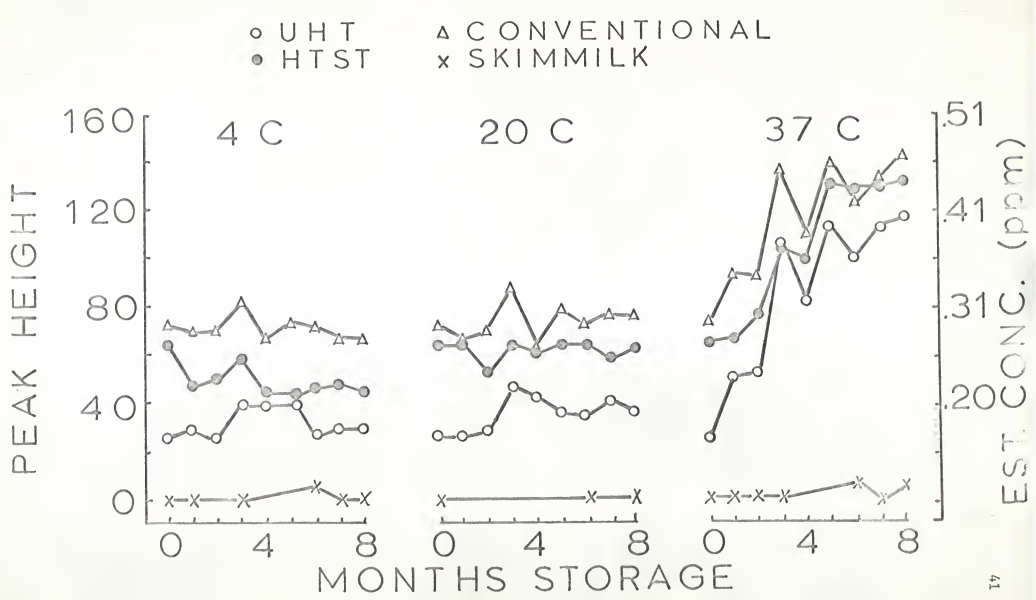


Figure 10. Monthly changes in 2-pentanone gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

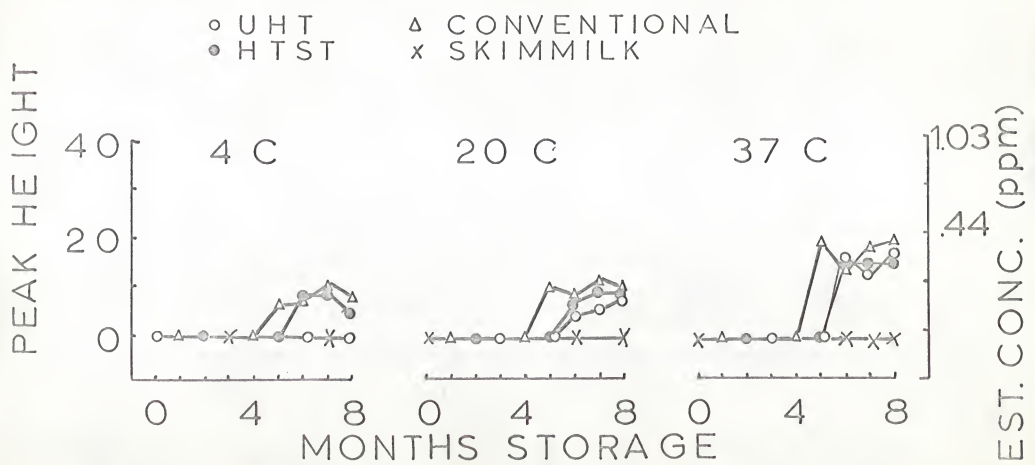


Figure 11. Monthly changes in 2-heptanone gas chromatographic peak heights and estimated concentrations in UHT, HTST, conventional and skimmilk stored at 4, 20 and 37 C.

alcohol since it was not affected by the basic hydroxylamine or the boric acid column reactions. After treatment of the milk with mercuric chloride, this peak was reduced by about 35%, which was similar to the reaction characteristics experienced with furan. On the basis of this circumstantial evidence, peak C was tentatively identified as furan.

