SOLID PHASE EXTRACTION OF STALE FLAVOR COMPONENTS FROM ULTRA-HIGH-TEMPERATURE PROCESSED MILK

by

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INTRODUCTION

Ultra-high-temperature (UHT) processed milk can be described as milk that has been processed to extremely high temperatures for short periods of time and aseptically packaged. The objective of this method of processing is to produce a commercially sterile product while minimizing the heat-induced chemical reactions that are responsible for flavor defects. Although there is no one definition for UHT milk, it is generally accepted that the processing temperature is from 135°C-150°C and held for a few seconds. Increasing the heat treatment will provide an even greater sterilization effect on bacteria with a decrease in bacterial spoilage, but is limited by the unacceptable chemical changes which are brought about by the heat processing. These chemical reactions can cause adverse effects on the color, flavor and nutritional attributes of UHT processed milk. UHT milk processing time and temperature parameters, therefore, provide a good balance between a maximum sterilization of bacteria and minimum chemical reaction rate.

Shelf-stability at ambient storage temperatures is UHT processed milk's greatest advantage over fresh pasteurized milk. It maintains its high nutrient value without a requirement for refrigeration. Renner and Berlage-Weinig (1983) summarized that UHT milk has color, flavor, and nutritive value equal to pasteurized milk and superior to conventionally sterilized milk. Because of these factors, UHT milk could provide a good solution for the preservation of the large

milk surpluses in the United States and could also be used to exploit new markets where nutrition, storage, or distribution problems are commonplace. These new markets may include underdeveloped countries, the military, and camping enthusiasts (Jelen, 1982). Replacing or substituting UHT milk for fresh pasteurized milk by consumers would be an even more fundamental use of this technology.

The potential cost and energy savings when compared to fresh pasteurized milk could represent a significant amount. A reduction in cost and energy could be realized at the wholesale, retail, and consumer level. The need to purchase refrigeration equipment such as compressors, insulated warehouses, display cases, refrigerated trucks and even home refrigeration could be reduced or eliminated. Additionally, the reduction or elimination of this equipment would result in a significant decrease in energy costs as well. Finally, distribution costs could be lowered by reducing the frequency of replenishment by increasing stockage levels. This could reduce personnel, equipment and energy costs for the manufacturer and retailer by allowing for more efficient production scheduling while minimizing down time.

Although UHT processed milk has been widely accepted by consumers in Europe, it has not gained the same popularity in the United States. This lack of acceptance can be partially credited to cost and lack of serious marketing. However, the flavor of UHT milk is probably the most limiting factor. UHT milk is criticized as having an off-flavor (cooked or stale) when compared to fresh

pasteurized milk as a result of the various chemical reactions that have taken place. American consumers, however, may be overly sensitive to the flavor of UHT milk because they have been conditioned to equate the taste of fresh pasteurized milk as the standard for what fresh milk should taste like. This conditioning has been reinforced by the availability of fresh milk supplies, refrigeration equipment, and the advanced distribution systems present in this country.

Since the off-flavor of UHT processed milk serves as a serious impediment towards consumer acceptance and marketing success, the study in this area is both relevant and beneficial. The purpose of this study is to determine the cause of stale flavor development in UHT processed milk. Specific objectives of our research are to isolate and identify the chemical compounds responsible for stale flavor development in UHT processed milk utilizing C_{18} Sep-Pak cartridges as a tool for entrapping or removing these compounds.

REVIEW OF LITERATURE

I. Definitions

There are many definitions available for ultra-high-temperature (UHT) milk. Burton (1988) described the UHT process as one in which the product is heated to a temperature of 135-150°C in continuous flow using a heat exchanger. The International Dairy Federation defines UHT milk as having been subjected to a continuous flow heating process at a high temperature for a short time. The PHS/FDA Grade A milk Ordinance and Code requires that the products be heated to not less than 137.8°C (280°F) with at least a 2 second holding time (Wainess, 1982). Although no two definitions may be exactly alike, it is generally accepted that UHT milk has been heat-treated to extremely high temperatures for short periods of time and aseptically packaged. The time-temperature standards are usually in the range of 135-149°C for 2 to 8 seconds (Hsu, 1970).

II. History

The development of UHT processed milk is extensively reviewed (Burton, 1988; Westhoff, 1978). Jonas Nielsen had pioneered the first recorded UHT processing plant by 1913 and later developed an aseptic canning system. In the late

1940's, plants started using higher processing temperatures and shorter holding times to give products better bacteriological quality which resulted in less change of color, flavor, and nutritive value of the milk. UHT milk was produced and distributed as early as 1940 in the United States and marketed in Switzerland in 1953 Burton (1988).

"Fresh-tasting," long shelf-life milk was produced by sterilizing milk in bottles in the 1940's and in cans in the 1950's (Hsu, 1970). In 1951, a rectangular polyethylene-coated paper bond container, with an aluminum foil laminate barrier was introduced in Switzerland by Tetra Pak, Sweden (Mehta, 1980). The aseptic systems, first by Tetra Pak along with the development of a wide variety of heat exchangers has led to the present state of aseptic processing worldwide (Burton, 1988).

III. Consumption

UHT processed milk has been available for over 30 years in Europe. This processing method with aseptic filling of milk and milk products is now practiced in more than 60 countries (Burton, 1988). In Germany, approximately 40% of the milk consumption in 1983 was in the form of UHT milk (Anon, 1983). In 1986, however, Italy led the way with 56.9%; Portugal 50.9%; Spain 39.8%; Germany 38.6%; and Switzerland 32.3% (Burton, 1988). In the United States, consumption

of UHT processed milk is less than 1% of the total milk sales. This is a result of poor consumer acceptability brought about by the off-flavors associated with UHT milk as well as other factors already discussed.

The largest user in the United States is the military which consumed UHT processed milk for practically all of their training exercises. The following table compares UHT milk consumption (%) in 1986 to 1975 for Western Europe.

Country	1986	1975
Austria	4.6	2
Belgium	52.3	15
Denmark	5.4	2
Finland	1.5	1
France	54.4	15
Germany	38.6	25
Greece	9.1	-
Italy	56.9	42
Netherlands	10.7	3
Norway	0.2	-
Portugal	50.9	-
Spain	39.8	10
Sweden	0.9	-
Switzerland	32.3	26
United Kingdom	2.5	1

Source: (Burton, 1988)

The above data shows large increases in consumption for some countries and little or no increase for others. This may be due to different consumer preferences, cost considerations, and effective marketing strategies among the various countries.

IV. UHT Processing Systems

The two primary methods used for the manufacture of UHT processed milk today are direct and indirect systems. Figure 1 below provides a summary of the systems.

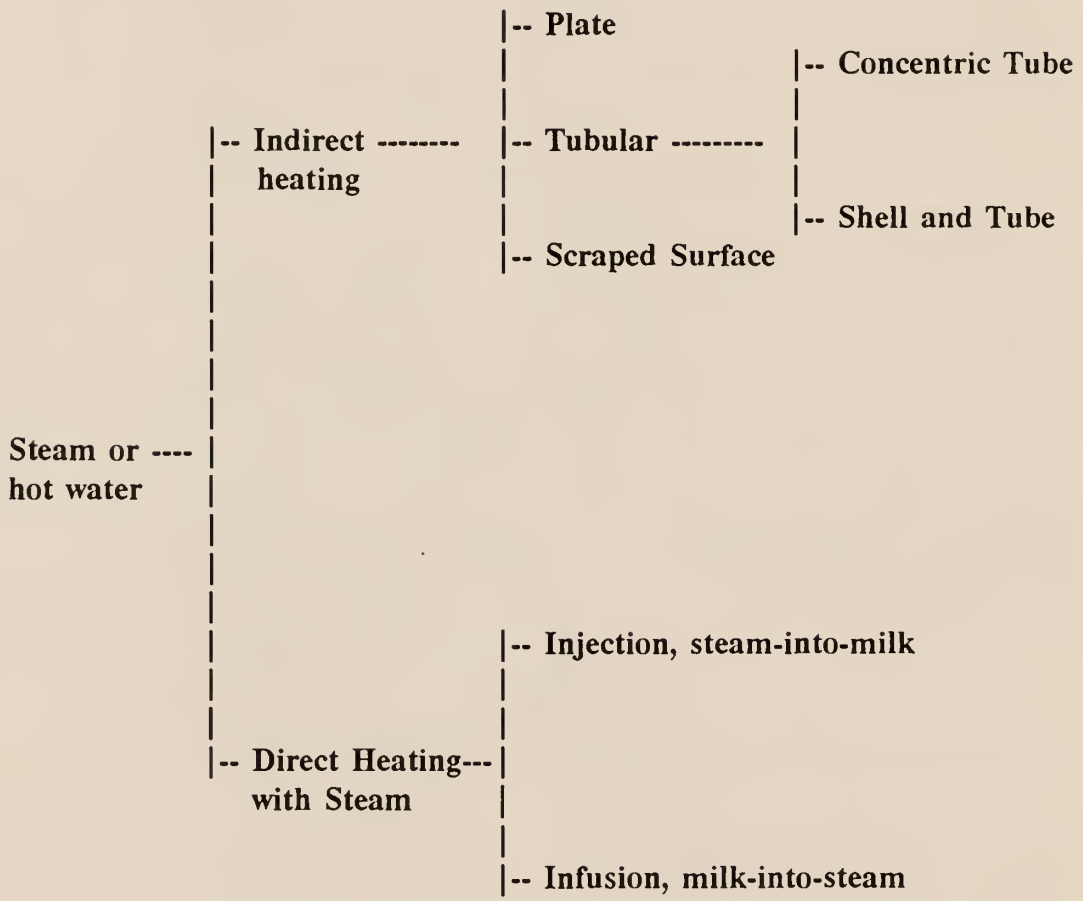


Figure 1. Types of UHT Sterilizing Processes (Burton, 1988).

In direct heating systems, the product is mixed directly with steam under pressure, so that the steam is condensed and its latent heat of vaporization is

transferred to heat the product very rapidly (Mehta, 1980; Burton, 1988). Steam can either be injected into the milk or milk can be sprayed into an atmosphere of steam (Infusion). Condensation of the steam into the milk causes dilution of the product which is compensated using a "flash-down" vacuum treatment which removes the added water and cools the milk at the same time (Rerkrai, 1986).

In the indirect heating system, milk is heated through a physical heat-conducting barrier (usually of stainless steel) between the product and the heating medium of steam or pressurized hot water. Examples include tubular, plate and scraped surface indirect processes (Mehta, 1980).

V. Effects of UHT Processing and Storage

A. Nutrition

The properties of UHT-processed milk are influenced by changes that result from the heat treatment and those occurring after the heat treatment during storage. Overall, there is little change in the nutritional quality of UHT processed milk when compared to fresh pasteurized milk.

The nutritive value of protein in severely heated milk may be impaired, because of a drop in the availability of lysine brought about by the Maillard reaction. However, there is little or no adverse effect on the nutritional quality of proteins during UHT heat treatment (Burton, 1988; Renner and Berlage-Weinig

1983; Ford & Thompson, 1981). During storage, the Maillard reaction leads to a covalent polymerization of the caseins, and to an increased resistance to proteolysis. It is not likely that these changes have a significant effect on nutritional and organoleptic qualities.

Physical or chemical changes in milk fat do not appear to have nutritional consequences from UHT processing. There is some loss of fatty acids in the milk triglycerides, linoleic acid, 33%; linolenic acid, 13%; and arachidonic acid, 7% (Pol & Groot, 1960) and up to a 30% loss of individual free fatty acids (Waycombe & Lindsey, 1969). During storage, the concentration of free fatty acids increases in UHT milk; however, their increase appears to be dependent on the storage temperature and type of processing. Free fatty acids are produced more rapidly at high storage temperatures than at low; with higher-fat milks; and for direct than indirect processing (Schmidt & Renner, 1978a). This is probably caused by the survival of resistant lipases of milk or from those produced by psychrotrophic organisms. The free fatty acids eventually lead to off-flavors in the product when their concentration exceeds the threshold of detectability (Burton, 1988).

The loss of vitamins during processing is dependent on the severity of the heat treatment and exposure to light or oxygen before or after processing. Typical vitamin losses due to various heat treatments are given in Table 1.

Table 1. Typical vitamin losses by heat treatment (derived from Ford and Thompson, 1981).

Vitamin	Loss (%)		
	Past.	Steril	UHT
Thiamin	<10	30	10
Riboflavin	NS	NS	NS
Nicotinic Acid	NS	NS	NS
Vitamin B_6	<10	20	10
Vitamin B_{12}	<10	<90	10
Pantothenic Acid	NS	NS	NS
Biotin	NS	NS	NS
Folic Acid	<10	50	15
Ascorbic Acid	20	90	25
Vitamin A	NS	NS	NS
Vitamin D	NS	NS	NS
Vitamin E	NS	NS	NS
B-carotene	NS	NS	NS

NS = Not significant

During storage and in the absence of light, the fat soluble vitamins A, D, and E are stable at room temperature at least for 3 months (Ford et al., 1969). Some water soluble vitamins suffer some loss during storage in the dark. The data in Table 2 shows the losses of some of the water-soluble vitamins.

Table 2. Losses of some water-soluble vitamins during storage of UHT milk in the dark.

Vitamin	Loss (%)		
	A	B	C
Thiamin	NS	10	NS
Riboflavin	NS	10	10
Nicotinic Acid	NS	20	-
Vitamin B_6	50	35	-
Vitamin B_{10}	40	-	15
Pantothenic acid	NS	30	-
Biotin	NS	20	-

NS = Not significant
A = Ford et al. (1969). 3 months at 15-19°C
B = Gorner & Uherova (1980). 6 weeks at 20-25°C
C = Thomas et al. (1975). 9 weeks at 23°C

The greatest loss of vitamins during storage of UHT milk is ascorbic acid and folic acid. Losses of these vitamins are interlocked, and are dependent on the availability of oxygen (Ford et al., 1969; Thomas et al., 1975). If the O_2 content is 1 mg/liter or less, and the UHT is stored in the dark in containers which are impermeable to oxygen, both ascorbic and folic acid are stable and may show a loss of only about 20% over a 3-month period (Ford et al., 1969; Thomas et al., 1975).

There is no loss of minerals as a result of UHT processing and storage (Henry & Touthill, 1960; Pelet & Donath, 1974). The mineral content of UHT

processed milk is just as high as for fresh pasteurized milk.

B. Microbiological

UHT processing is intended to destroy all the microorganisms present in both vegetative and spore forming states or at least make them incapable of growth in the product, so that a long keeping quality is obtained without refrigerated storage (Burton, 1988). This does not mean that the product is sterile in an absolute sense because it may contain some microorganisms, however, the remaining viable microorganisms are inhibited under the normal storage conditions. The term "commercially sterile milk" has been frequently used to describe UHT milk (Lembke, 1972). Microorganisms are destroyed by heat when the microbial proteins coagulate and enzymes required for their metabolism are inactivated. Although most microorganisms and their spores are destroyed by the UHT process, a few may be resistant to the UHT heat treatment. Spores of the obligate thermophilic soil bacterium Bacillus stearothermophilus and spores of mesophilic bacilli and clostridia may survive UHT treatment.

Atwal et al. (1974) examined different types of bulk milk from a commercial plant and found that the most common resistant spore strains were B. subtilis and B. stearothermophilus.

C. Color

UHT processing increases the reflectance of milk due to the denaturation of serum proteins and their aggregation with casein resulting in a whiter product (Williams et al., 1955; Burton, 1955; Horak & Kessler, 1981; Kessler, 1981). Milk becomes browner with the increased severity of heating as a consequence of the Maillard reaction between the lysine of the milk proteins and lactose (Adrian, 1974). The Maillard reaction lowers the degree of reflectance and therefore lightness caused by an increase in the green and yellow component in the milk (Bosset et al., 1979). During storage, lightness further decreases with a reduction in the green component and an increase in the yellow component (Zadow, 1970; Ismail and El Deeb, 1973). Only a small change in color can be attributed as a result of UHT processing.

D. Sedimentation and Age Gelation

Sedimentation is caused by the denaturation of the milk proteins or precipitation of the salts in milk resulting from the intensive heat used in UHT processing. This sediment settles to the bottom of the carton with some of the sediment returning to solution during storage (Burton, 1988). The degree of sedimentation increases with the intensity of heat treatment (Thome et al., 1964).

UHT processed milk shows a greater tendency to thicken and coagulate during storage than pasteurized milk. The cause of age gelation is not fully

understood although a lot of literature is available on the subject (Harwalker, 1981). Hostettler and Imhof 1965 suggests that a casein-serum protein complex is involved while Muir (1984) considers the milk fat to be involved. Other workers attribute it to proteolytic enzymes that originated from the growth of psychrotrophic vegetative organisms in the cold-stored raw milk that survived the UHT heat treatment (Burton 1988). The enzymes produced by a pseudomonad can breakdown κ-casein to para-κ-casein during storage in a similar way as rennet. This destabilizes the casein micelles and leads to gel formation.

E. Flavor

UHT processed milk exhibits various flavor changes. When fresh, there is a strong heated or cooked flavor with a sulfurous odor (Shipe et al., 1978). After a few days, the cooked flavor disappears to leave a characteristic flavor comparable to fresh pasteurized milk with a slight cooked residue. On storage, a stale flavor develops and increases in intensity over time (Burton, 1988). Even when the flavor of UHT milk is at its best, it is judged as inferior to that of pasteurized milk (Perkins, 1985). Consumer judgments, however, are made by people using raw or pasteurized milk as their standard and may therefore be biased or programmed when choosing a response. Finally, towards the end of the UHT shelf-life and just prior to age gelation, a bitterness may be detectable.

VI. OFF-FLAVOR IN UHT MILK

Ashton et al. (1965) categorized the flavor changes in UHT milk into five periods. Immediately after processing, there is a strong boiled cabbage flavor. After two or three days, there is a strong cooked flavor. From five to twelve days, a creamy taste develops which resembles the flavor of pasteurized milk. From twelve to eighteen days, a flat and chalky taste develops. After nineteen days, a stale flavor develops.

A. Cooked Flavor

The flavor of UHT processed milk is relatively poor after production, but improves within the first few days of storage (Ashton, 1965). The initial flavor is referred to as "cooked" or "sulfurous" which decreases in intensity during storage giving way to a residual cooked flavor (Shipe et al., 1978; Heath, 1983).

The initial sulfurous flavor arises from the formation of free sulfhydryl groups released during the denaturation of the serum proteins, mainly β-lactoglobulin of milk (Hutton & Patton, 1952; Burton, 1988). This gives it a strong hydrogen sulfide smell described before as "boiled cabbage." The free sulfhydryl groups are oxidized by dissolved oxygen during the first few days of storage resulting in a decrease in intensity of the sulfurous smell and cooked flavor (Clarke, 1967). The rate of disappearance depends upon the dissolved oxygen content (Thomas et al., 1975) as

well as storage temperature (Patrick & Swaisgood, 1976). The oxidation is slower in direct processed UHT milk than indirect UHT processed milk which has higher levels of dissolved oxygen available to oxidize the free sulfhydryl groups (Jordan 1968). In indirect-UHT processed milk with an initial high or medium dissolved oxygen content, the sulfhydryl groups decreased rapidly (Thomas et al., 1975). Patrick and Swaisgood (1976) found that the -SH groups decreased due to oxidation more rapidly at room temperature than at refrigeration temperatures. There are several ways to reduce the cooked flavor in UHT milk according to the literature (Badings et al., 1978; Renner and Berlage-Weinig, 1983; Swaisgood, 1980; Ferretti et al., 1974).

B. Stale Flavor

The flavor of UHT milk begins to deteriorate detectably after two to three weeks storage at room temperature (Burton, 1955; Thomas et al., 1975; Schmidt & Renner 1978a). Jeon (1976) summarized the following terms to describe the stale flavor in UHT milk: "oxidative rancidity or cardboardy" (Ashton, 1965), "rancid or oxidized" (Zadow and Birtwistle, 1973), and "stale" (Kirk et al., 1968; Thomas et al., 1975). Other researchers have described stale flavor as "aged" (Muck et al., 1963), "old rubber" (Patel et al., 1962), "cereal-stale" (Bassette, 1958), "cardboard" and "gluey" (Henry and Toothill, 1960). Hansen and Swartzel (1982) and Shipe et al. (1978) used the term "lack of freshness" instead of stale.

Badings and Neeter (1980) isolated and identified at least 400 volatile compounds from pasteurized milk and UHT processed milk by a combination of gas chromatography and mass-spectrometry. These compounds represent vary different classes of chemical compounds according to various researchers. Carbonyl compounds (Harper & Huber, 1956), dimethyl sulfide (Bassette et al., 1966), alkanols, fatty acids, lactones, esters, sulfur compounds, nitrogen compounds, aliphatic and aromatic hydrocarbons (Badings and Neeter, 1980). Jeon (1976) identified 8 methyl ketones ($C_{3,\ 5,\ 7\text{-}11,\ 13}$), 6 n-alkanals ($C_{5\text{-}10}$), 4 aliphatic alcohols ($C_{3\text{-}6}$), 2,3-butanedione, 2-furfurals, benzaldehyde, toluene, ethylbenzene, and methyldisulfides. Scanlon et al. (1968) identified $C_{3\text{-}5,\ 7\text{-}11,\ 13}$ n-methylketones, $C_{8,\ 10,\ 12}$ delta-lactones, benzaldehyde, furfural phenylacetaldehyde, vanillin, oct-1-en-3-o1, n-heptanal, 2-butoxy-ethanol, maltol, acetophenone, benzothiazole and diacetyl. Rerkrai (1986) reported that acid degree valve (ADV), dissolved oxygen and titratable acidity correlated closely with the stale flavor in UHT milk. Increases in concentrations of acetaldehyde, propanal, 2-hexanal, 2-pentanone, 2-hexanone and 2-heptanone paralleled the stale flavor (Rerkrai, 1986).

Many psychrotrophic lipases and proteases are heat stable and are able to hydrolyze lipids causing lipolyzed flavor during storage (Adams and Brawley, 1981; Driesen, 1983; Christen and Wang, 1985; Mottar, 1981). Also, heat stable lipases from pseudomonas bacteria that survived the high temperature may contribute to lipolyzed flavor (Adams and Brawley, 1981; Driessen and Stadhouders, 1974). Both

heat resistant proteases and lipases can appear in milk as the result of bacterial growth and lead to off-flavor development during storage (Burton, 1988). According to Schmidt and Renner (1978a), free fatty acids are produced in storage as a result of the survival of resistant lipases from either the natural lipases of milk or those produced by psychrotrophic organisms leads to off-flavors. Although many compounds have been identified in UHT milk to date, which of the many compounds responsible for stale flavor development is not yet known.

C. Isolation

Isolation of the stale flavor compounds is extremely difficult because of the large number of compounds that have been identified in milk. Many of these compounds have diverse functional groups, low sensory thresholds, and that often, flavor components in minute concentrations contribute more towards flavor than those in higher concentrations.

VII. Factors Affecting Flavor of UHT Milk

A. Raw Milk Quality

According to Rerkrai (1986), in order to produce UHT milk which is close to pasteurized milk in physical, chemical, and organoleptic characteristics, three major factors must be taken into account. They are the quality of the raw milk, the quality of the UHT milk immediately after processing, and the quality of stored UHT milk. Flavor defects due to lipolysis can be minimized by reducing the activities of the serum lipases in raw milk or lipases from bacterial origin that survive the UHT process. Bacterial lipase levels can be restricted by avoiding contamination of the raw milk with pseudomonads and lowering the storage temperature before heat treatment (Burton, 1988; Zadow, 1980; Kosaric et al., 1981).

B. Storage time and temperature

Chemical reactions and stale flavor development in UHT processed milk increase when stored at higher temperatures (Mogensen and Poulsen, 1980; Kirk et al., 1968). It has been confirmed by many researchers that refrigeration minimizes the changes which occur in UHT milk during storage (Bassette and Jeon, 1983; Aoki et al., 1977; Gorner et al., 1977; Hansen and Swartzel, 1982). Zadow (1984) found that the heat treatment resulted in a rapid increase in the rate of

Maillard reaction with the rate continuing in storage depending on storage conditions. Burton (1988) believes that the Maillard reaction occurring due to protein-lactose interaction during storage contributes to the decline in the acceptability of UHT milk. Mehta and Bassette (1980) observed that stale flavor developed more rapidly when stored at a higher temperature than a lower temperature. They also reported that increased propanal, n-pentanal, and n-hexanal paralleled the stale flavor intensity (Mehta and Bassette, 1978). Bassette and Jeon (1983) found increases in concentrations of volatile compounds primarily aliphatic aldehydes (acetaldehyde, n-pentanal and n-hexanal) at room temperature with little change during refrigeration temperatures. Similar results were reported by Wadsworth (1984) and Jeon et al. (1978). Rerkrai (1986) reported that stale flavor of UHT milk developed sooner at room temperature than at refrigerated temperature and that concentrations of acetaldehyde, propanal, n-hexanal, 2-pentanone, 2-hexanone and 2-heptanone increased more at room temperature than refrigeration temperature.

During storage of UHT milk at room temperature for 2-3 months, free fatty acids are produced from the triglycerides which lead to detectable flavor changes (Schmidt & Renner, 1978b). The production of free fatty acids are most likely caused by the action of lipases which have survived the UHT process (Burton, 1988). This enzymatic activity could be either from the natural lipase in milk or from the lipolytic and proteolytic activity from the growth of psychrotrophic bacteria

in the raw milk before processing that survived the UHT processing. Rerkrai (1986) measured milk fat lipolysis of UHT milk using Acid Degree Value (ADV) and found that changes in ADV paralleled very closely to stale flavor development.

Lipid oxidation may not be a major factor in the staling of UHT processed milk. Rerkrai (1986) and Wadsworth and Bassette (1985) found that lipid oxidation values as measured by Thiobarbituric Acid (TBA) method did not correlate with stale flavor development.

C. Dissolved Oxygen

Thomas et al. (1975) studied the effect of dissolved oxygen content in indirect UHT milk and found that UHT milk with initial higher oxygen content resulted in the disappearance of the cooked flavor faster than milk with a lower dissolved oxygen content. They also found that, although the cooked flavor disappeared faster, stale and oxidized flavors developed sooner. This was confirmed by work done by Wadsworth and Bassette (1985). UHT processed milk with lower initial dissolved oxygen content was less acceptable due to a cooked flavor persisting in the milk a longer time (Rerkrai, 1986). The speed of disappearance of the cooked flavor depends on the initial dissolved oxygen content. The reason behind this is that the dissolved oxygen oxidizes the free -SH groups released during the denaturation of β-lactoglobulin. These free -SH groups are responsible for the cooked or hydrogen sulfide flavor. According to Thomas et al., (1975) free -SH

groups disappeared in two days if the dissolved oxygen content is 9 mg/liter, while it took 3 weeks if the dissolved oxygen level is 1 mg/liter to oxidize 65% of the available free -SH groups.

MATERIALS AND METHODS

Grade A raw, pasteurized, and ultra-high-temperature (UHT) milk samples were obtained for analysis from the following sources. All of the raw and fresh pasteurized milk samples were provided by the Kansas State University dairy bar and maintained at a refrigerated temperature. The UHT milk samples in 8 oz/236 ml cartons were provided by Real Fresh, Inc., Vasilia, CA 93277, Plant No. 06-858 and stored at room temperature. All chemicals and standards used in the experiments were analytical grades. Sep-Pak filters used for the isolation of organic compounds from milk were purchased from Waters Associates, Milford, MA 01757. Other materials and methods will be described in the following sections.

I. Evaluation of Solid Phases

As a preliminary study, various solid phase filters from Waters Associates, Maple Street, Milford, MA 01757, were tested to determine their effect in removing the stale flavor in UHT milk. The following filters were tested:

Sep-Pak C_{18} Cartridge

Sep-Pak Silica Cartridge

Sep-Pak Florisil Cartridge

Sep-Pak Alumina A Cartridge

Sep-Pak Alumina N Cartridge

Sep-Pak Alumina B Cartridge

Sep-Pak NH_2 Cartridge

Sep-Pak CN Cartridge

Sep-Pak Diol Cartridge

Sep-Pak filters were mounted on a 5 ml glass syringe and 2 ml of 95% ethyl alcohol was passed through to activate the filter followed by 10 ml of distilled water. Stale UHT milk (17 ml) was slowly passed through the filter at an approximate flow rate of 3 ml/min. The milk that was passed through the filter was evaluated for stale aroma and flavor by an informal but 3-membered experienced taste panel. Results were expressed as either positive or negative for the presence of stale flavor and used as a screening tool.

II. **Organoleptic Analysis**

A. Introduction

Based on results of the preliminary study with various solid phase filters, fresh whole pasteurized milk, UHT stale milk, and UHT stale milk that were eluted through a C18 Sep-Pak and were evaluated by a trained sensory panel for cooked aroma intensity, stale aroma intensity, cooked flavor intensity, and stale flavor intensity.

B. Training Session

Reference samples of fresh, stale and cooked flavor milk were used in training a 6 membered taste panel to familiarize them with these flavors. During the training sessions, panelists discussed flavor intensities and characteristics of the samples and agreed upon defects and scores.

C. Organoleptic Evaluation of Samples

Samples of fresh whole pasteurized, UHT stale, and UHT stale milk filtered through a C18 Sep-Pak were introduced to a 6 membered taste panel. The samples were evaluated for cooked aroma intensity, stale aroma intensity, cooked flavor intensity and stale flavor intensity. Evaluations were made on three separate days with stale and cooked reference samples provided each time. Panelists were asked to judge aroma by smelling and flavor by tasting the samples. Cooked or stale aromas and flavors were determined or scored using a 5-point intensity scale (1 = none to 5 = extremely intense). Comments were encouraged to describe off-aromas or flavors. Fig. 2 provides an example of the format used by the taste panel to score the UHT milk.

UHT Milk Taste Panel

Sample I.D.						
Cooked aroma intensity[1]						
Stale aroma intensity[1]						
Briefly describe stale aroma						
Briefly describe mouthfeel						
Cooked flavor intensity[1]						
Stale flavor intensity[1]						
Briefly describe stale flavor						

[1] **Intensity scale**
1 none
2 slight
3 moderate
4 definite
5 extremely

Figure 2. Example of the format used by the taste panel to score UHT milk.

III. Analysis of Free Fatty Acids

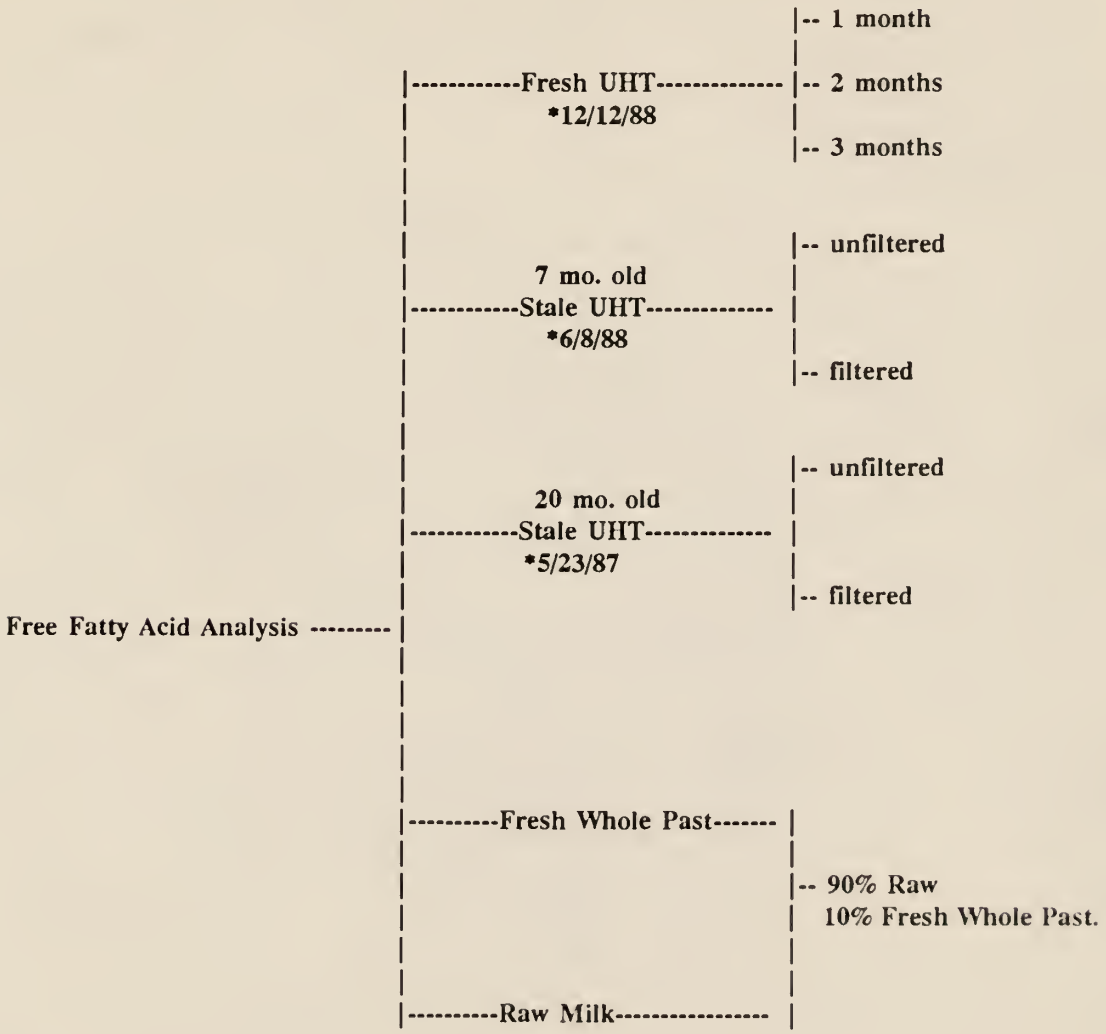
A. Introduction

Duplicate milk samples were prepared and analyzed for the presence and quantity of free fatty acids using a gas chromatographic method developed by Deeth et al. (1983). All the experiments were repeated twice. A description of these samples is as follows:

Identification	Process Date
Fresh UHT (Code No. 83471)	12/12/88
Stale UHT - 7 months (Code No. 12954)	6/8/88
Stale UHT - 20 months (Code No. 96842)	5/23/87
Fresh whole pasteurized	1/15/89
Raw milk	1/15/89
90% Raw/10% Fresh pasteurized	1/15/89

In addition to the description provided, the fresh UHT milk was examined at one, two and three month intervals. The two stale samples were analyzed in both a filtered and unfiltered form using a C_{18} Sep-Pak filter (filtering procedure discussed in Part I, Materials and Methods). The milk containing 90% raw milk and 10% fresh whole pasteurized was refrigerated for eight hours prior to examination.

Fig. 3 Summary of milk samples analyzed for free fatty acids



* Process Date

B. Extraction Procedure

A gas chromatographic method for determination of free fatty acids in milk developed by Deeth. et al. (1983) was utilized for all the samples discussed. Alumina, Neutral, (Brockman Activity, 80-200 mesh) (38.4 g) was deactivated by adding 1.6 ml distilled water in a 50 ml glass beaker and stirred vigorously with a glass rod for approximately 5 minutes. The alumina was covered with para-film and set aside at least two hours before use. A 10 ml milk sample was added to a 50 ml screw-capped glass tube containing a mixture of 20.7 ml ice cold diethyl ether, 3.3 ml HCl, and 1 ml of a tridecanoic acid (C_{13}) standard solution. The C_{13} standard solution was prepared by weighing 0.10 g C_{13} acid using an analytical balance and dissolving it into a 100 ml volumetric flask containing diethyl ether. Two ml of this solution was withdrawn using a 2 ml pipet and added to a 10 ml volumetric flask containing diethyl ether. One ml of this solution was added to the milk sample. The milk and diethyl ether - HCl solution was agitated gently for 10 min with a mechanical agitator and then centrifuged (2,000 rpm) for 10 min at 0°C using a Beckman Model J-21B Centrifuge. After centrifugation, 15 ml of the ether layer was collected using a pasteur pipet and transfered to a 50 ml screw-capped glass tube containing 15 ml hexane and 1 g sodium sulfate. This was mechanically agitated for 10 min. A small glass chromatography column (10 mm i.d.) was prepared by packing approximately 0.5 g of glass wool tightly into the bottom of the tube with a glass rod and adding 1 g of deactivated alumina into the tube. The

ether - hexane solution was then slowly passed through the column at a rate of approximately 3 ml/min and collected. The ether-hexane solution collected was passed through the column a second time. A 1:1 solution of hexane and diethyl ether was prepared and 5 ml of this solution was passed through the column. Another 5 ml of the hexane-ether solution was passed through the column and the eluate was discarded. The alumina in the column containing the absorbed free fatty acids was dried by applying a vacuum to the bottom of the column outlet for 5 min. The dry alumina was transferred into a small screw-capped glass tube and 1 ml of a solution containing iso-propyl ether with 6% formic acid was added. The tube was mechanically agitated for 10 min and centrifuged at 2000 g for 5 min, again using a Beckman Model J-21B Centrifuge. The isopropyl ether layer was transferred into a small glass vial and 4 μl was injected into the gas chromatograph.

C. GC Analysis

Free fatty acids were determined by analyzing the prepared samples using a Hewlett-Packard 588OA Series gas chromatograph equipped with a flame-ionization detector and Hewlett-Packard 588OA Series GC Terminal (Level Four Data System). The fatty acids were separated on a Nukol fused silica capillary column with polyglycol liquid phase (15 m x 0.53 mm i.d.) made by Supelco, Inc., Bellefonte, PA. The operating conditions were programmed as follows:

Oven Temperature Profile:

Initial value	=	100°C
Initial time	=	1.0 min

Level 1

Program rate	=	15.00°C/min
Final value	=	220°C
Final time	=	12.00 min
Post value	=	220°C
Post time	=	3.00 min

Other operating conditions:

Helium carrier flow rate	=	27 ml/min
Hydrogen flow rate	=	28 ml/min
Oxygen flow rate	=	430 ml/min
Chart speed	=	0.5 cm/min
Attenuation	=	2↑ x 1
Peak width	=	.08
Threshold	=	-1
% Offset	=	10

D. Identification and Quantification

The free fatty acids in the milk samples were identified by injecting known analytical standards (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{18}:1) into the gas chromatograph and comparing the peak retention times for the standards to the peak retention times for the milk samples. The concentrations of free fatty acids in the milk samples were determined by taking a ratio of the peak area of the free fatty acid in the milk and that of the C_{13} fatty acid standard added to the milk during sample preparation. Because of the large number of samples and trials as well as considering the variability of the data, the areas were adjusted to reflect an average area for the C_{13} standard. This was necessary to allow for the comparison of the different chromatograms. The following equations depict the adjustment of peak areas for each chromatogram.

(1) $$\frac{\text{Average Area of } C_{13} \text{ Standard}}{\text{Area of } C_{13} \text{ Standard}} = \text{Correction Factor}$$

(2) $$\text{Correction Factor} \times \text{Area of Free Fatty Acid} = \text{Adjusted Area of Free Fatty Acid}$$

Four ml of each sample was injected into the gas chromatograph. A chromatogram along with an Area % Compensated Analysis Report was obtained and utilized for the identification and quantification of the free fatty acids in the samples.

IV. Analysis of Neutral Volatile Compounds

A. Introduction

Duplicate samples of UHT fresh milk, stale milk, and stale milk filtered through a C_{18} Sep-Pak were steam distilled and the distillates analyzed for volatile compounds. The C_{18} Sep-Pak filters used to filter the stale milk samples were eluted with organic solvents to flush out the trapped compounds and then the eluants were analyzed. The samples were analyzed by gas chromatography and mass spectrometry (GC-MS) to determine the presence and identification of volatile compounds. The experiments were repeated three times and a comparison was made among the different samples and the differences in results were evaluated. Fig. 4 depicts a flow diagram of the experiments.