A large increase in the concentration of furan during storage at 37 C is indicated in Fig. 5. The increase in this compound was considerably more rapid in the skimmilk than in the other samples. Little change was observed in the peak heights of furan from samples stored at 4 C and 20 C. Usually greater concentrations of the furan were found as the length of heating during processing of the concentrated whole milk was increased.

Peak D was identified as acetone since there was excellent agreement in retention time with pure acetone, and both were removed by reacting with acid hydroxylamine, but were not affected by the mercuric chloride treatment or the boric acid column. The graphs in Fig. 6 represent the changes in acetone peak heights during storage of the milk. Few consistent changes were observed in the 4 C and 20 C storage samples, but a gradual increase in acetone concentration was noted with increases in storage time at 37 C storage. The concentration of acetone in the skimmilk was considerably lower than in the other milks.

2-methyl furan was believed to be represented by peak E. Both peak E and authentic 2-methyl furan have retention times of 5.1 min and are completely removed by the mercuric chloride treatment, but not affected by the other selective reactions. It would not be surprising to find furans in heated or aged milk since many of the furan compounds have been associated with the Maillard browning degradation, which proceeds under these conditions (27).

Only in the HTST milk at 7 months storage was any 2-methyl furan detected in the milk samples stored at 4 C and 20 C. These peaks were at minimum detectable levels of concentrations. Fig. 7 shows the changes in 2-methyl furan concentration during storage of the milk. The samples stored at the higher temperatures showed increases in 2-methyl furan content as storage time was increased. This compound, like furan, increased more rapidly in the skimmilk than in the whole milk samples.

Peak D agreed in retention time with butanone and both peak D and that of butanone were removed by acid hydroxylamine. Therefore, this peak was identified as butanone. The peak heights of butanone in the milk are shown in Fig. 8. The levels of this compound encountered in all of the milk stored at 4 C and 20 C were in the range of the amounts often found in raw milk. A consistent increase in butanone was observed in all 37 C storage samples throughout this study.

Peaks G and L were identified as alcohols since they were removed by the boric acid column, but not affected by the other selective reactions that were used. As shown in Fig. 2, peak G was a shoulder at the end of peak F. It was not always possible to determine if this was a shoulder representing a compound or just a tailing effect after peak F. The retention time of peak G agreed with that of ethyl alcohol. Peak L was identified as n-butyl alcohol since its retention time was the same.

Other than being difficult to distinguish at some monthly examinations, the ethyl alcohol peak did not change much during storage of any of the samples. As illustrated in Fig. 9, n-butyl alcohol was found primarily in the HTST and conventional milk. Consistent concentration changes in butanol were not found in these milk samples. No explanation could be

found for the variation in peak heights of this compound from month to month or its absence in the UHT milk and skimmilk. This variation was especially prevalant in the H-20 storage samples, in which case the peak height was either zero or in the 26 to 34 range. Several replicate vapor analyses were made of samples that had either a peak height of zero or about 30 to determine if this could be instrument or sampling variation, or if the compound was actually totally absent or present in the initially observed range in each of the particular milk samples. Consistent peak heights were obtained from each milk sample, which indicated variation from sample to sample in the HTST milk rather than an actual monthly variation. Bingham (7) found a peak in sterilized milk which agreed in retention time with both n-butyl alcohol and 2-heptanone. Otherwise, this compound has not been reported in sterilized milk.