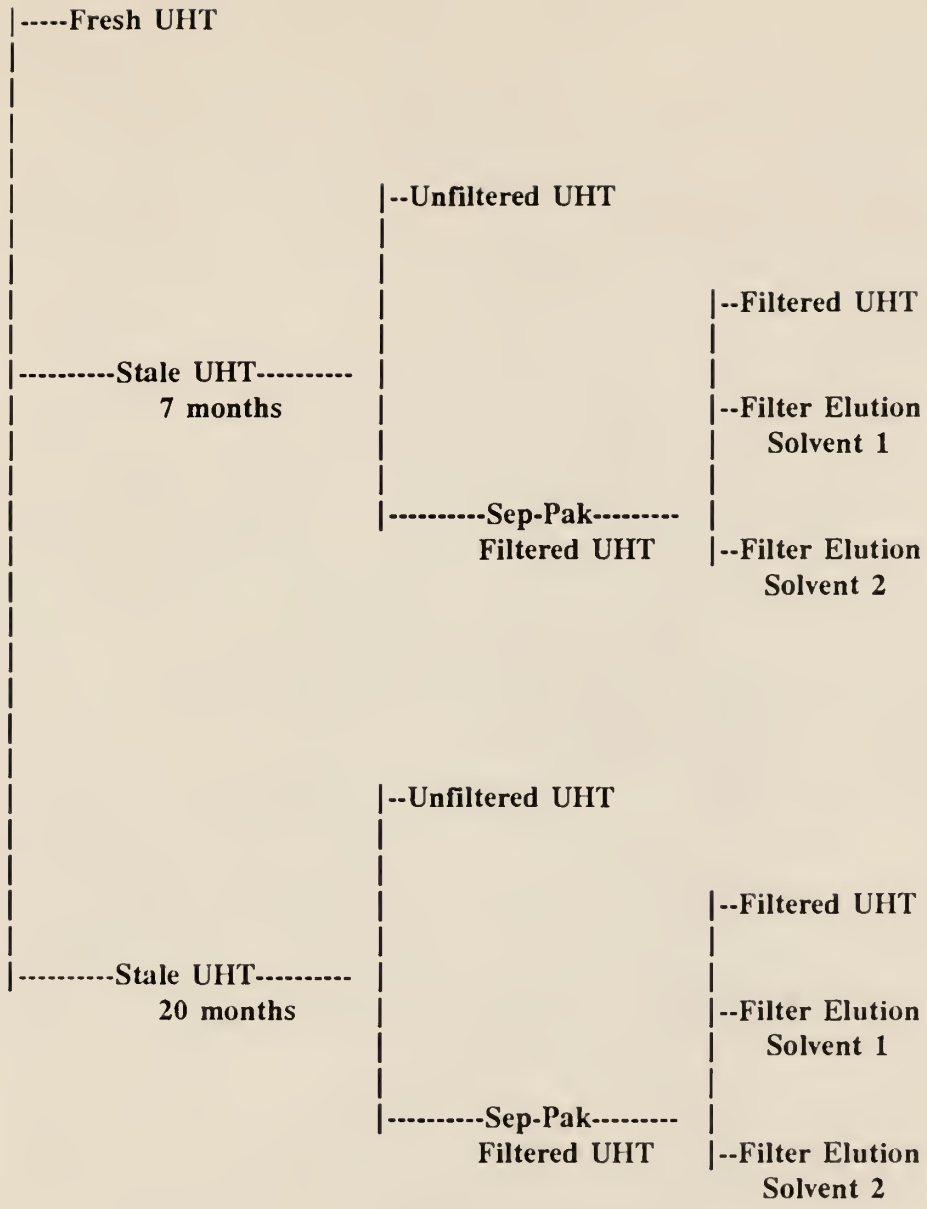


Figure 4. Samples Analyzed for Volatile Compounds

B. Extraction Procedures

The UHT milk samples were prepared by adding fifty milliliters of milk and 1 ml of a defoamer-internal standard solution into a Kemmerer-Hallet type distillation flask. The filtered milk samples, however, were first passed through a C_{18} Sep-Pak and then placed into the distillation flask. The samples were steam distilled in a micro-Kjeldahl distillation unit with ice water circulating in the condenser. The defoamer-internal standard solution was prepared by adding one gram of defoamer (10% active silicone ingredient from Christian Hansen's Laboratory, Inc., Milwaukee, WI) into a 100 ml steam distillation flask containing forty-five ml of double-distilled water, which had been treated with potassium permanganate. The solution was gently boiled for 20 min to strip off and remove the volatile compounds present in the defoamer. The solution was cooled to room temperature and made to a volume of 100 ml with double-distilled permanganate water. This solution contained 1000 ppm defoamer. Seventy-five ml of the 1000 ppm defoamer solution and 1 ml of a 1-butanol internal standard solution were mixed and made to a final volume of 100 ml by adding double distilled permanganate water. The 1-butanol internal standard solution was made by weighing 0.3750 g of 1-butanol and dissolving into 45 ml double distilled permanganate water. This was made to a volume of 100 ml and 1 ml of this standard solution was added to the defoamer making a total solution of 750 ppm defoamer and 37.5 ppm 1-butanol.

Five milliliters of distillate were collected in approximately five minutes using a 15 ml graduated conical test tube placed in an ice water bath. The distillate was rapidly removed and poured into a separatory funnel containing 20 ml of redistilled cold ethyl ether maintained in the refrigerator. This procedure was repeated a total of twenty times, collecting approximately 100 ml of distillate in the separatory funnel with cold ethyl ether for each individual sample. The distillate and ether solution were mixed gently by rotating the separatory funnel back and forth (180° angles) for approximately five minutes. The solution was allowed to stand for ten minutes in the refrigerator and then the water phase (bottom layer) was separated from the ether phase (top layer) by draining the water from the bottom of the funnel. The ethyl ether phase (10-15 ml) was then placed in a 25 ml glass beaker containing 1.0 g of anhydrous sodium sulfate to remove excess water. The ethyl ether was poured into a 15 ml graduated conical test tube and concentrated down to 1 ml, 0.3 ml, and finally, 0.1 ml concentrations by evaporating the ether with a gentle nitrogen stream. The concentrated samples were transferred into a 1 ml graduated serum vial and 1 microliter was injected into a gas chromatograph and mass spectrometer.

A total of 60 Sep-Paks were used during the preparation of the Sep-Pak filtered UHT milk samples. These Sep-Pak cartridges were eluted with organic solvents and the eluates were analyzed for the presence of compounds that were trapped in the filter. The 60 Sep-Pak cartridges were each eluted with 5 ml methylene chloride (Solvent No. 1) followed by 5 ml ethyl ether (Solvent No. 2) and

collected in two separate beakers. The solvents with extracted compounds from the Sep-Pak filters were concentrated down to 1 ml, 0.3 ml, and 0.1 ml, respectively, and 1 microliter samples were injected into the gas chromatograph and mass spectrometer.

C. GC and MS Analysis

Neutral volatile compounds were determined by analyzing the prepared samples on a Hewlett-Packard Model 588OA Gas Chromatograph equipped with a flame ionization detector as well as a Hewlett-Packard Model 5970B Mass Selective Detector combined with a Hewlett-Packard Model 589OA Gas Chromatograph. A 5% phenyl methyl silicone crosslinked fused silica capillary column (50 m x 0.2 mm i.d. x 0.33 μm film thickness) manufactured by Hewlett-Packard was utilized for the separation of the volatile compounds in both instruments. The operating conditions for the GC were programmed as follows:

Oven temperature	=	40°C
Injection port temperature	=	230°C
Detector temperature	=	230°C
Oven temperature profile	=	
Initial value	=	40°C
Initial time	=	1.00 min

Level 1

Program rate	=	5.00°C/min
Final value	=	100°C
Final time	=	0.10 min

Level 2

Program rate	=	10.00°C/min
Final value	=	180°C
Final time	=	0.10 min

Level 3

Program rate	=	15.00°C/min
Final value	=	210°C
Final time	=	15.00
Post value	=	220°C
Post time	=	3.00
Helium carrier flow rate	=	0.7 ml/min
Hydrogen flow rate	=	28 ml/min
Oxygen flow rate	=	430 ml/min
Make-up gas	=	20 ml/min
Chart speed	=	0.5 cm/min
Attenuation	=	2↑ x 0
Peak width	=	0.08

Threshold	=	-1
% offset	=	10

The operating conditions for the GC-MS were programmed as follows:

Acquisition Parameter

Solvent delay 7.00 min.

	Start time	Low mass	High mass	Scan threshold	a/d samples	Scans per second
1.	3.00	40.0	500.0	500	2	0.93
2.		50.0	550.0	1000	2	0.86
3.		50.0	550.0	1000	2	0.86

Plot #1 Total ion scale 1000000
Plot #2 Total ion scale 2000000

Temperature Program and Heated Zones

Run time: 38.20 min.
Equilibretion time: 0.50 min.
Purge time off: 0.75 min

Level	Initial Temp	Initial Time	Rate C°/Min	Final Temp	Final Time	Total Time
1	40	1.00	5	100	0.10	13.10
2			10.0	100	0.10	21.20
3			15.0	210	15.00	38.20

	Actual	Set Point	Limit
Oven	40	40	300
Inj Port	230	230	300
Transfer line	280	280	290

Run Table

Valves Splitless Off	0.75 min
Group 1	3.00 min
Mass Spec On	7.00 min
Stop Run	38.20 min

D. Identification and Quantification

The neutral volatile compounds in the milk samples were tentatively identified by analyzing the individual spectrums from the Total Ion Chromatogram (TIC) and comparing the spectrums with those listed in the automated National Bureau of Standards (NBS) library. These were later confirmed by injecting analytical standards of aldehydes, ketones, and free fatty acids from Supelco, Inc., Bellefonte, PA. 16823 into the GC and GC-MS. Identification was considered as confirmed when the retention time of the unknown compounds matched the retention time of the analytical standards. The concentration of the neutral volatile compounds in the milk samples was determined by comparing the peak area of compounds in the sample to the peak area of the 1-butanol standard added to the milk during sample preparation.

One microliter of each sample was injected into the GC and GC-MS. A chromatogram along with an Area % Computed Analysis Report was obtained and utilized for the identification and quantification of the neutral volatile compounds in the samples.

V. Stale Flavor Replication

A. Introduction

Comparisons were made among the GC and GC-MS chemical profiles of the UHT fresh, UHT stale, and UHT stale milk filtered through a C18 Sep-Pak. Compound concentrations were determined for each sample by using a reference compound as an internal standard. The differences in compound concentration between the fresh UHT and the stale UHT milk were determined. Compounds reflecting significant differences in concentrations were selectively added directly to fresh UHT milk in an attempt to replicate stale flavor development. Samples were evaluated for stale flavor development by a trained taste panel.

B. Replication Procedure

GC and GC-MS chemical profiles were used to determine compound identification and concentrations for UHT fresh and UHT stale milk. Differences in compound concentrations between the UHT fresh and UHT stale milk were determined in the following manner:

UHT Stale Milk Concentration (p.p.m./p.p.b)	-	**UHT Fresh Milk Concentration** (p.p.m./p.p.b)	=	**Difference** (p.p.m./p.p.b)

Compounds reflecting significant differences in concentrations were added directly to fresh UHT milk to investigate their effect on stale flavor development.

The following samples were prepared:

Identification	Compound added	Concentration *ppm/ppb
Sample 1	octanoic acid (C_{18})	*34.0
Sample 2	decanoic acid (C_{10})	*80.0
Sample 3	octanoic acid (C_8)	*34.0
	decanoic acid (C_{10})	*80.0
Sample 4	n-hexanal	0.5
	2-heptanone	60.0
	n-heptanal	0.8
	2-nonanone	50.0
	n-nonanal	0.20
	2-butanone	0.09
	2-decanone	0.36
	2-undecanone	4.0
	2-tridecanone	1.0
Sample 5	octanoic acid (C_8)	*34.0
	decanoic acid (C_{10})	*80.0
	n-hexanal	0.5
	2-heptanone	60.0
	n-heptanal	0.8
	2-nonanone	50.0
	n-nonanal	0.20
	2-butanone	0.09
	2-decanone	0.36
	2-undecanone	4.0
	2-tridecanone	1.0

C. Organoleptic Analysis

UHT fresh milk samples with added concentrations of selected compounds were evaluated by a trained six member taste panel. Stale and fresh UHT milk samples were provided as references for their aroma and flavor characteristics. The taste panel evaluated the five samples in three separate sessions over a three day period. Stale and fresh UHT samples were provided as a reference during each session. Panelists were asked to judge aroma by smell and flavor by tasting the samples. Stale aromas and flavors were scored using a 5-point intensity scale (1 = none to 5 = extremely intense). Comments were requested for stale aromas and flavors. The format used to score the samples is shown in Figure 2.

RESULTS AND DISCUSSION

I. Evaluation of Solid Phases

Results from the informal taste panel are provided in Table 3.

Table 3. Results from the evaluation of solid phases.

Type Solid Phase	Result
Sep-Pak Alumina A Cartridge	-
Sep-Pak Florisil Cartridge	-
Sep-Pak Silica Cartridge	-
Sep-Pak C_{18} Cartridge	+
Sep-Pak Alumina N Cartridge	-
Sep-Pak Alumina B Cartridge	-
Sep-Pak NH_2 Cartridge	-
Sep-Pak CN Cartridge	-
Sep-Pak Diol Cartridge	-

(+) = stale flavor removed
(-) = stale flavor not removed

The data indicates that when passing stale UHT processed milk through C_{18} Sep-Pak cartridges, the stale flavor was removed. The other filters that were tested showed no decrease in stale flavor intensity. This finding suggests that the stale flavor components in UHT processed milk were entrapped in the C_{18} Sep-Pak

cartridges. By utilizing this technique, it was then possible to analyze the differences in chemical composition between UHT stale and UHT stale filtered milk. Also, the compounds entrapped in the C_{18} Sep-Pak filter, thought to be responsible for stale flavor development, could also be subjected to analysis. The C_{18} Sep-Pak cartridge therefore provides a significant step forward toward the successful isolation and subsequent identification of the stale flavor components in UHT processed milk.

II. Organoleptic Analysis

Results from the organoleptic analysis of fresh pasteurized milk, stale UHT milk, and stale UHT filtered through a C_{18} Sep-Pak are shown in Table 4.

Table 4. Means of organoleptic flavor scores.

Defect	Sample: Pasteurized Whole	UHT	UHT Sep-Pak Trt
	Scores[1]		
Cooked Aroma Intensity	1.3	3.2	2.8
Stale Aroma Intensity	1.5	2.4	1.7
Cooked Flavor Intensity	1.3	3.0	2.8
Stale Flavor Intensity	1.2	4.1	2.1

[1] Scores: 1 = none to 5 = extremely intense

Figure 5 Summary of Organoleptic Results

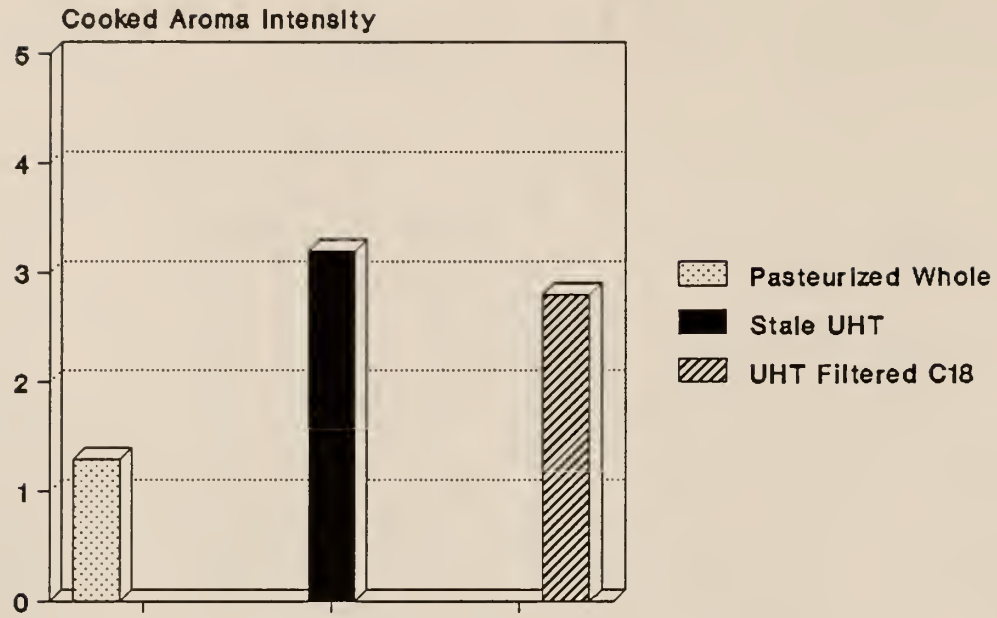
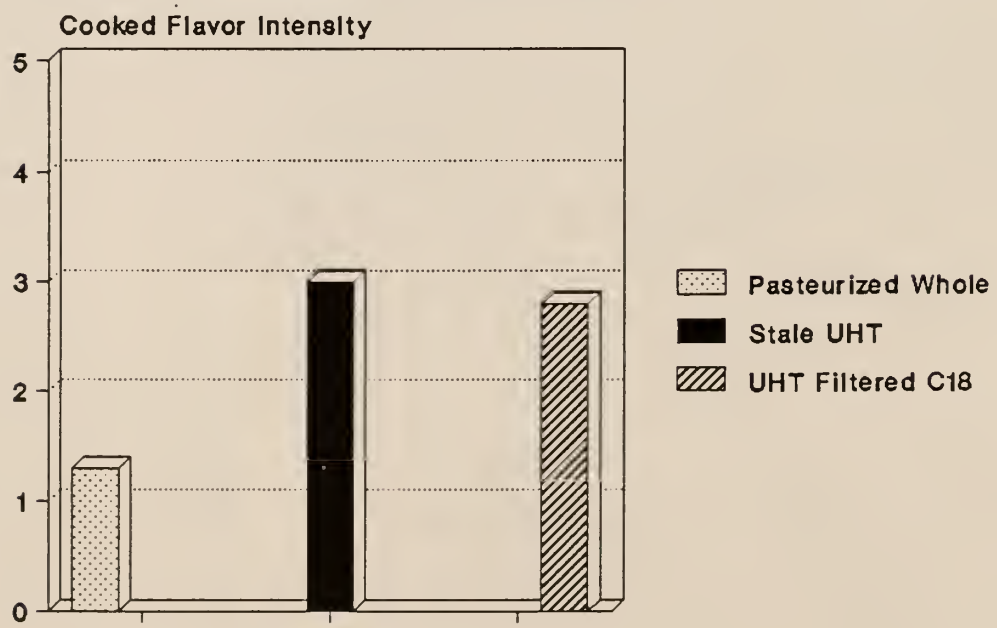


Figure 6 Summary of Organoleptic Results



Cooked aroma and flavor results are represented graphically in Figures 5 and 6. Cooked aroma and flavor were not detected for the fresh pasteurized milk, but were moderately detected for both the stale and stale filtered UHT samples. The presence of the cooked aroma and flavor in the UHT samples is consistent with that found by other researchers (Ashton, 1965; Hostettler, 1972; Clarke, 1967; Burton, 1988). This cooked or sulfurous flavor develops because of the extremely high temperature used in UHT processing, which results in the formation of free -SH groups released during the denaturation of the serum proteins, mainly β-lactoglobulin (Hutton and Patton, 1952; Burton, 1988). Pasteurization temperatures were not high enough to denature the serum proteins in fresh pasteurized milk which explains why the cooked aroma and flavor were not detected for that sample. As shown by the graphs, the C_{18} Sep-Pak filter did not remove the cooked aroma and flavor compounds (free -SH groups) in the filtered UHT milk.

Stale aroma and flavor results are represented graphically in Figures 7 and 8. Stale aroma and flavor was not detected in the fresh pasteurized milk and only slightly detected in the stale UHT milk that was filtered through the C_{18} Sep-Paks. For the stale UHT samples, however, a slight to moderate stale aroma but an intense stale flavor was found. By comparing the samples, it is evident that the Sep-Pak cartridges were effective in removing the stale flavor and aroma from the stale UHT milk to a level comparable to fresh pasteurized milk.