Peaks I and M (Fig. 2) were identified as carbonyl compounds by their positive reactions with acid hydroxylamine. The former peak agreed in retention time with both 2-pentanone and n-pentanal. The latter peak had the same retention time as 2-heptanone and n-heptanal when using the carbowax 20M column. However, the retention times of the authentic aldehydes and ketones were different when a Apiezon-L column was used for separations. The milk samples were analyzed by the Apiezon-L column and peaks were found that had the same retention times as the ketones, but not the aldehydes. Therefore, peaks I and M were believed to represent primarily, if not entirely, 2-pentanone and 2-heptanone respectively. The peak heights of the pentanone and heptanone during storage of samples are included in Fig. 10 and 11 respectively. Increases in concentration of pentanone with storage time, were observed in the concentrated whole milk samples stored at 37 C. However, no consistent changes were found at the other temperatures

of storage. Essentially no pentanone or heptanone were found in any of the skimmilk samples. A consistent increase in heptanone concentration was observed in all of the concentrated whole milk, but did not occur until after the fourth month of storage.

The concentration of the compounds represented by peaks H, J and K was too low to determine accurately their reaction characteristics. Consequently they could not be identified. Peak K appeared only in the chromatographs of the skimmilk and is not shown in Fig. 2.

Effect of Processing on Volatile Compounds. When possible, milk samples were collected after various processing stages and gas chromatographic patterns were determined. The effect of manufacturing on volatile compounds in conventionally sterilized milk before storage is illustrated in Fig. 12.

Although the volatile compounds of the specific raw milk used in manufacturing the sterilized milks were not determined, the acetone (Peak D) peak height from the conventionally forewarmed milk (Fig. 12) was 4 to 10 times greater than those normally encountered in raw milk. The vacuum concentration operation was successful in removing most of the volatile compounds from the conventionally forewarmed milk as shown in graph two of Fig. 12. The most noticeable change in this milk occurred when it was sterilized, when large increases in acetaldehyde, methyl sulfide, acetone and pentanone (Peaks A, B, D and I respectively) were observed. It has been demonstrated (16, 22, 31, 40) that many of these compounds are formed when isolated milk fat is heated.

Results similar to those described for the conventionally manufactured milk were observed when the HTST, UHT and skimmilk were processed, except less of the carbonyls and more of the methyl sulfide and the compound believed to be furan were observed in the skimmilk.

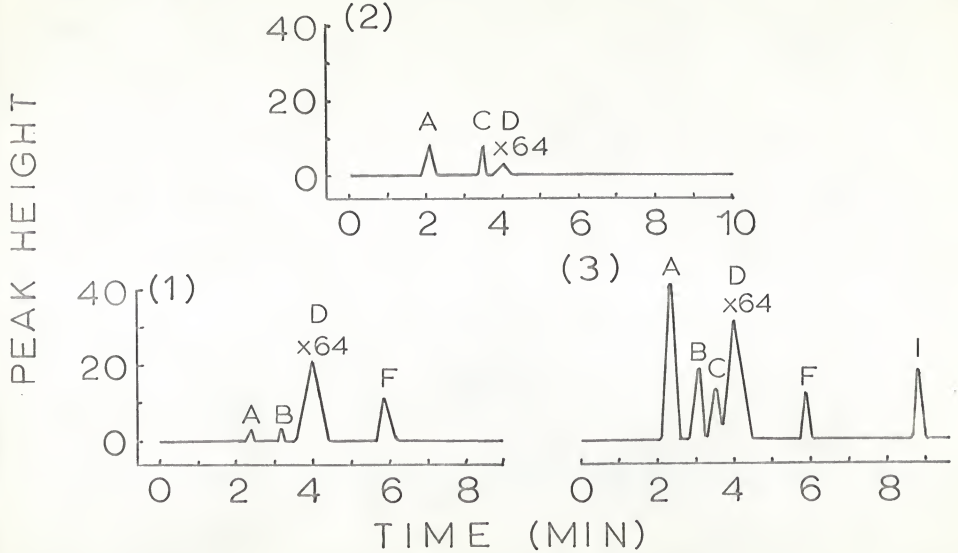


Figure 12. Gas chromatographic patterns of conventionally processed milk after (1) forewarming, (2) vacuum condensing and (3) subsequent sterilization (all peaks have attenuation factors of x 4 unless otherwise indicated above peak).

To verify further the formation of volatile compounds by heat, raw milk was heated in the laboratory at 118 C in an autoclave and 98 C in flowing steam and gas chromatographic analyses were performed. These results are shown in Table 8. Increases in most of the carbonyl compounds, methyl sulfide and furan were observed as the level of heat treatment was increased. The butanone concentration, which was higher than normally found in raw milk, was not increased by the heat treatments. It should be noted that the milk heated in the 118 C autoclave was sealed in cans while the milk heated at 98 C was in bottles with loose fitting lids during heating. Some of the valatile compounds probably escaped from the latter milk during the heat treatment.

Table 8. Effect of heat on gas chromatographic peak heights of volatile compounds in unconcentrated milk.

	Heat treatment						
Compound	Raw Milk	10 min 118 C	20 min 118 C	30 min 118 C	15 min 98 C	30 min 98 C	60 min 98 C
	(Peak Heights)[a]						
Acetaldehyde	5	14	36	155	41	51	71
Methylsulfide	0	9	38	62	27	40	67
Furan	0	0	0	38	0	0	0
Acetone	2281	2286	2354	3102	2511	2435	2458
Butanone	212	200	194	228	220	214	214
Peak H	0	0	0	0	0	0	6
Pentanone	0	0	14	80	7	17	31
Heptanone	0	0	0	12	0	0	0

[a] % of full scale recorder deflection x attentuation factor.

In practically all lots of milk processed for the storage study the concentration of the ketones (Fig. 6, 8, 10, and 11) were consistently higher in the samples which received the most severe heat treatment during processing, and lower amounts in the milk receiving less heat. Also, the ketones in the skimmilk, except for butanone, were lower than in the milk containing fat, or nonexistent. These data would indicate that most of

these ketone precursors are in the lipid fraction of the milk. Langler and Day (16) found that these ketones were produced in a similar manner when milk fat was heated in the presence of water.

Other Chemical Analyses

Rancidity. The acid degree value (ADV) results are summarized in Table 9. Few changes were observed in ADV of milk samples stored at 4 C throughout the 8 months storage period, but slight increases were noted in all samples stored at 20 C, and large increases observed in 37 C storage samples. The type of heat process affected the ADV very little in the overall experiment.

Table 9. Monthly acid degree values of sterile milk during storage.

Sample[a]	Storage time (months)								
	0	1	2	3	4	5	6	7	8
U-4	1.44	1.33	1.66	1.82	1.90	1.69	1.65	1.51	1.53
U-20	1.44	1.45	1.76	1.77	1.98	1.71	1.71	1.67	1.78
U-37	1.44	1.63	2.09	1.96	2.73	2.39	2.65	2.75	3.15
H-4	1.50	1.47	1.66	1.77	1.84	1.60	1.56	1.51	1.59
H-20	1.50	1.47	1.66	1.82	2.03	1.64	1.80	1.72	1.80
H-37	1.50	1.65	2.09	2.05	2.64	2.17	2.74	2.68	3.08
C-4	1.56	1.42	1.76	1.67	1.88	1.53	1.45	1.49	1.64
C-20	1.56	1.47	1.81	1.91	1.93	1.69	1.67	1.69	1.75
C-37	1.56	1.51	1.90	2.08	2.59	2.28	2.51	2.64	2.99

[a] U, H and C indicates process and 4, 20 and 37 indicates storage temperature.