Fig. 7 Summary of Organoleptic Results

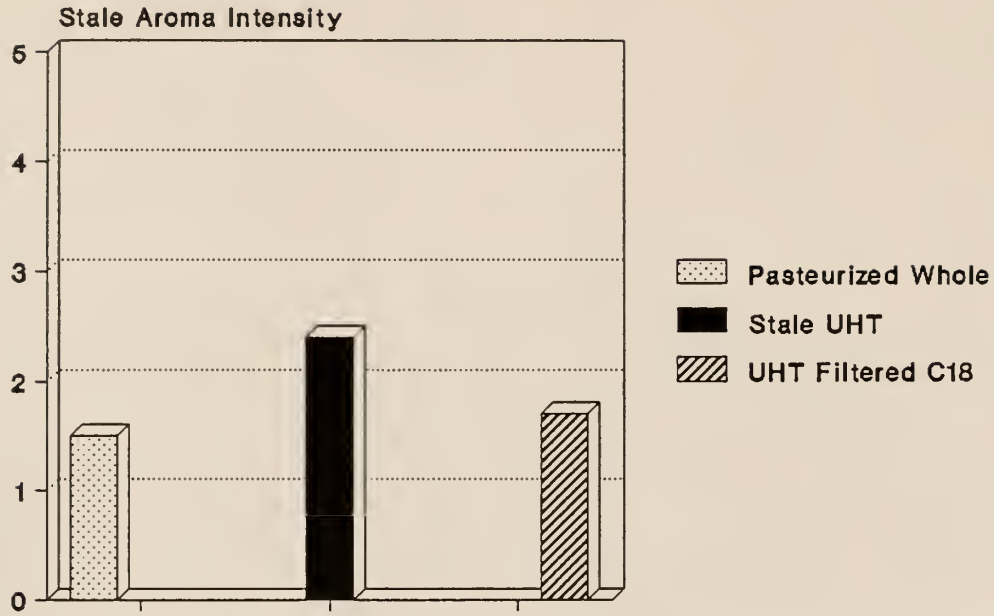
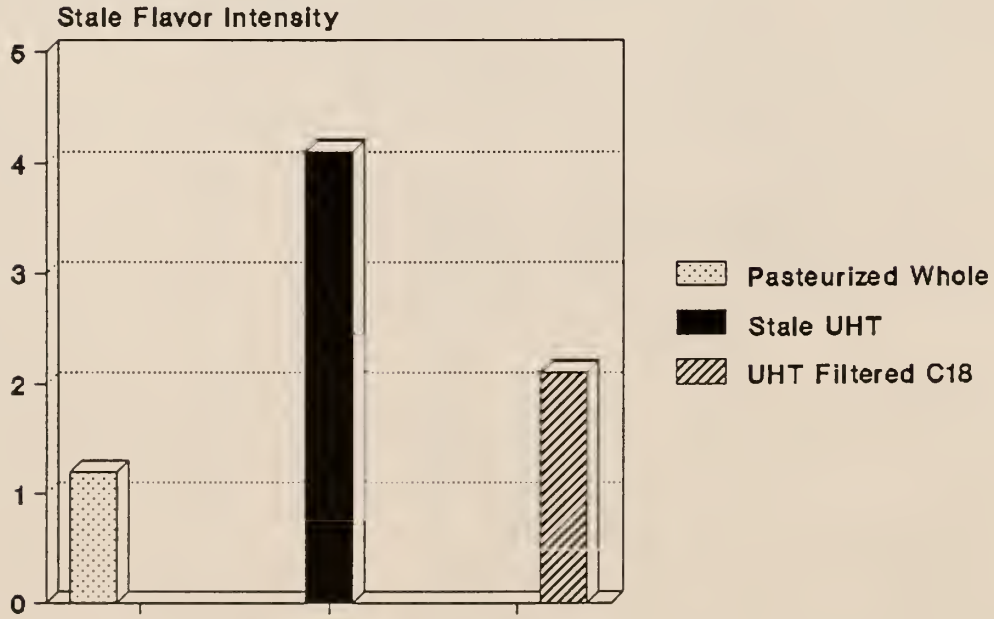


Figure 8 Summary of Organoleptic Results



III. Analysis of Free Fatty Acids

The free fatty acid concentrations found in various milk samples are listed in Table 5. In general, the data shows that the stale UHT milk samples and 90% raw/10% fresh pasteurized sample had much higher free fatty acid concentrations than the fresh UHT samples. Low fatty acid concentrations were found in fresh UHT milk with similar patterns also found in fresh whole pasteurized and raw milk. For the stale Sep-Pak filtered milk, the data was mixed. Concentrations for some of the free fatty acids were high while others were low. Low concentrations for stale filtered milk indicate that these compounds were removed by the C_{18} Sep-Pak filter. The following paragraphs provide a detailed comparison of the various milk samples analyzed.

Butyric acid (C_4) concentrations in the various samples are represented graphically in Figure 9. Low C_4 levels were found in fresh UHT, fresh pasteurized and raw milk. Higher concentrations were found in the stale UHT, Sep-Pak filtered, and 90% raw/10% fresh pasteurized samples. The similar C_4 concentrations found between the stale and Sep-Pak filtered samples indicate that the Sep-Pak filters did not remove butyric acid.

Table 5 Free fatty acid concentrations in various milk samples (ppm).

Samples	C4	C6	C8	C10	C12	C14	C16	C18	C18:1
Fresh UHT	7.67	9.14	7.50	12.96	16.56	40.01	116.28	47.38	132.37
7 mo. Stale UHT	50.96	49.88	41.71	93.34	108.10	315.82	977.60	222.82	798.09
7 mo. Stale UHT C18 Filtered	47.82	19.66	6.72	9.06	52.79	304.01	1021.71	209.23	768.79
20 mo. Stale UHT	29.49	29.07	37.10	61.50	65.21	149.71	439.19	132.19	533.50
20 mo. Stale UHT C18 Filtered	19.13	9.92	3.43	4.55	23.35	98.63	346.75	122.87	410.99
Fresh Whole Pasteurized	13.08	13.64	11.24	12.66	20.61	66.85	151.82	47.52	169.00
Raw	9.22	15.57	7.97	13.93	22.05	54.14	179.94	44.51	159.96
90% Raw 10% Fresh Pasteurized	68.93	68.79	55.51	106.92	125.42	253.56	600.55	108.52	573.23

Figure 9. Comparison of butyric acid (C4) concentrations (ppm) in various milk samples.

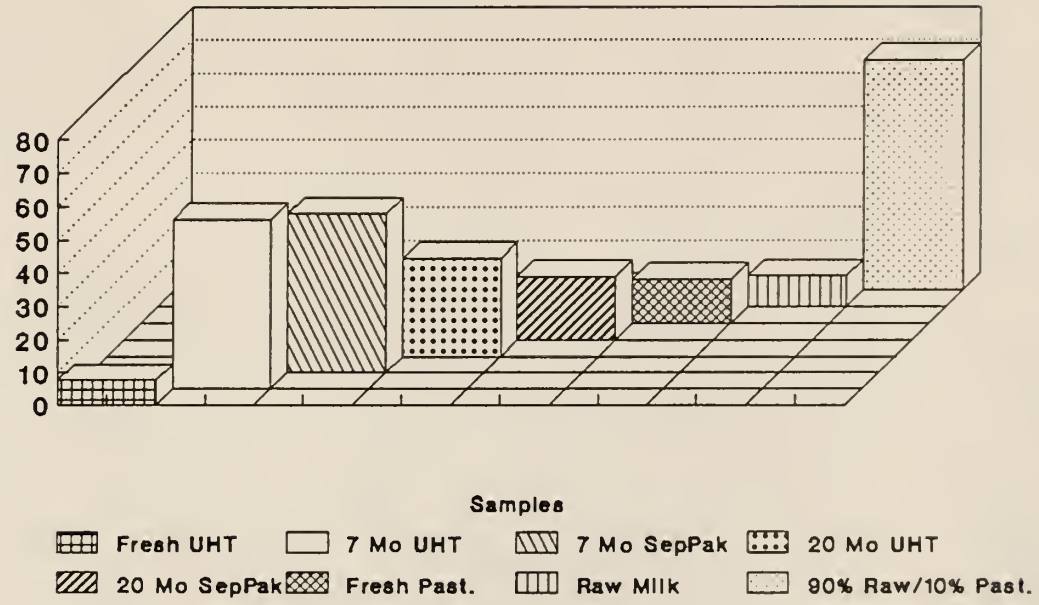
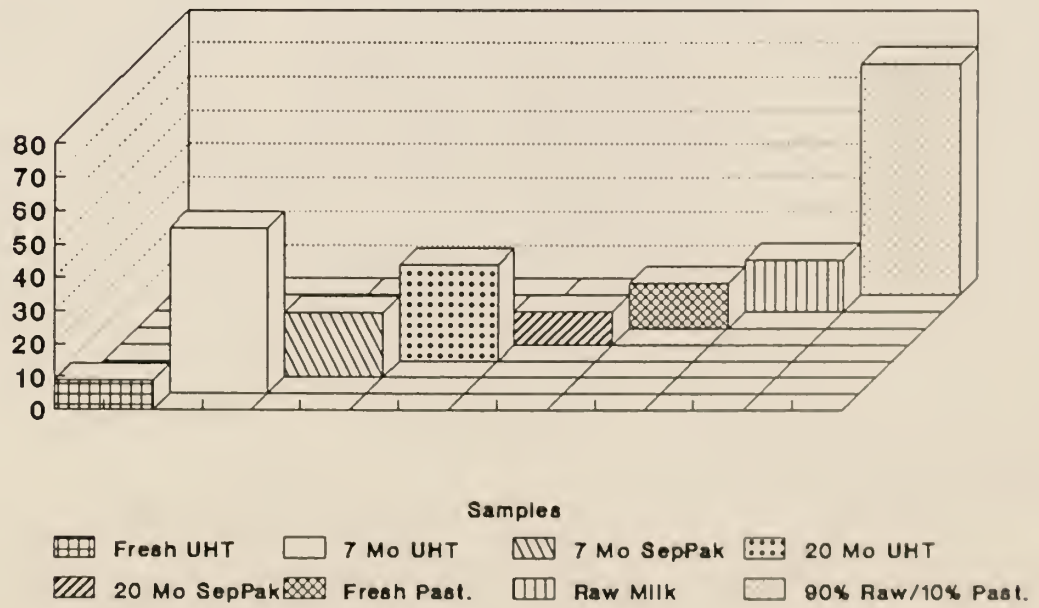


Figure 10. Comparison of caproic acid (C6) concentrations (ppm) in various milk samples.



Caproic acid (C_6) concentrations in the various samples are represented graphically in Figure 10. Low C_6 levels were found in the fresh UHT, fresh pasteurized and raw milk samples. High levels were found in the stale UHT and 90% raw/10% fresh pasteurized samples. However, moderate C_6 levels were found in the Sep-Pak filtered samples. This data indicates that the Sep-Pak filters were effective in removing approximately 60% of the caproic acid (C_6) in the stale milk samples.

Caprylic acid (C_8) concentrations in the various milk samples are graphically represented in Figure 11. Low C_8 concentration levels were observed in the fresh UHT, fresh pasteurized, raw milk, and Sep-Pak filtered samples. High concentrations were found in the stale and 90% raw/10% fresh pasteurized milk samples. The data shows a significant difference in C_8 concentrations between the stale UHT and filtered UHT samples. This indicates that the Sep-Pak filters were effective in removing almost all of the caprylic acid (C_8) from the stale UHT samples. A closer look at the differences in C_8 concentration between the stale and filtered UHT samples are provided in Figure 12. When filtered through the C_{18} Sep-Pak, approximately 85% of the caprylic acid (C_8) is removed. The filtered UHT sample shows concentration levels close to the fresh UHT sample as well as the fresh pasteurized and raw milk samples.

Figure 11. Comparison of caprylic acid (C8) concentrations (ppm) in various milk samples.

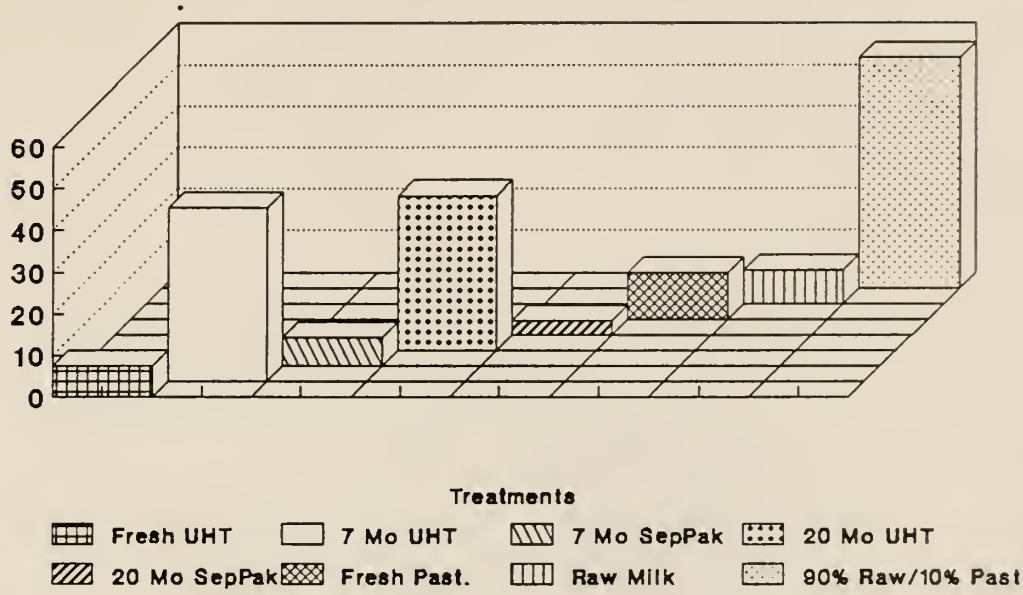
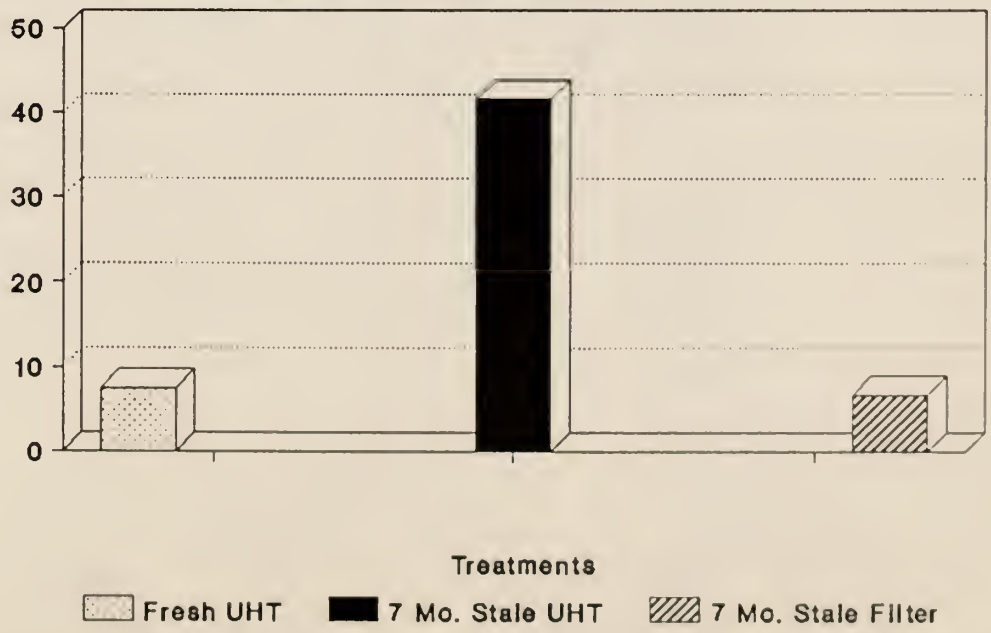


Figure 12. Comparison of caprylic acid (C8) concentrations (ppm) in fresh, stale, and stale Sep-Pak filtered UHT milk



Capric acid (C_{10}) concentrations in various milk samples are graphically shown in Figure 13. A similar trend was found for C_{10} as for C_8. Low C_{10} concentration levels were observed for fresh UHT, fresh pasteurized, raw milk, and Sep-Pak filtered samples. High concentrations were found in the stale and 90% raw/10% pasteurized samples. The sharp contrast between the high concentrations in stale UHT and low concentrations in filtered UHT indicate that the Sep-Pak filters effectively removed the capric acid (C_{10}) from the stale UHT milk. A closer look at the difference in C_{10} concentration between the stale and filtered samples are provided in Figure 14. Approximately 90% of the C_{10} is removed when the stale UHT is passed through the Sep-Pak filter. The filtered milk shows C_{10} concentration levels very similar to fresh UHT milk.

Lauric acid (C_{12}) concentrations in various milk samples are graphically shown in Figure 15. Low C_{12} concentration levels were observed for fresh UHT, fresh pasteurized, and raw milk samples. High concentrations were found in the stale and 90% raw/10% pasteurized samples. Moderate levels were found in the Sep-Pak filtered samples indicating that the filter removed some of the lauric acid. A closer look at the differences in C_{12} concentration between the stale and filtered samples are provided in Figure 16. Approximately 50% of the C_{12} is removed from the stale UHT milk when passed through the Sep-Pak.

Figure 13. Comparison of capric acid (C10) concentrations (ppm) in various milk samples.

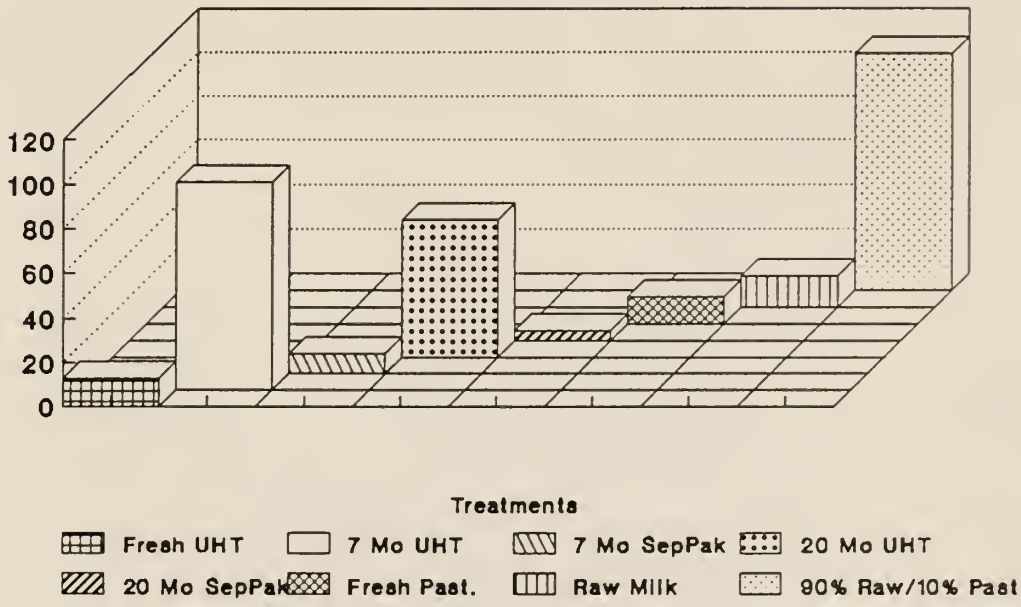


Figure 14. Comparison of capric acid (C10) concentrations (ppm) in fresh, stale, and stale Sep-Pak filtered UHT milk

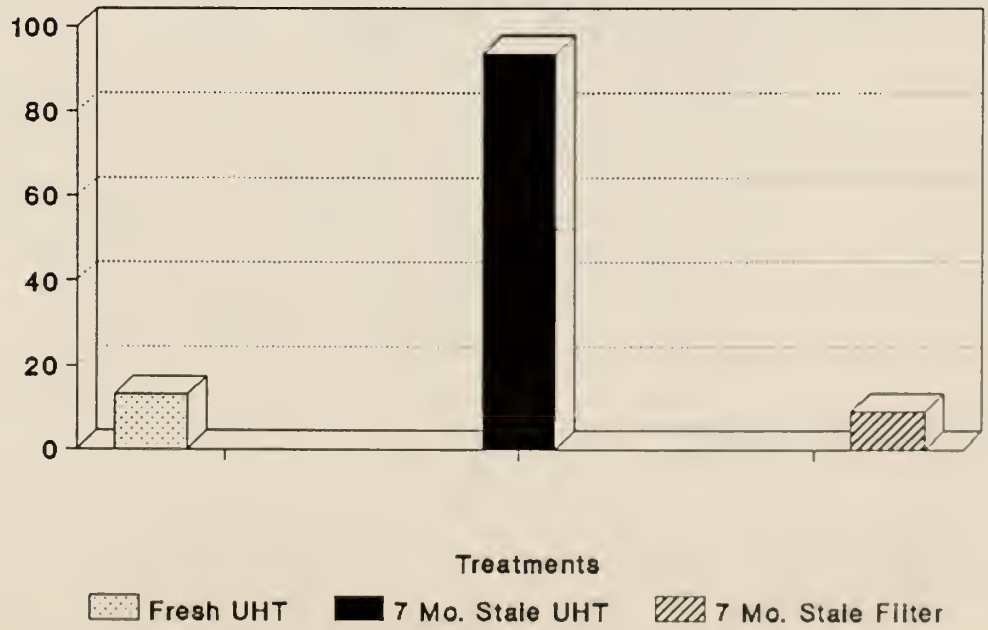


Figure 15. Comparison of lauric acid (12) concentrations (ppm) in various milk samples.

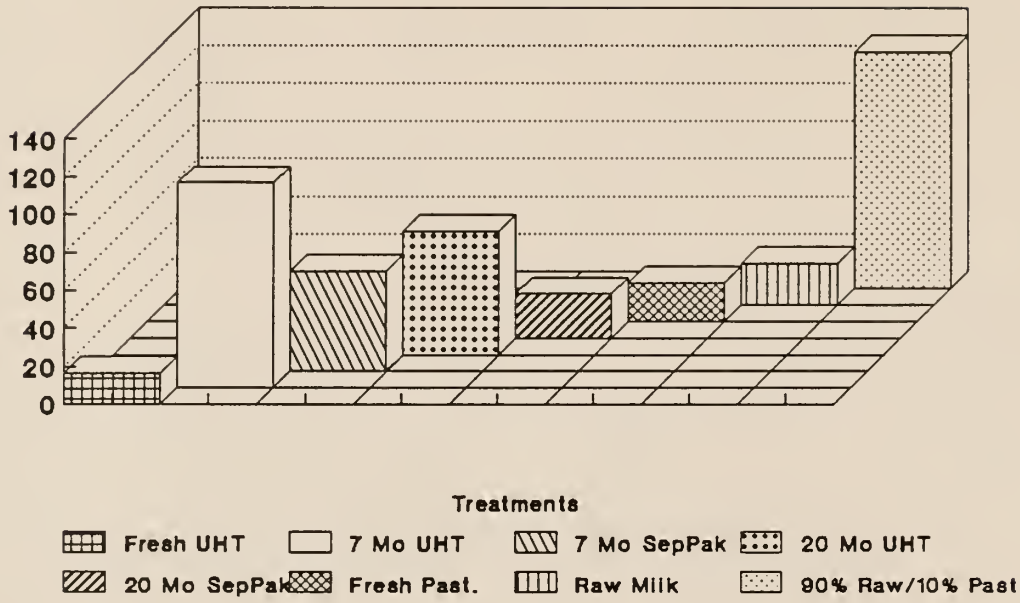


Figure 16. Comparison of lauric acid (C12) concentrations (ppm) in fresh, stale, and stale Sep-Pak filtered UHT milk

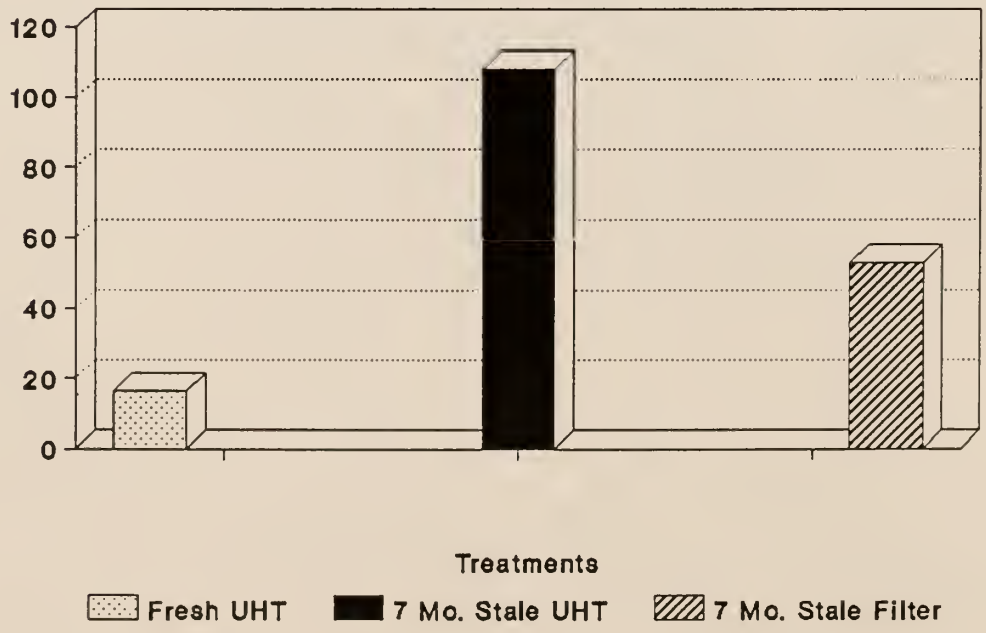


Figure 17. Comparison of myristic acid (C14) concentrations (ppm) in various milk samples.

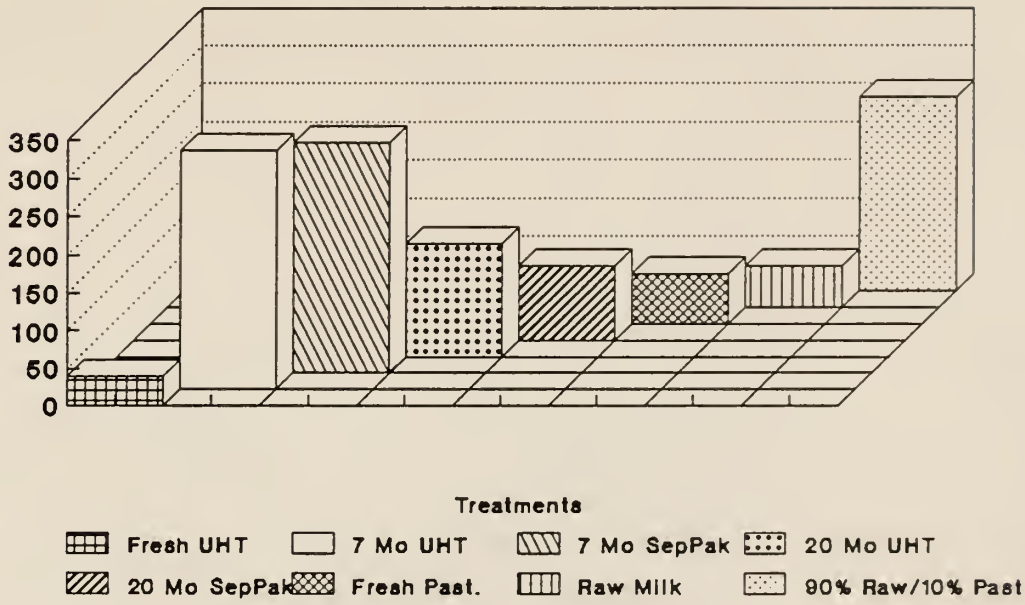
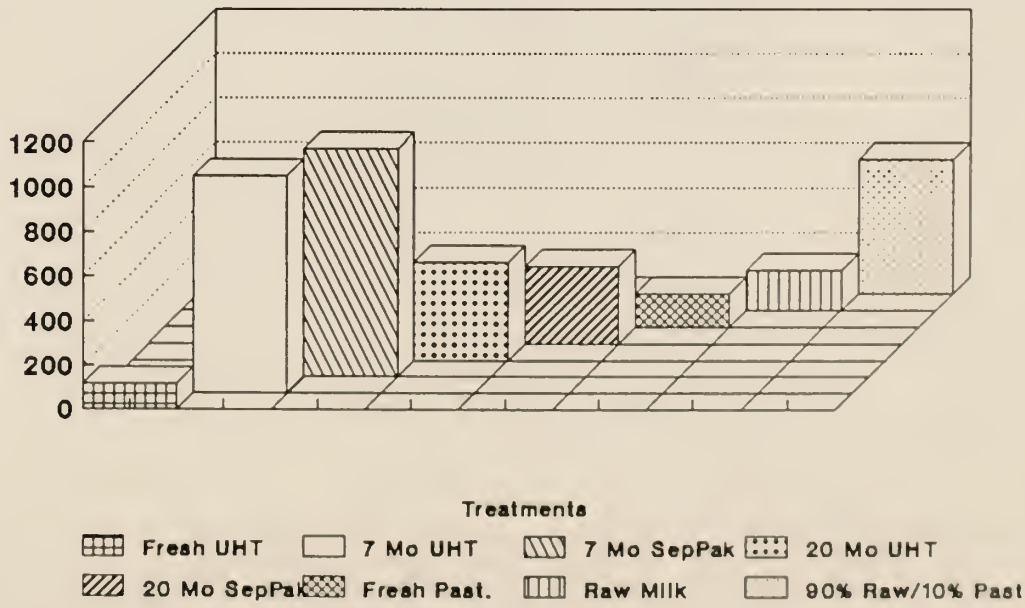


Figure 18. Comparison of palmitic acid (C16) concentrations (ppm) in various milk samples.



The remaining free fatty acids analyzed all showed similar trends. Myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), and oleic acid ($C_{18:1}$) concentrations in various milk samples are shown in Figures 17 to 20, respectively. Low concentration levels for these fatty acids were observed for fresh UHT, fresh pasteurized, and raw milk samples. High concentrations were found in the stale UHT, Sep-Pak filtered UHT, and the 90% raw/10% pasteurized milk samples. These free fatty acids were not removed by the Sep-Pak filter.

The observation that free fatty acid concentrations increase for stale UHT milk is supported by various researchers. Schmidt and Renner, (1978) found that free fatty acids were produced in UHT milk stored at room temperature for 2-3 months. Rerkrai (1986) reported that the Acid Degree Value (ADV) which is a measure of milk fat lipolysis paralleled the development of stale flavor in UHT milk. Other researchers (Burton, 1988) reported that the production of free fatty acids is most likely caused by the action of lipases which have survived the UHT process and that this could be either from the natural lipase in milk or from the lipolytic and proteolytic activity from the growth of psychrotropic bacteria before processing that survived the UHT process.

The data from the free fatty acid analysis of the various milk samples indicates increased concentrations for all of the free fatty acids from fresh UHT to stale UHT milk. This conclusion is supported by the analysis of free fatty acid concentrations in UHT milk during 30, 60, and 90 day intervals. Results from this

Figure 19. Comparison of stearic acid (C18) concentrations (ppm) in various milk samples.

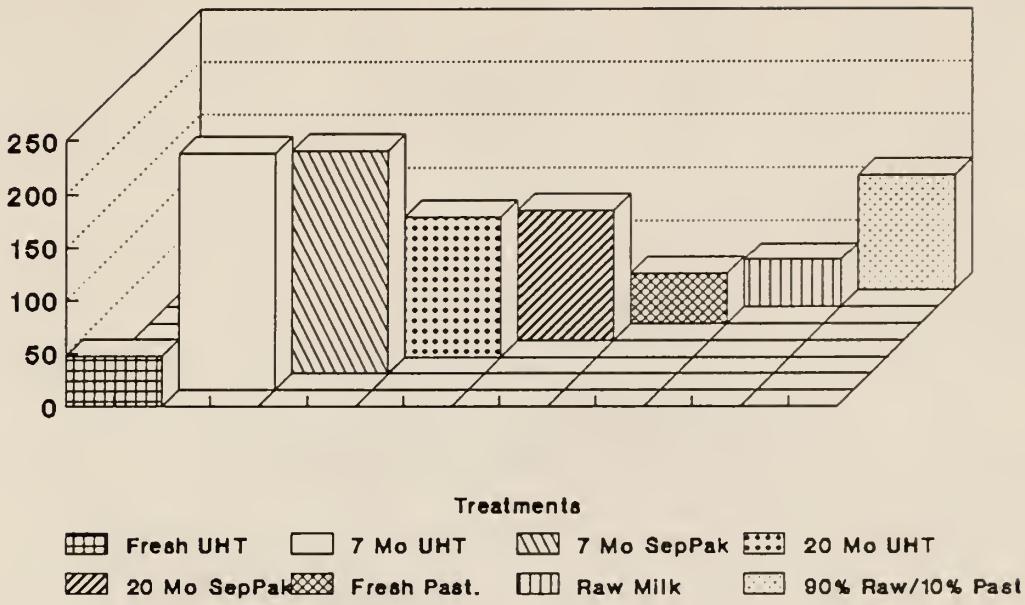


Figure 20. Comparison of oleic acid (C18:1) concentrations (ppm) in various milk samples.

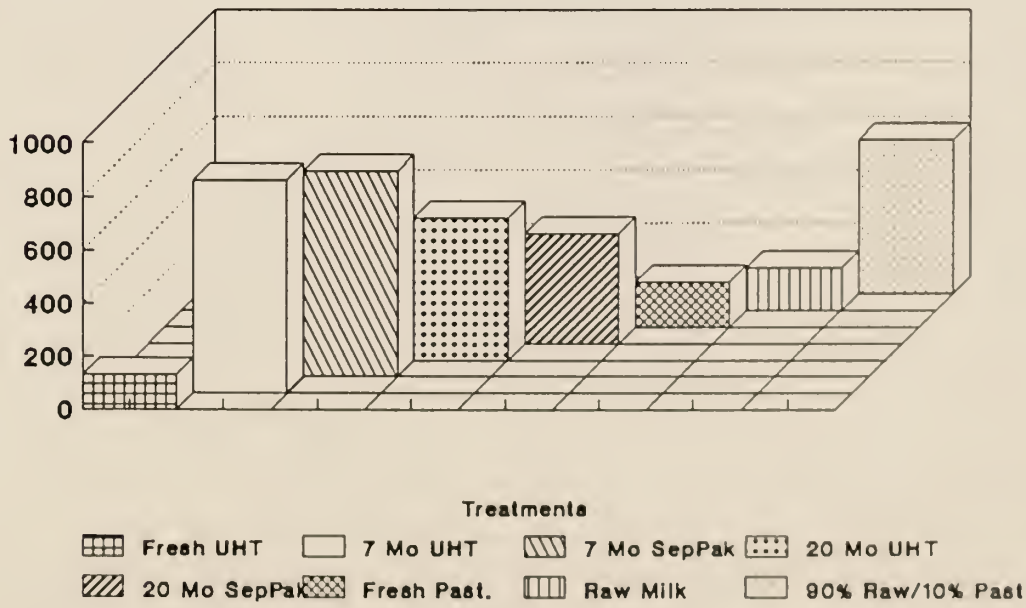


Table 6 Changes in UHT milk free fatty acid concentration (ppm) during storage.

Samples/Days	C4	C6	C8	C10	C12	C14	C16	C18	C18:1
30	7.67	9.14	7.50	12.96	16.56	40.01	116.28	47.38	132.37
60	38.4	36.0	24.0	42.0	54.8	154	732.4	159.2	436.4
90	69.2	58.8	45.2	63.6	86.0	256.8	814.4	199.6	538.0

Figure 21. Changes in butyric acid (C4) concentrations (ppm) over a 90 day period.

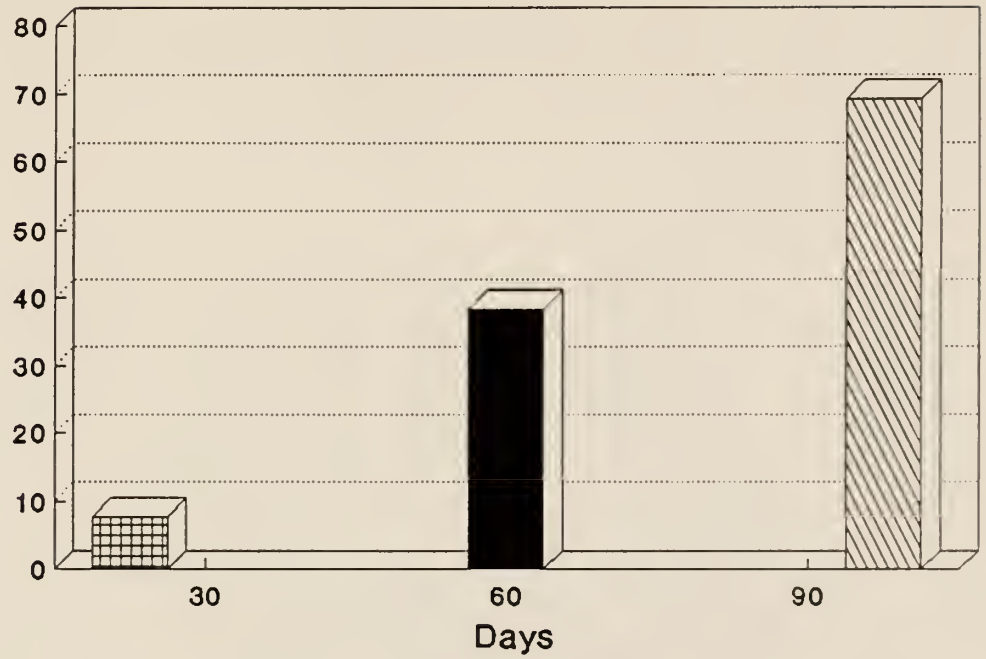


Figure 22. Changes in caproic acid (C6) concentrations (ppm) over a 90 day period.

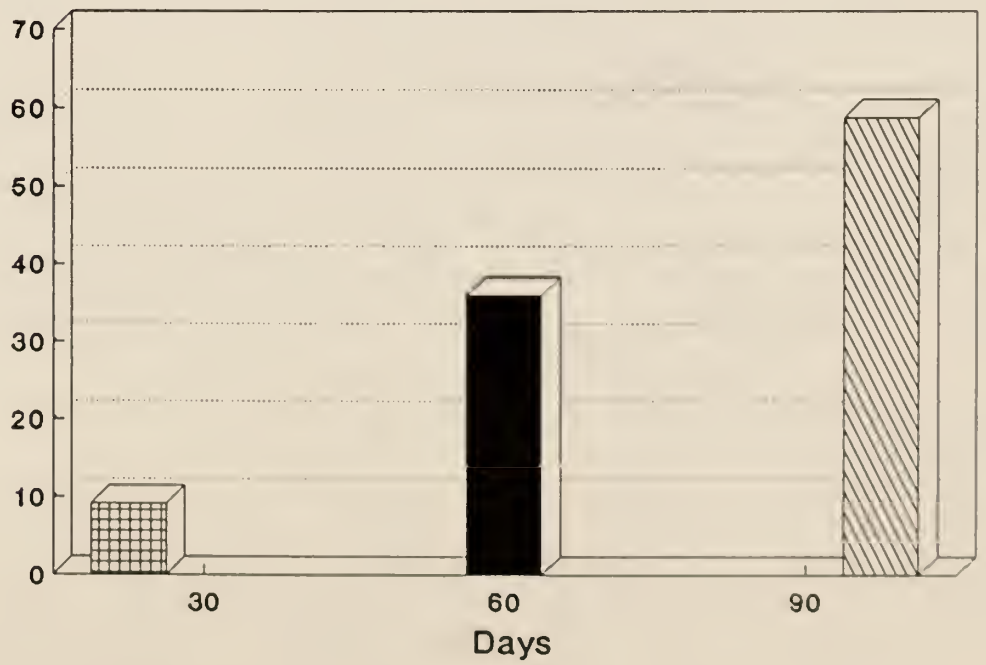


Figure 23. Changes in caprylic acid (C8) concentrations (ppm) over a 90 day period.

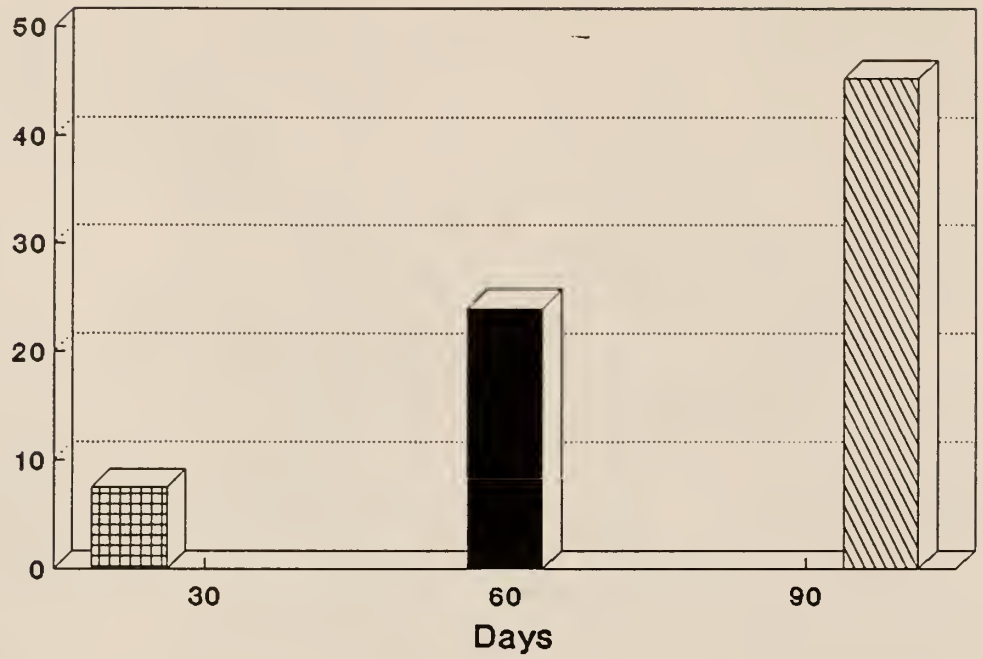


Figure 24. Changes in capric acid (C10) concentrations (ppm) over a 90 day period.