By fresh milk standards (34), all of the milk samples in this study would be considered rancid on the basis of the ADV. It was not determined if these high ADV represented only free fatty acids or if other fat soluble acidic compounds were present. It is interesting to note that Sundararajan

et al. (33) described aged sterile milk as having an acidy-type flavor, among other defects. A panelist in this present study also described aged milk samples as having an acid-type defect. Very seldom is sterile milk criticized for being rancid, but other defects could conceivably mask or alter this defect somewhat.

In an attempt to determine if the ADV of the milk samples used in this study were abnormally high for sterile milk, two samples of commercial evaporated milk, processed by different manufacturers, were obtained from a retail market and their ADV determined. These samples had ADV of 1.29 and 1.55, which was in the same general range as the experimental milk.

Using a solvent extraction procedure Adams, et al. (1) found ADV of evaporated milk, stored four years at refrigeration temperature, to range from 0.392 to 1.052. The average ADV of the stored samples in the 4 year study were about 50% higher than the ADV of freshly processed milk, as determined by the same procedure.

To determine if lipid hydrolysis could be brought about by heat at levels approximating that used for processing evaporated milk raw whole milk was heated in flowing steam and in an autoclave. The ADV results on these samples are presented in Table 10. These data indicate that hydrolysis as measured by ADV is not proceeding under these quiescent heating conditions.

With the storage samples it is likely that some triglyceride hydrolysis proceeded at higher temperature storage conditions, but the high initial ADV of the sterile milk samples is not yet understood. Any free fatty acid formation, especially shorter carbon chain acids, would surely be important in the flavor of the milk.

Table 10. Acid degree values of heated raw milk samples.

Sample	Heat treatment		ADV
	Temp. (C)	Time (min)	
1	0	0	0.98
2	118[a]	10	0.97
3	"	20	0.89
4	"	30	0.93
5	98[b]	15	0.89
6	"	30	0.92
7	"	60	0.89

[a] Chamber temperature.

[b] Milk temperature.

Browning. The results indicating the degree of browning, as measured by the 2-hydroxmethylfurfural (HMF) content, are presented in Tables 11 and 12. Data are presented in light absorbance units and are not calibrated directly into concentration of HMF, but increases in light absorption indicate an increase in HMF content.

Although the results of the browning test varied considerably from month to month, an increase in both free (Table 11) and bound (Table 12) HMF was observed in all milk samples during 37 C storage. The increases were less in the UHT processed milk than in the other milk samples which received more heat during processing. Very few consistent changes were found in the 4 and 20 C storage samples. Adams et al. (1) observed only a slight color change in evaporated milk stored at refrigeration temperatures for 4 years.

After the fifth month of this study it was observed that the TBA-HMF reaction apparently was not complete in the test for browning at the end of the 40 C incubation period and color development continued even at room temperature. To control this variation, all absorption readings for the sixth through the eighth month were made within 5 min after removing the

Table 11. Changes in free HMF light absorption values of sterile milk during storage at three temperatures.

Sample[a]	Storage time (months)								
	0	1	2	3	4	5	6	7	8
U-4	.031	.026	.053	.035	.038	.039	.041	.033	.034
U-20	.031	.034	.051	.031	.036	.041	.035	.032	.032
U-37	.031	.035	.064	.058	.057	.081	.091	.093	.111
H-4	.045	.036	.074	.055	.069	.056	.054	.044	.047
H-20	.045	.036	.073	.052	.054	.067	.065	.047	.047
H-37	.045	.047	.083	.-80	.086	.117	.129	.113	.135
C-4	.066	.051	.083	.058	.067	.066	.070	.054	.059
C-20	.066	.047	.077	.065	.086	.067	.068	.055	.057
C-37	.066	.059	.095	.093	.087	.116	.124	.119	.127
S-4	.052	nd[b]	nd	nd	nd	nd	.063	.045	.047
S-20	.052	nd	nd	nd	nd	nd	.068	nd	.057
S-37	.052	nd	nd	nd	nd	nd	.103	.100	.130

[a] U, H and C indicates process, S indicates skimmilk, and 4, 20 and 37 indicates storage temperature.