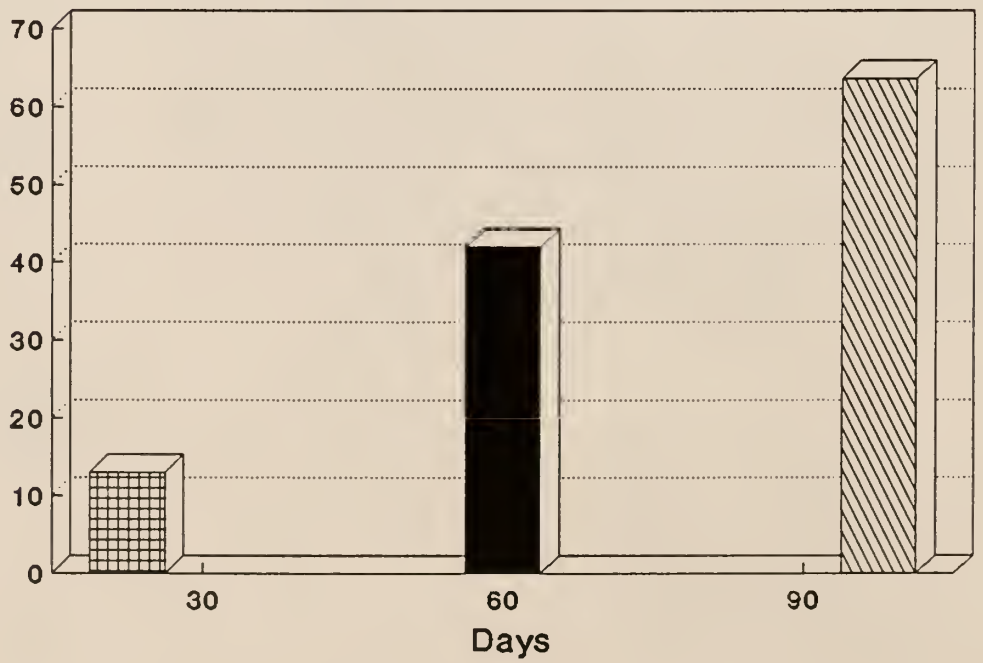


Figure 25. Changes in lauric acid (C12) concentrations (ppm) over a 90 day period.

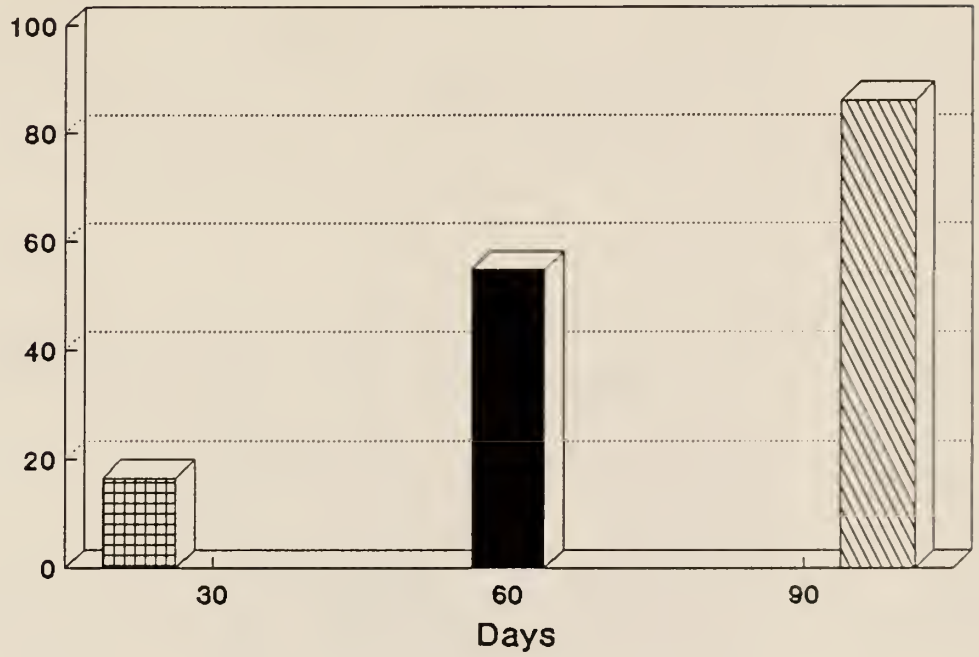


Figure 26. Changes in myristic acid (C14) concentrations (ppm) over a 90 day period.

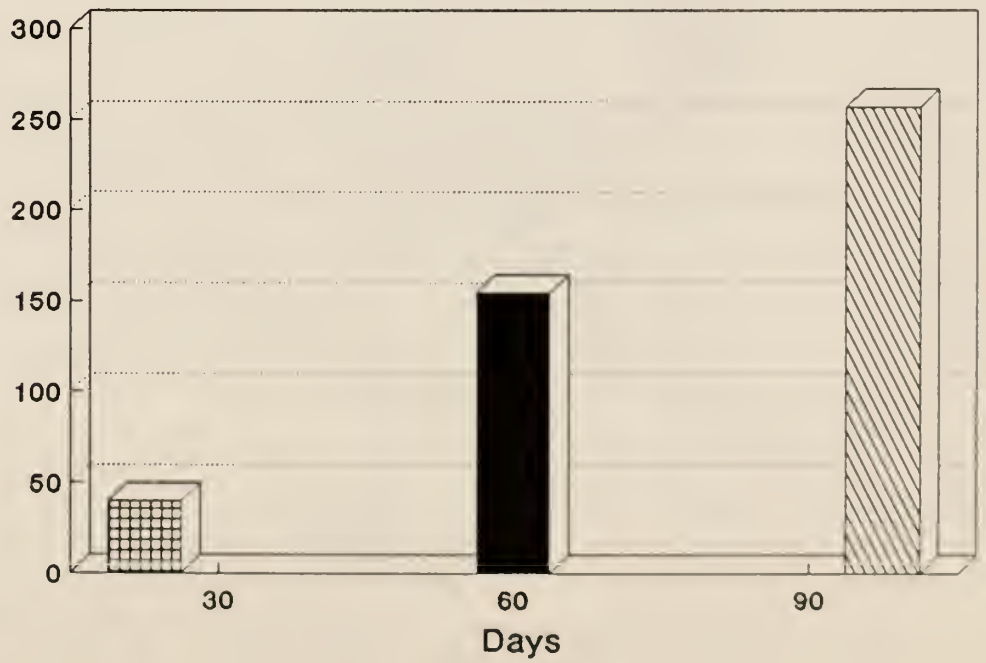


Figure 27. Changes in palmitic acid (C16) concentrations (ppm) over a 90 day period.

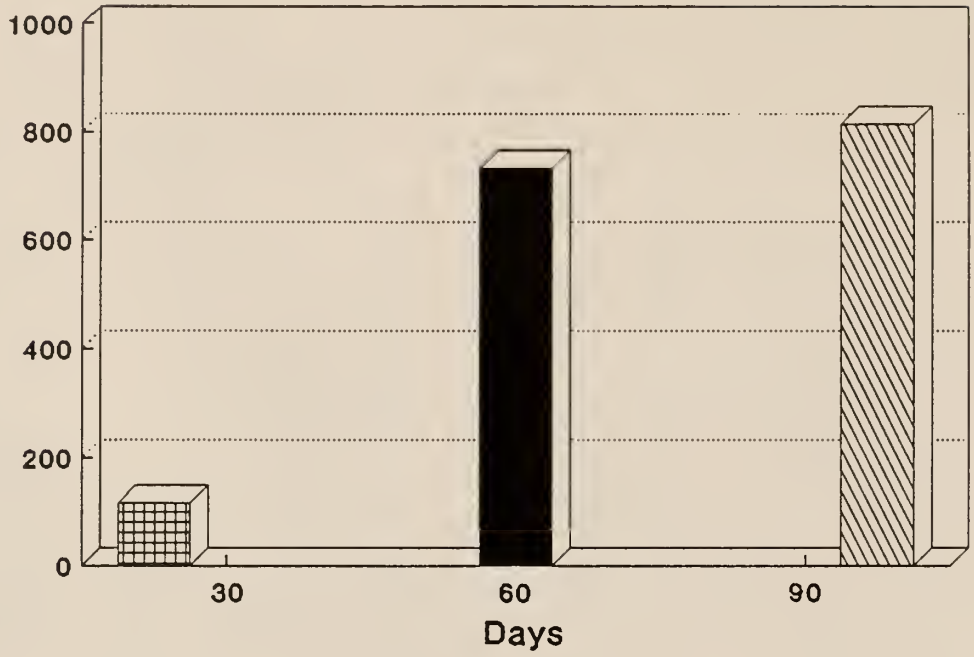


Figure 28. Changes in stearic acid (C18) concentrations (ppm) over a 90 day period.

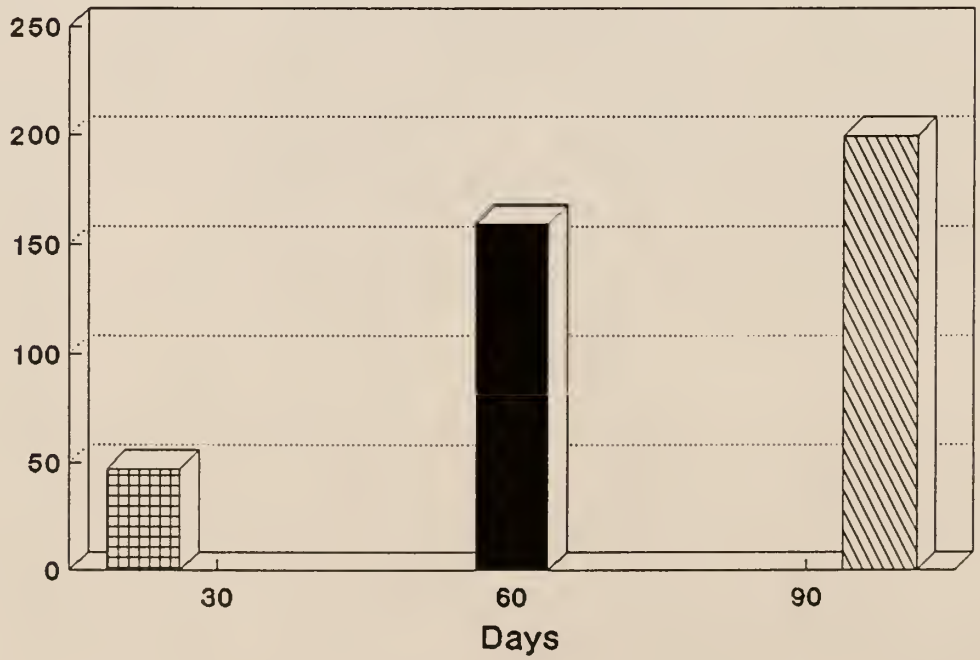
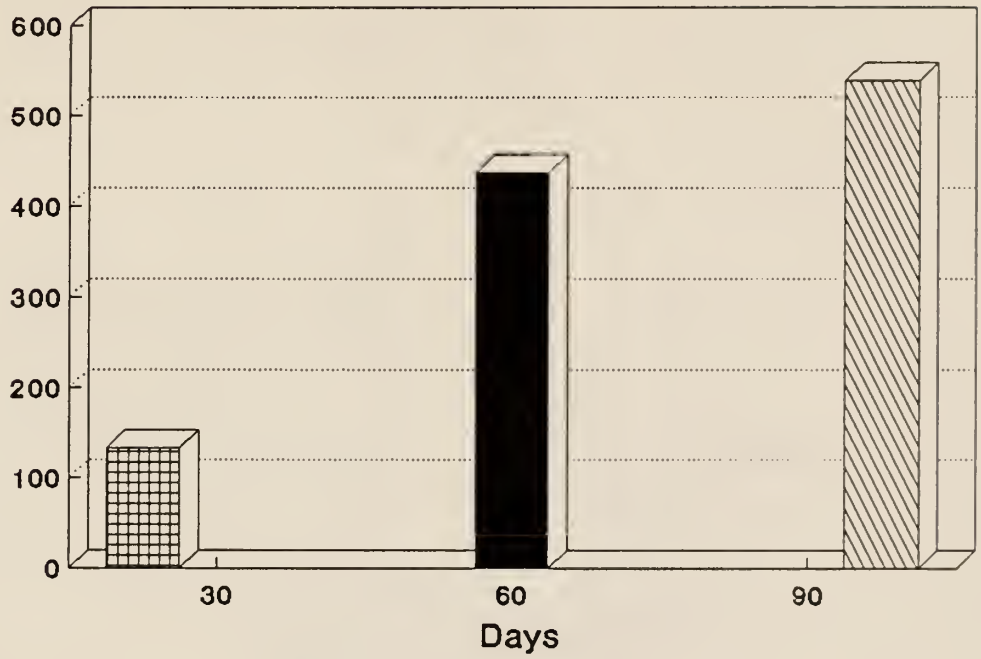


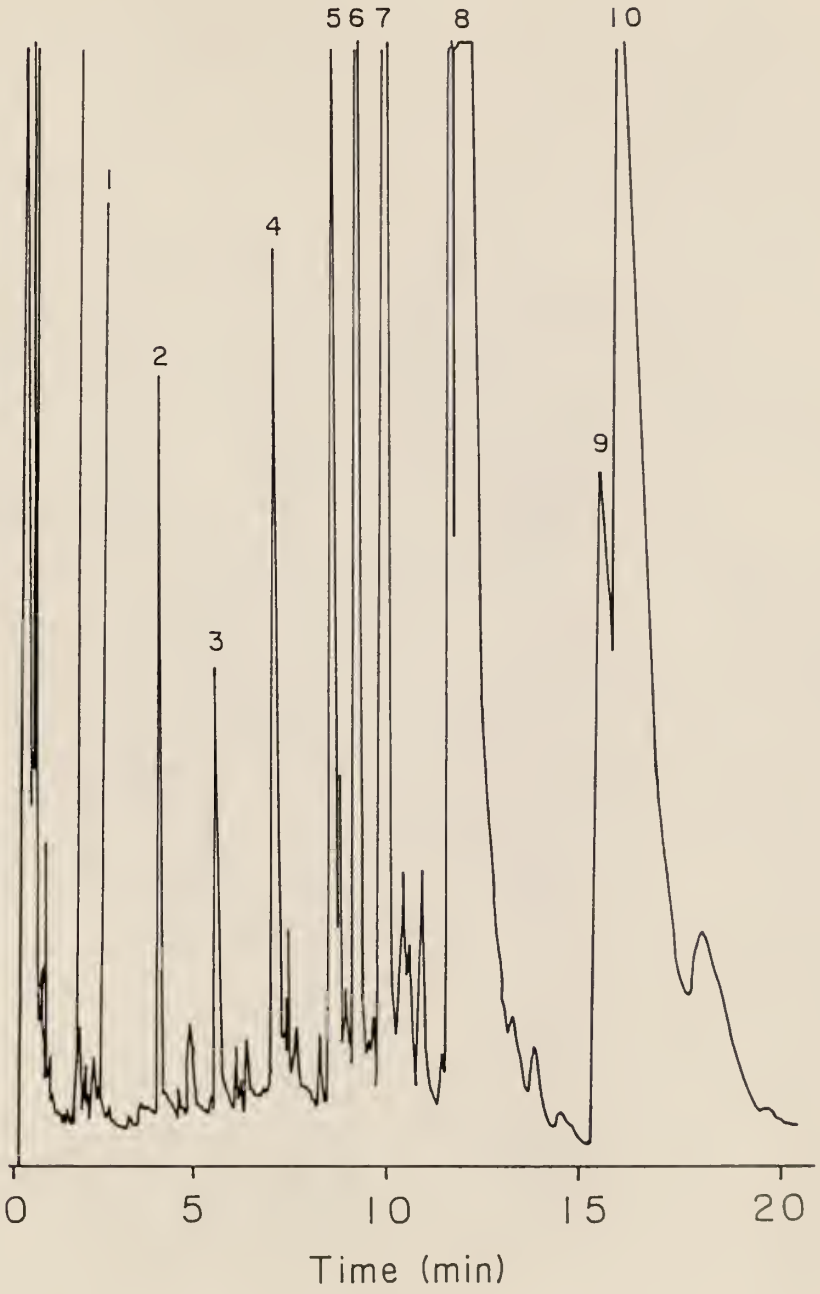
Figure 29. Changes in oleic acid (C18:1) concentrations (ppm) over a 90 day period.



analysis are presented in Table 6. This data is represented graphically in Figures 21 to 29. From this information, it is evident that all of the free fatty acids substantially increased in concentration over the observed period. Additionally, there appears to be a linear relationship for these increases.

Many psychotropic lipase and proteases are heat stable and are able to hydrolyze lipids causing lipolyzed flavor during storage (Adams and Brawley, 1981; Driesen, 1983; Christen and Wong, 1985; Mottar, 1981). Although some researchers agree that free fatty acids in UHT milk may cause flavor defects, little or no research to date has been conducted to support this fact. The finding that capric acid (C_{10}) may play a major role in staling has not been reported in previous experiments and could result in a major breakthrough for UHT flavor research. Chromatograms of the free fatty acid analysis of the various milk samples are provided in Figures 30 to 38.