[b] Not determined.

Table 12. Changes in bound HMF light absorption values of sterile milk during storage at three temperatures.

Sample[a]	Storage time (months)								
	0	1	2	3	4	5	6	7	8
U-4	.194	.106	.192	.124	.231	.270	.211	.244	.211
U-20	.194	.152	.199	.121	.233	.286	.222	.266	.252
U-37	.194	.168	.237	.218	.443	.520	.342	.515	.525
H-4	.291	.264	.300	.255	.315	.385	.221	.337	.282
H-20	.291	.226	.249	.172	.335	.345	.247	.310	.313
H-37	.291	.231	.266	.344	.465	.575	.470	.590	.619
C-4	.453	.314	.329	.260	.442	.410	.317	.392	.338
C-20	.453	.271	.301	.251	.380	.351	.250	.359	.365
C-37	.453	.274	.296	.376	.545	.536	.404	.600	.568
S-4	.275	nd[b]	nd	nd	nd	nd	.250	.355	.318
S-20	.275	nd	nd	nd	nd	nd	.212	nd	.319
S-37	.275	nd	nd	nd	nd	nd	.393	.524	.525

[a] U, H and C indicates process, S indicates skimmilk, and 4, 20 and 37 indicates storage temperature.

[b] Not determined.

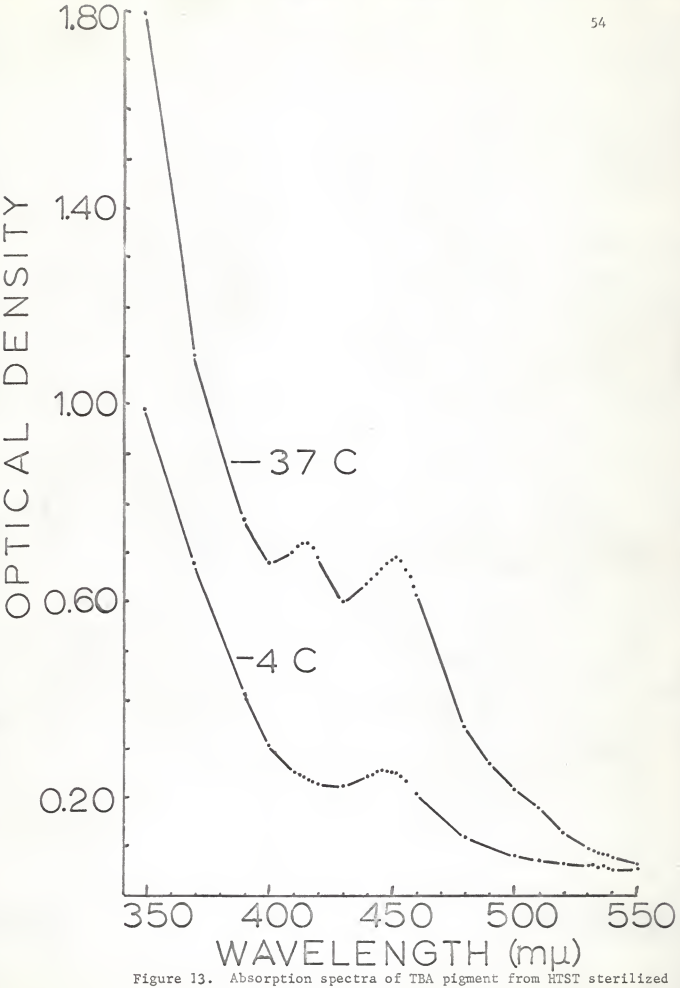


Figure 13. Absorption spectra of TBA pigment from HTST sterilized milk after 8 months storage at 4 and 37 C.

the addition of stabilizing salts. Therefore, such salts were not added to the milk. No visible protein coagulation was observed in any of the samples after sterilization or at any time during storage.