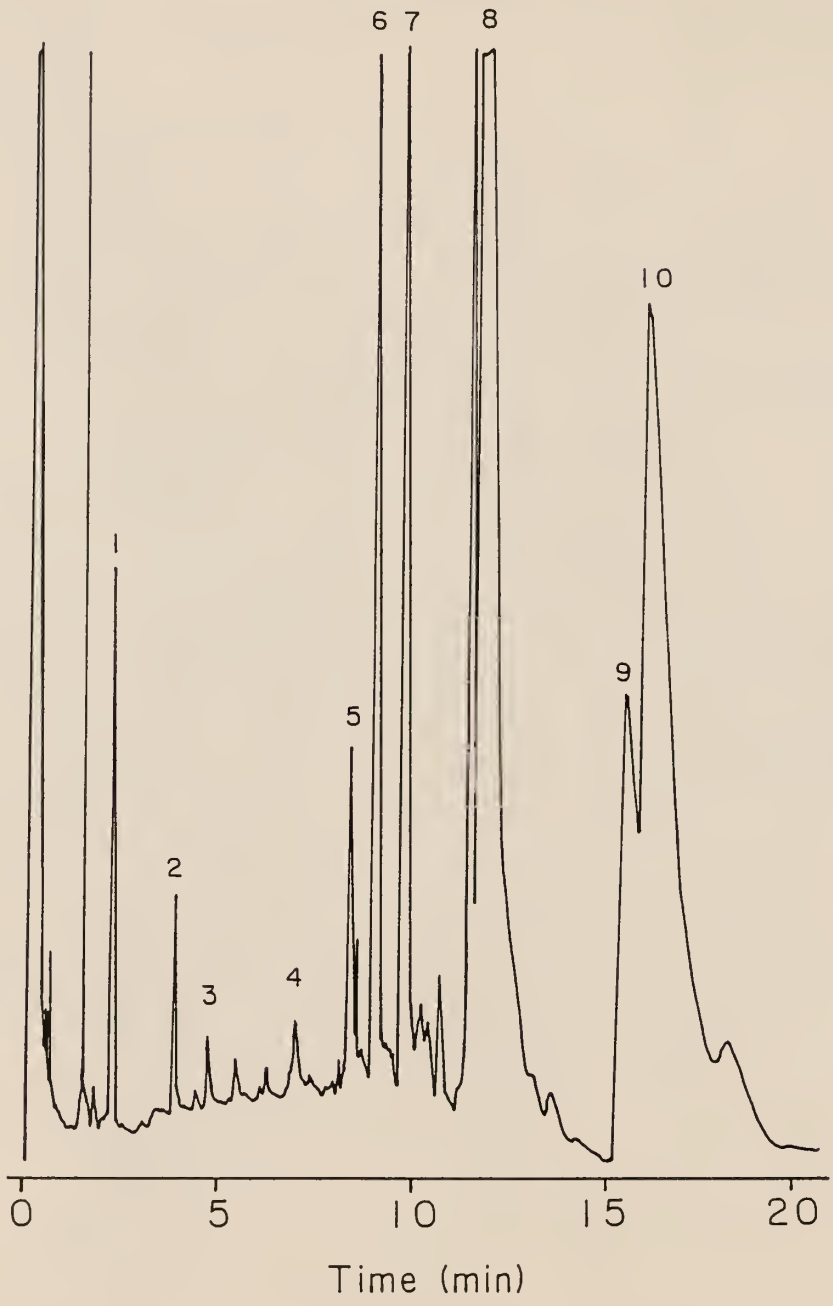
Figure 30. GC Chromatogram of 7 month old stale UHT milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

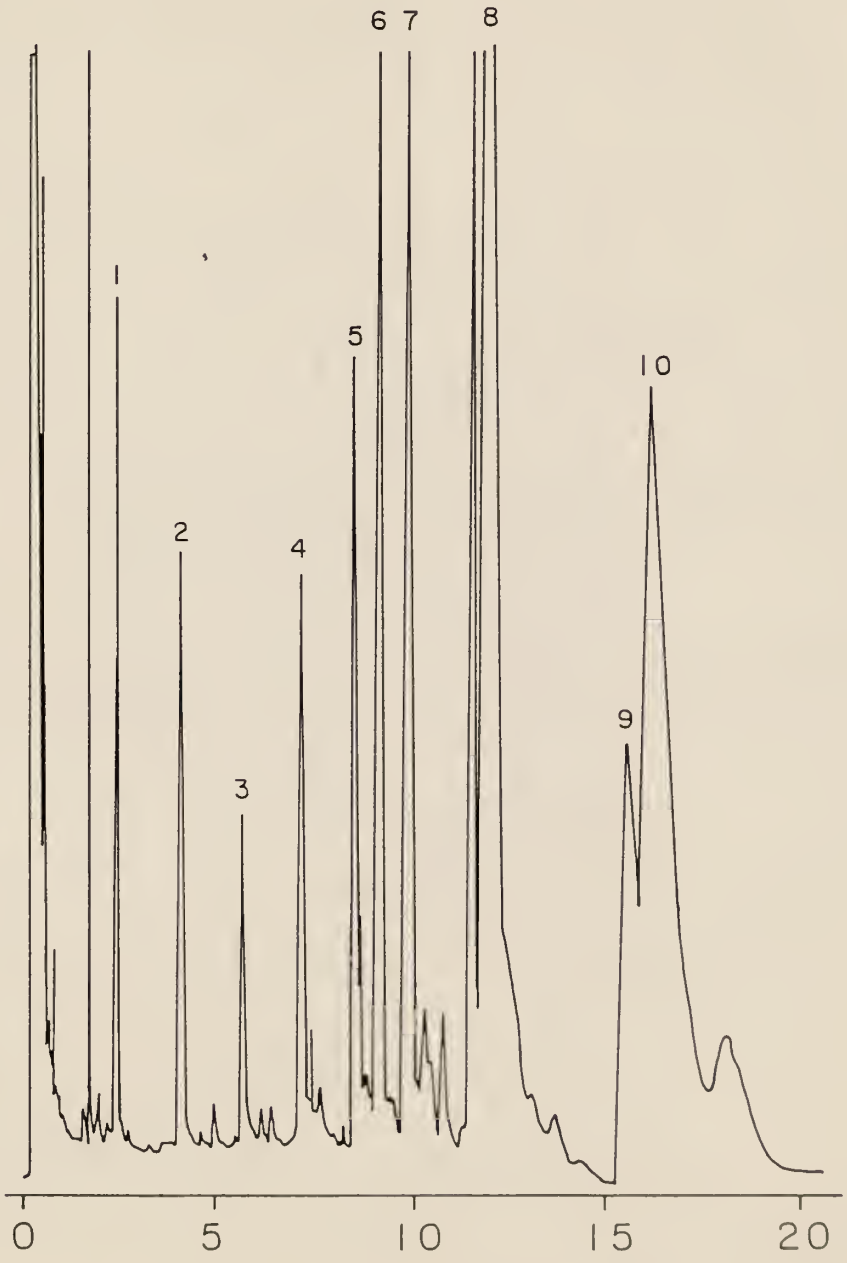
Figure 31. GC Chromatogram of 7 month old stale milk filtered through C_{18} Sep-Pak.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

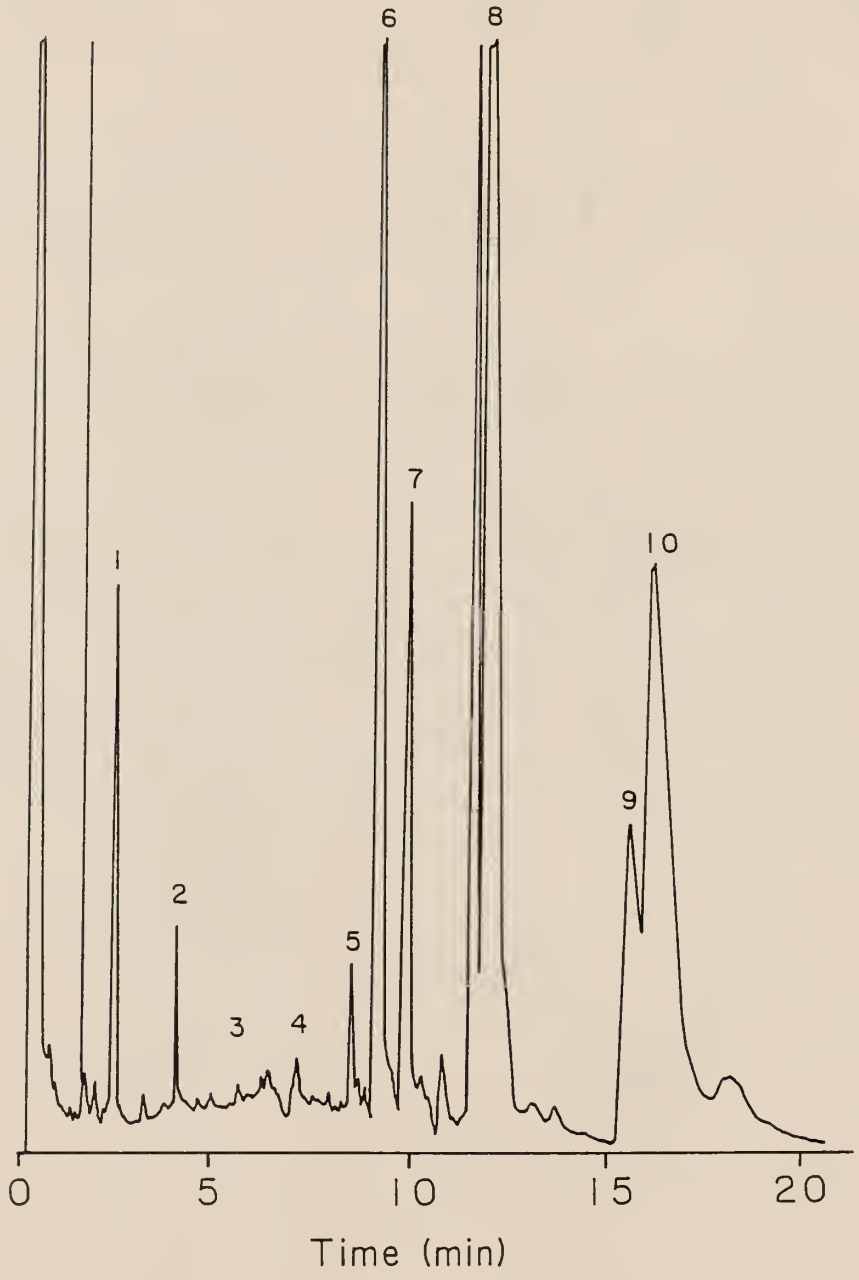
Figure 32. GC Chromatogram of 20 month old stale UHT milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

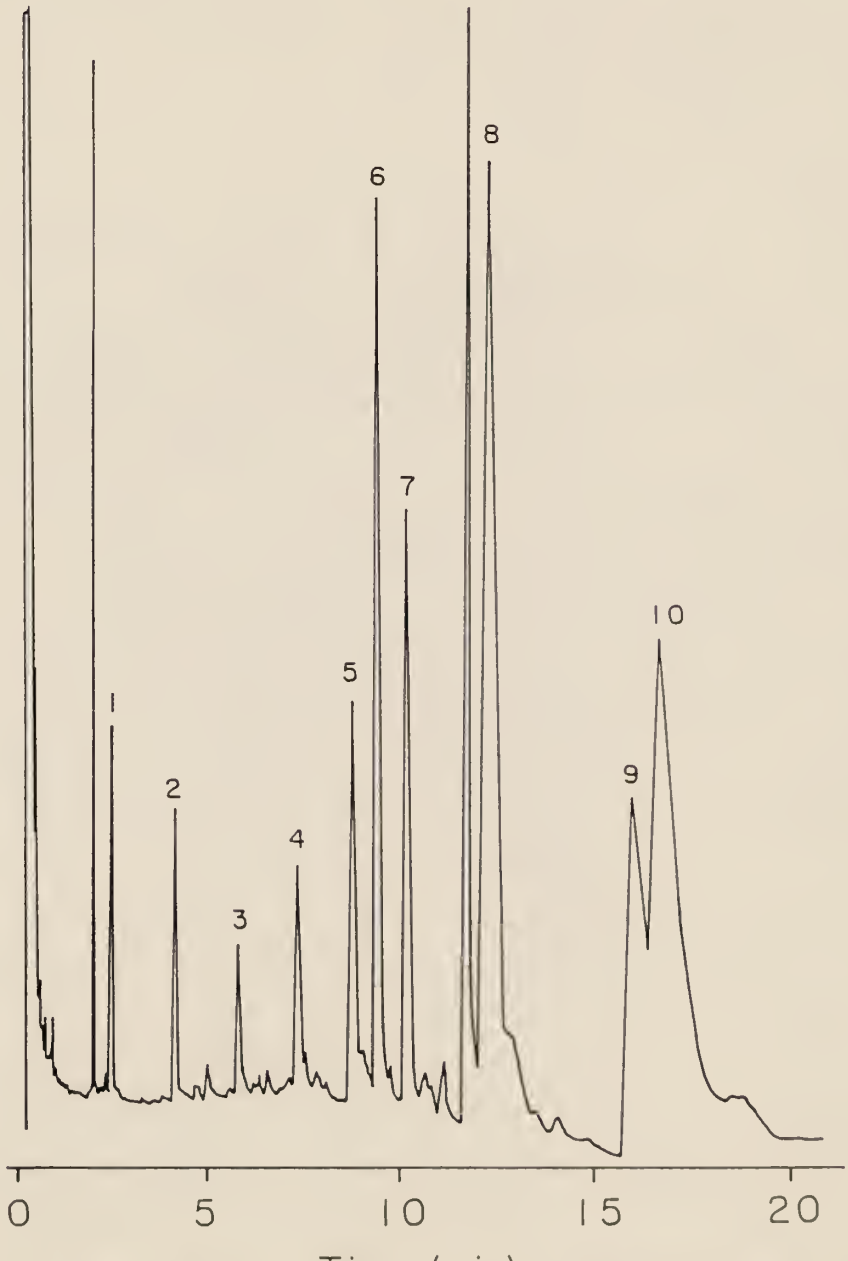
Figure 33. GC Chromatogram of 20 month old stale UHT milk filtered through C_{18} Sep-Pak.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

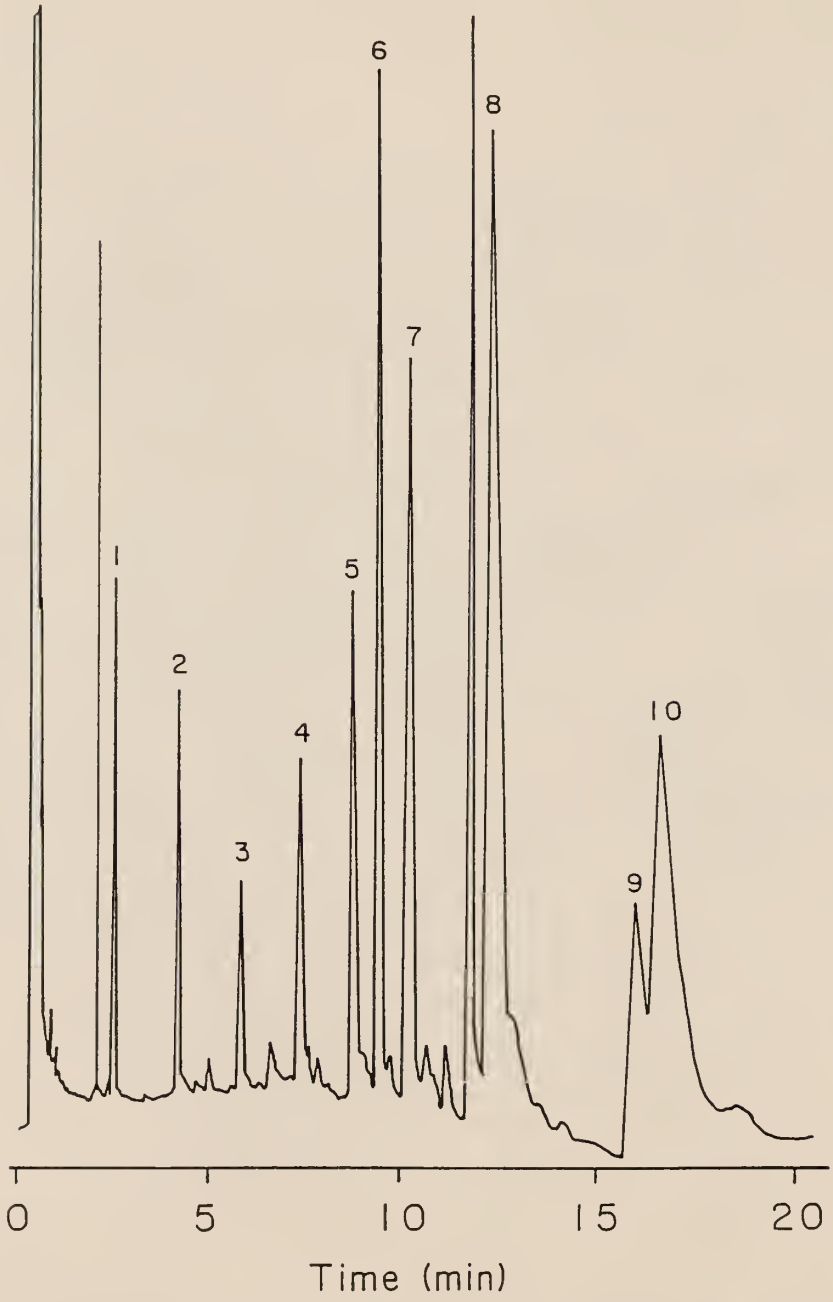
Figure 34. GC Chromatogram of fresh pasteurized milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

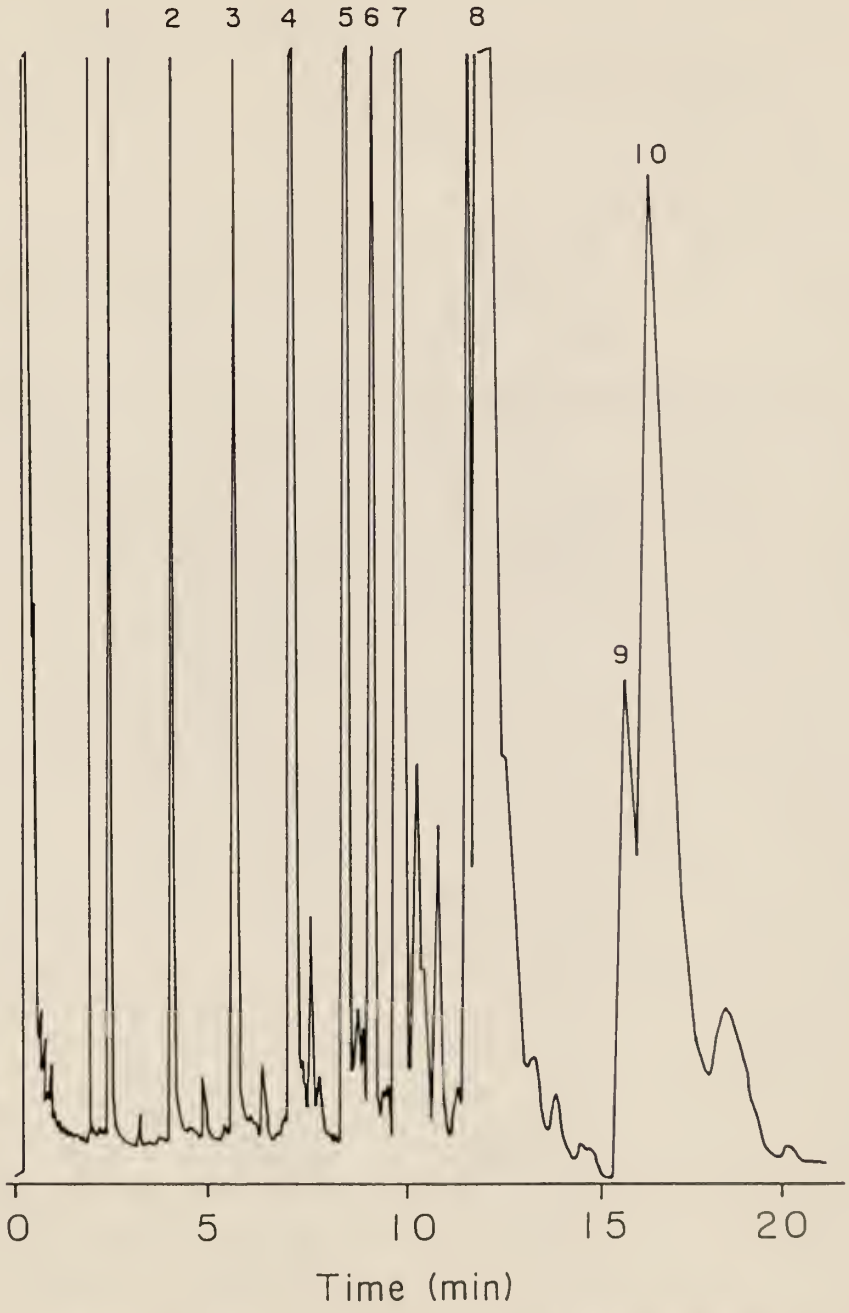
Figure 35. GC Chromatogram of raw milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