Table 13. Amount of fat separation in sterile during storage.

Sample[a]	Months storage		
	0	6	8
		(grams)	
U-4	0	0.5	1.0
U-20	0	1.0	9.1
U-37	0	10.0	20.7
H-4	0	0.5	8.1
H-20	0	13.0	19.3
H-37	0	32.8	34.1
C-4	0	0	1.0
C-20	0	2.0	17.5
C-37	0	33.5	36.6

[a] U, H and C indicates process and 4, 20 and 37 indicates storage temperature.

Initial viscosities and their changes during storage are shown in Table 14. Only slight viscosity changes were observed in any of the groups of milk. The greatest change was in the C-37 lot where the viscosity decreased from 17.5 to 9.8 centipoise. The viscosity decreases were probably related to the separation of the fat from the rest of the milk at higher storage temperatures. Normally the fat could not be re-dispersed in the milk without agitation, which was avoided as much as possible during these measurements. No large monthly increases or gelation trends were observed in any of the groups of milk.

The amount of sediment found in the bottoms of the milk cans during their monthly examination is shown in Table 15. Practically no sediment was observed in any of the milk samples during the quiescent storage term. It

Table 14. Viscosity changes in sterile milk during storage.

Sample[a]	Months storage								
	0	1	2	3	4	5	6	7	8
	(centipoise)								
U-4	9.5	10.2	9.6	11.5	9.0	9.2	12.8	13.2	16.2
U-20	9.5	10.3	9.8	10.5	9.5	9.7	10.5	10.8	11.2
U-37	9.5	9.0	8.5	8.5	8.5	8.0	9.5	9.7	8.5
H-4	10.2	10.5	9.5	10.5	8.7	9.0	11.5	11.0	11.5
H-20	10.2	9.5	9.5	9.0	11.3	10.2	9.0	8.8	9.3
H-37	10.2	9.0	9.8	10.0	9.5	9.4	10.5	8.0	7.2
C-4	17.5	20.0	17.6	20.6	14.5	15.0	15.5	16.2	16.5
C-20	17.5	16.0	13.5	15.0	10.5	13.8	12.2	14.6	15.6
C-37	17.5	13.0	11.6	11.6	13.0	12.5	11.2	10.0	9.8

[a] U, H and C indicates process and 4, 20 and 37 indicates storage temperature.

Table 15. Sedimentation changes in sterile milk during storage.

Sample[a]	Months storage								
	0	1	2	3	4	5	6	7	8
	(mm)								
U-4	0	0	sl[b]	0	0	0	0	0	sl
U-20	0	0	sl	sl	sl	sl	sl	1.0	1.0
U-37	0	0	sl	sl	2.0	2.0	2.0	2.0	2.0
H-4	0	0	0	0	0	0	0	0	0
H-20	0	0	0	sl	0	sl	sl	sl	sl
H-37	0	0	0	sl	sl	sl	2.0	2.0	1.0
C-4	0	0	sl	0	0	0	0	0	0
C-20	0	0	0	sl	sl	sl	sl	sl	sl
C-37	0	0	0	0	sl	sl	2.0	1.0	1.0

[a] U, H and C indicates process and 4, 20 and 37 indicate storage temperature.

[b] Less than 1.0 mm sediment.

is particularly important to note the amounts of sediment in the UHT processed milk as this defect is often severe in this type of milk. Swanson (34) has reported the heat treatment of the concentrate decreased the amount of sediment formed in some types of sterilized milk.

Overall the milk samples studied in this experiment possessed the physical properties normally found in these types of milk.

CONCLUSIONS

Based on the results of this experiment the following conclusions are made:

1. Processing treatments significantly affected the flavor and browning characteristics of the milk, both initially and during storage, but had little effect on ADV. The quality of the milk was in the decreasing order of UHT, HTST and conventionally processed milk.

2. Storage temperature was a critical factor in the rate of flavor deterioration, browning changes and ADV changes, where lower storage temperatures showed fewer changes.

3. No consistent relationships were found between changes in volatile compounds measured and flavor scores or flavor defects.

4. Some ketones were produced when milk containing the lipid phase was heated.

5. During storage few changes were observed in the physical properties of the milk except fat separation was prevalent at 20 and 37 C storage in all types of processed milks.

6. The need exists for refinement of methods for measuring chemically the degree of oxidation and browning, and for development of other methods of isolating flavor compounds which give good or bad flavor characteristics.

7. The best flavor and keeping quality were obtained by UHT processing and 4 C storage for less than 4 months.

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SOME CHEMICAL AND FLAVOR CHANGES OF STERILE CONCENTRATED MILK DURING STORAGE

by

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B. S., Oklahoma State University, 1961

AN ABSTRACT OF A MASTER'S THESIS

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This study was designed to determine the effect of processing conditions, storage temperature and storage time on the organoleptic, and some of the chemical and physical characteristics of sterile 2:1 concentrated whole milk.

Concentrated milk samples were sterilized by three methods using different time-temperature relationships, ultra-high temperature (UHT - 144 C for 4.0 sec), high-temperature short-time (HTST - 112.8 C for 4.9 min plus 125.6 C for 2.7 min), and conventional (117.2 C for 12.3 min). Less severe total heating is required for sterilization by the UHT method, followed by HTST and conventional processes. Each type of processed milk was stored at each of three temperatures, 4, 20 and 37 C for 8 months.

Initial and monthly evaluations of flavor quality, gas chromatographic measurements of changes in volatile compounds, rancidity and browning were made on each of the nine groups of milks. Occasional evaluations also were made on concentrated skimmilk (2:1) processed by the HTST method and stored at each temperature.

Organoleptic results indicated that samples which received the least severe heat treatment during sterilization, and the lowest storage temperature were of the highest flavor quality. After about 4 months storage, differences in the flavor scores between types of processed milk were small at each particular temperature of storage. All types of milk were defined as having a cooked flavor initially. All 37 C storage samples were criticized for having extremely high scorched and stale flavors. The UHT processed milk was criticized as being slightly oxidized at 4 and 20 C storage. Other defects noted in the milk samples during storage were astringent, chalky and high acid.

Gas chromatographic analysis of the milk revealed that acetaldehyde, dimethyl sulfide, acetone, ethyl and n-butyl alcohol, butanone, 2-pentanone, 2-heptanone and compounds believed to be furan and 2-methyl furan were found in at least some of the milk samples. Selective reactions to remove specific classes of chemical compounds, and comparison of retention times with authenic compounds were used for identification purposes. Three unidentified gas chromatographic peaks were found in some of the milk.

Dimethyl sulfide, furan and 2-methyl furan were in greater concentrations in the skimmilk. At 37 C storage, the increase in furan and 2-methyl furan was rapid. The acetone, pentanone and heptanone were much greater in the whole milk samples, than in the skimmilk, which would indicate they were formed from some fraction of the lipid phase. Butyl alcohol was found in HTST and conventionally processed milks, and was quite erratic from month to month as was the acetaldehyde. Volatile compounds were usually in greater concentrations in milks receiving the most severe heat treatment during processing and the greater temperature of storage.

Although the milk did not possess a rancid flavor, the acid degree values (ADV - 1.33 to 3.15) were in the range normally considered rancid in fresh market milk. These high ADV were noted in all types of processed samples initially and did not change much at 4 and 20 C storage. Increases in ADV were observed in all samples stored at 37 C.

Results of tests for the browning reaction varied somewhat throughout the study. The milk samples receiving the most severe heat sterilization and higher temperatures of storage showed more tendency to brown.

Viscosities of the milks ranged from 8.0 to 20.6 centipoise over the entire storage of all samples. Little fat separation was found in the 4 C