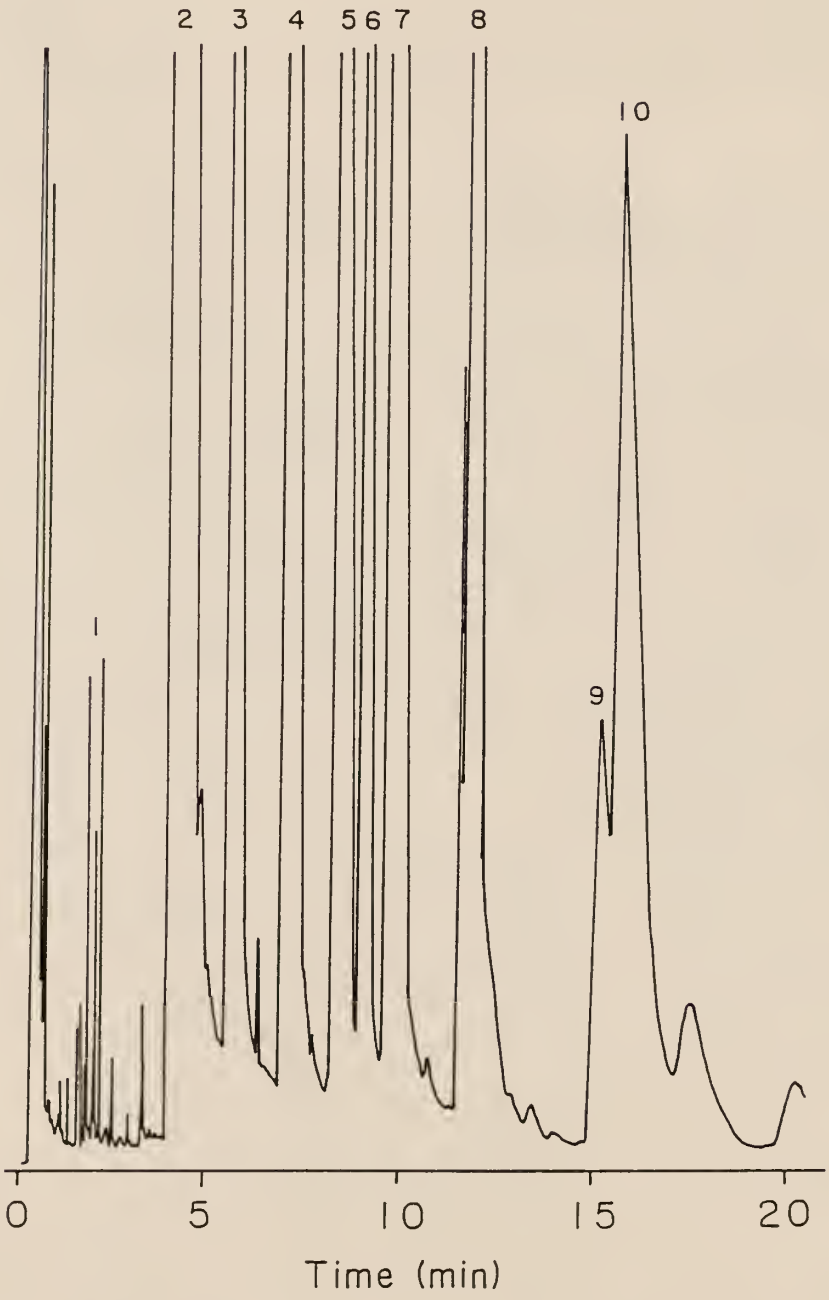
Figure 36. GC Chromatogram of 90% raw/10% pasteurized milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

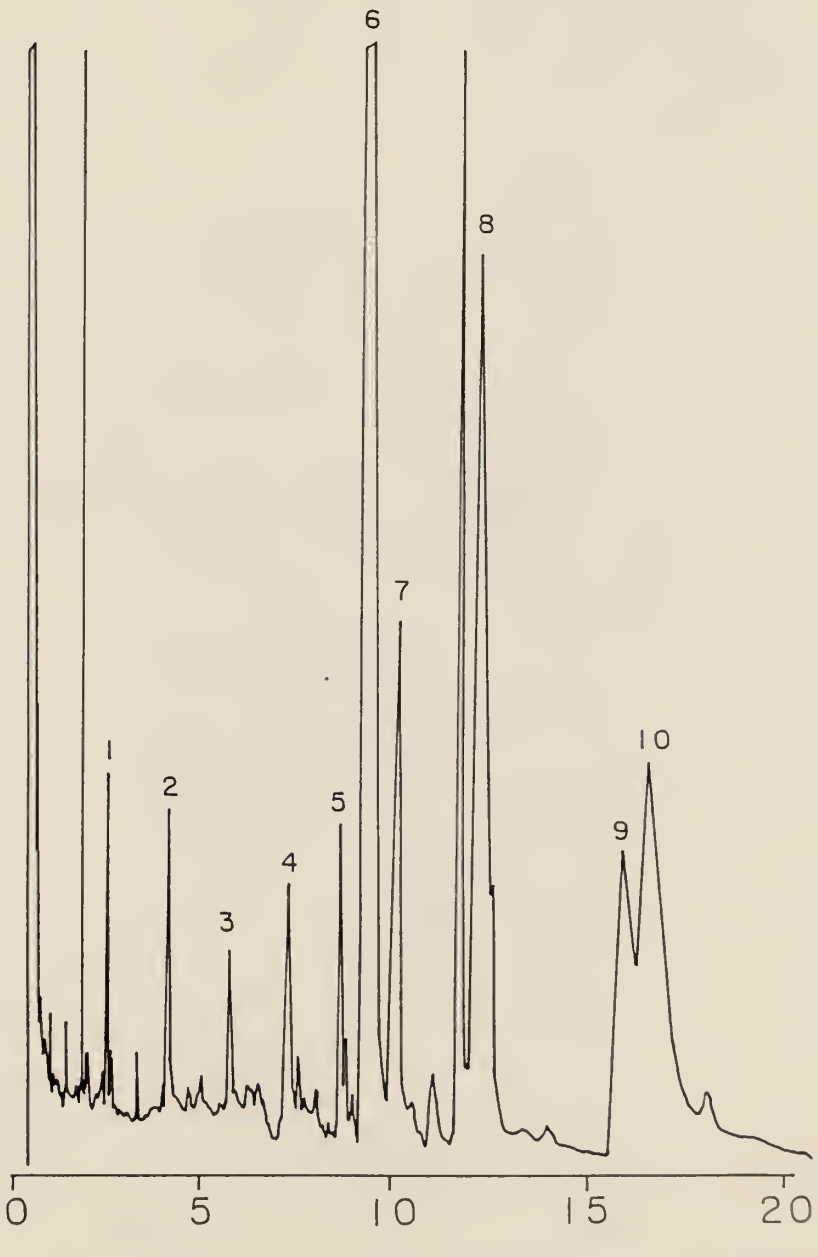
Figure 37. GC Chromatogram of free fatty acid standards.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

Figure 38. GC Chromatogram of fresh UHT milk.



Peak No.

1 = Butyric acid;
2 = Caproic acid;
3 = Caprylic acid;
4 = Capric acid;
5 = Lauric acid;
6 = Tridecanoic acid (internal standard)
7 = Myristic acid;
8 = Palmitic acid;
9 = Stearic acid;
10 = Oleic acid

IV. Analysis of Neutral Volatile Compounds

Data from the analysis of neutral volatile compounds is provided in Tables 7, 8, and 9. The results show the identification of numerous compounds in the fresh, stale, and Sep-Pak filtered milk samples as well as the compounds that were eluted from the Sep-Paks. The compounds identified in the milk samples include the ketones; 2-butanone, 2-pentanone, 2-heptanone, 6-methyl, 2-heptanone, 2-nonanone, 2-decanone, 2-undecanone, and 2-tridecanone. The aldehydes identified were n-hexanal, benzaldehyde, n-heptanal, n-octanal and n-nonanal. Other compounds identified include benzothiazole as well as C_8, C_{10}, C_{12}, free fatty acids. The identification of these compounds is consistent with prior research (Harper and Huber, 1956; Bassette et al., 1966; Badings and Neeter, 1980; Jeon, 1976; Scanlon et al., 1968; Rerkrai, 1986). Although numerous compounds were identified in the milk samples, only some had increased in concentration from the fresh to the stale samples. Concentrations increased were n-hexanal, 2-heptanone, 2-nonanone, 2-nonanal, 2-butanone, 2-decanone, and 2-tridecanone. These compounds are probably the result of lipid oxidation.

Results from the Sep-Pak treated stale UHT milk showed that only 2-heptanone and 2-nonanone decreased in concentration from the Sep-Pak filtering. When the Sep-Pak filters were eluted with organic solvents, 2-heptanone, 2-nonanone, octanoic acid (C_8), decanoic acid (C_{10}), and dodecanoic acid (C_{12}) were

identified. This indicates that the C_{18} Sep-Pak filters are effective in removing these compounds. Based on the fact that passing stale UHT milk through C_{18} Sep-Paks removed the stale flavor, and the compounds trapped in the filter have been identified, it is now possible to take a closer look at these compounds by attempting to replicate the results. The chromatograms for the samples are provided in Figures 39 to 49.

Table 7. Compounds identified in 7 mo. stale UHT milk.

	Stale (ppm)	Sep-Pak treated	Trapped in Sep-Pak
1-butanol	0.75	0.75	-
1-pentanone	0.019	-	-
n-hexanal	0.014	*	-
2-heptanone	0.214	-	+
n-heptanal	0.010	-	-
benzaldehyde	0.013	*	-
n-octanal	0.005	0.006	-
2-nonanone	0.183	0.053	+
n-nonanal	0.011	0.008	-
2-decanone	0.047	*	+
benzothiazole	*	*	-
2-undecanone	0.151	0.155	-
2-tridecanone	0.225	*	-
octanoic acid	*	*	+
decanoic acid	*	*	+
dodecanoic acid	*	*	+

* = identified but not quantifiable
- = not found in sample
+ = found in Sep-Pak

Table 9. Compounds identified in 20 mo. stale UHT milk.

	Stale (ppm)	Sep-Pak Treated	Trapped in Sep-Pak
1-butanol	0.75	0.75	-
2-butanone	0.075	-	-
n-hexanal	0.006	0.001	-
2-heptanone	0.561	0.097	+
n-heptanal	0.008	0.006	-
benzaldehyde	0.024	0.007	-
2-heptanone 6-methyl	0.763	-	-
n-octanal	0.004	0.003	-
2-nonanone	0.436	0.038	+
n-nonanal	0.033	0.019	-
benzothiazole	-	*	-
2-decanone	*	0.001	-
2-undecanone	0.118	0.039	-
2-tridecanone	*	*	-
octanoic acid	*	*	+
decanoic acid	*	*	+
dodecanoic acid	*	*	+

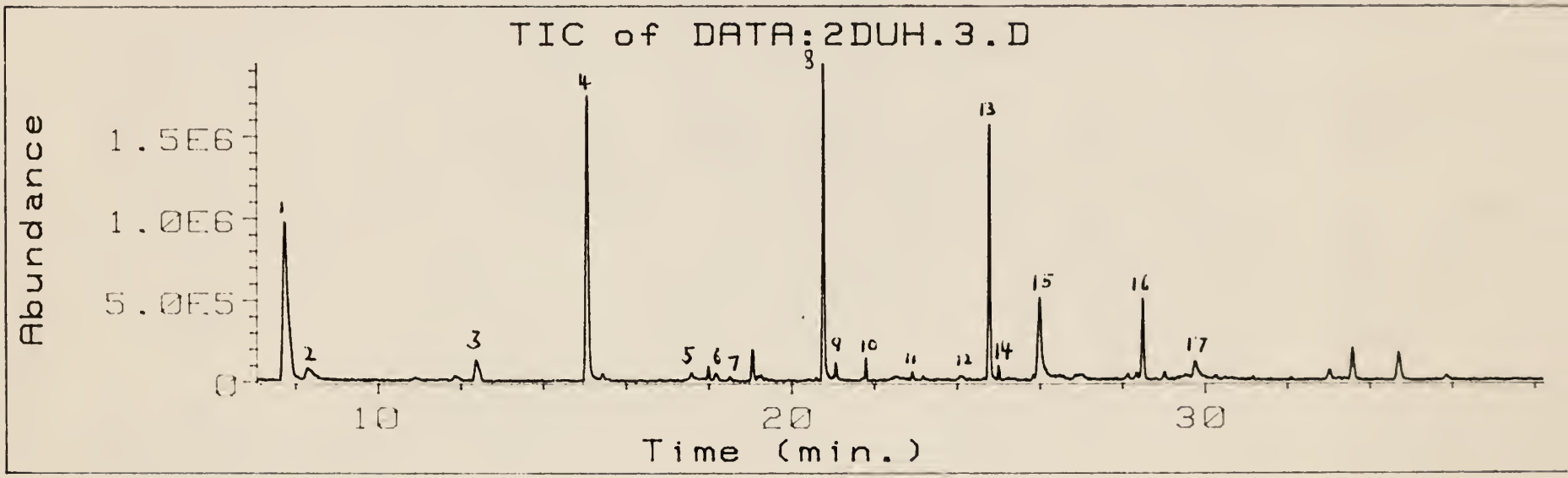
* = identified but not quantifiable
- = not found in sample
+ = found in Sep-Pak

Table 8. Compounds indentified in fresh UHT sample.

	Fresh (ppm)
1-butanol	0.75
2-butanone	0.010
2-pentanone	0.018
hexanal	0.0135
heptanal	0.0092
benzaldehyde	0.0125
octanal	0.0065
2-nonanone	0.133
nonanal	0.0108
decanone	0.4664
undecanone	0.147
tridecanone	0.224
octanoic acid	*
decanoic acid	*
dodecanoic acid	*

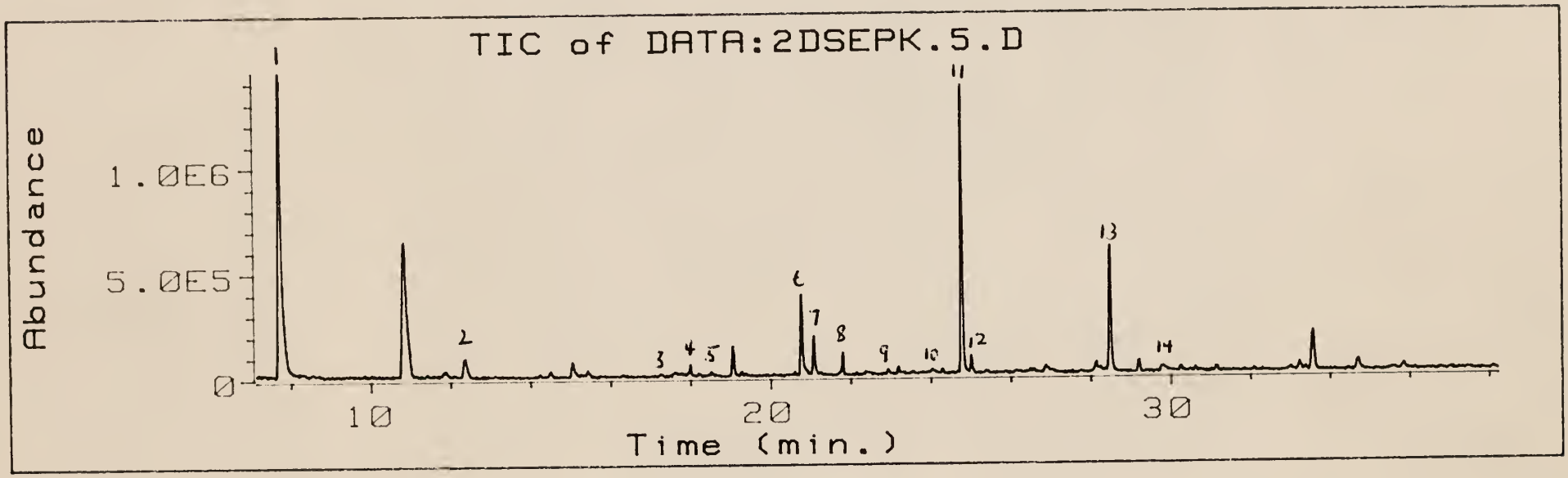
* = identified but not quantifiable

Figure 39. Chromatogram for 7 mo. stale UHT sample



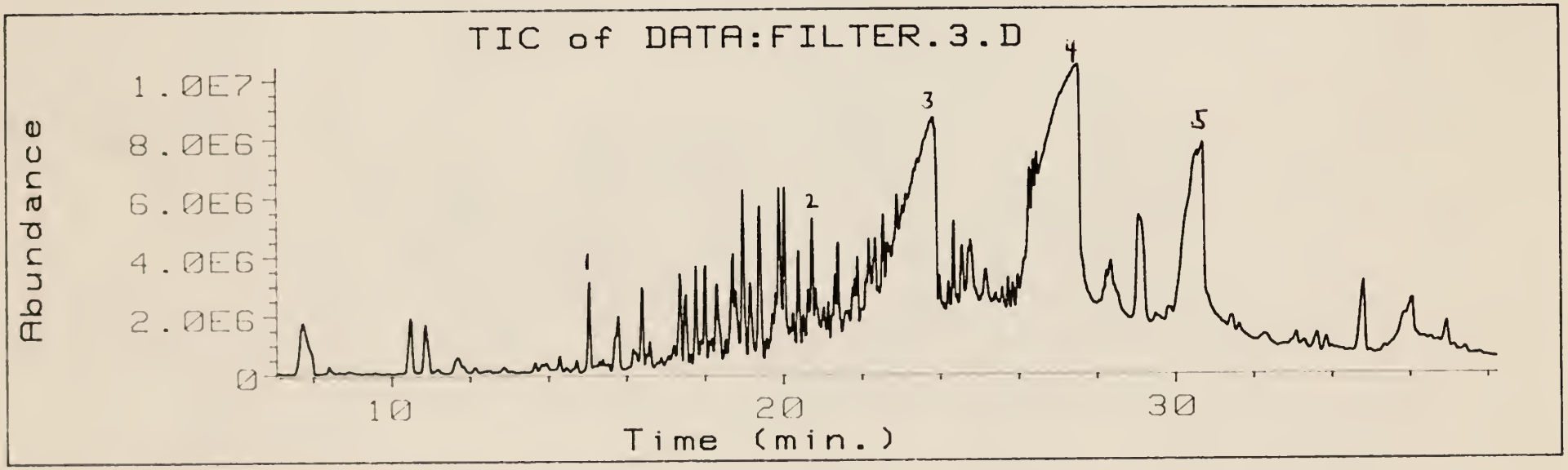
1 = 1-butanol
2 = pentanone
3 = hexanal
4 = 2-heptanone
5 = benzaldehyde
6 = cyclotetrasilxane
7 = octanal
8 = nonanone
9 = nonanal
10 = cyclopentasiloxane
11 = 2-decanone
12 = benzothiazole
13 = 2-undecanone
14 = cyclohexasiloxane
15 = decanoic acid
16 = tridecanone
17 = dodecanoic acid

Figure 40. Chromatrogram of 7 mo stale UHT sample filtered through Sep-Paks



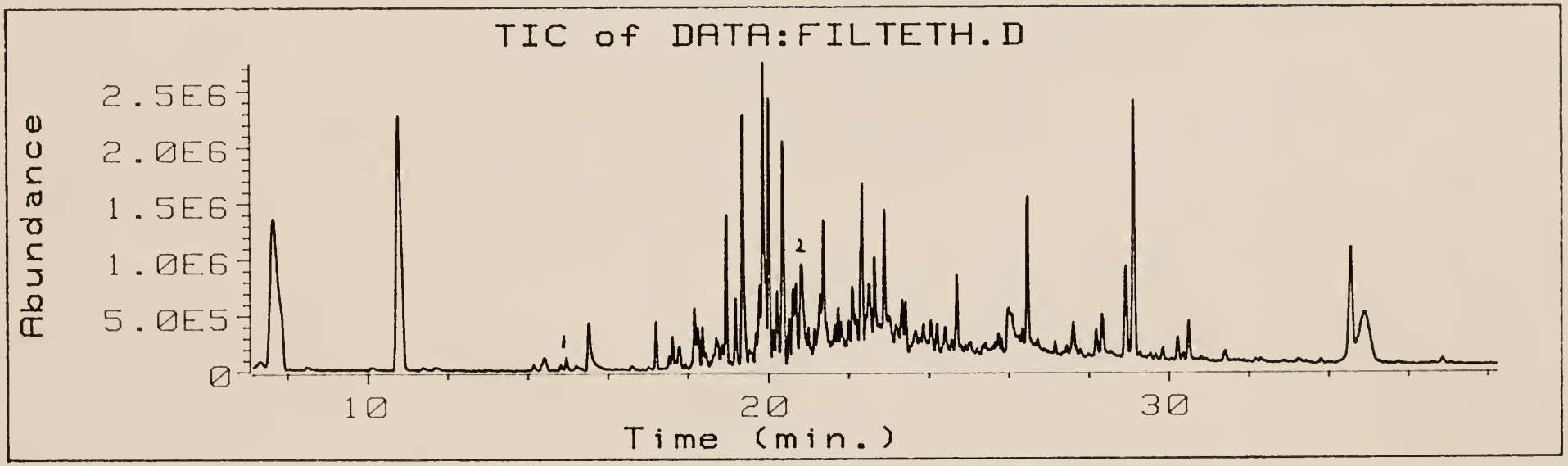
1 = 1-butanol
2 = hexanal
3 = benzaldehyde
4 = cyclotetrasiloxane
5 = octanal
6 = 2-nonanone
7 = nonanal
8 = cyclopentasiloxane
9 = 2-decanone
10 = benzothiazole
11 = undecanone
12 = cyclohexasiloxane
13 = tridecanone
14 = dodecanoic acid

Figure 41. Chromatogram for methylene chloride elution of Sep-Paks 7 mo stale UHT sample



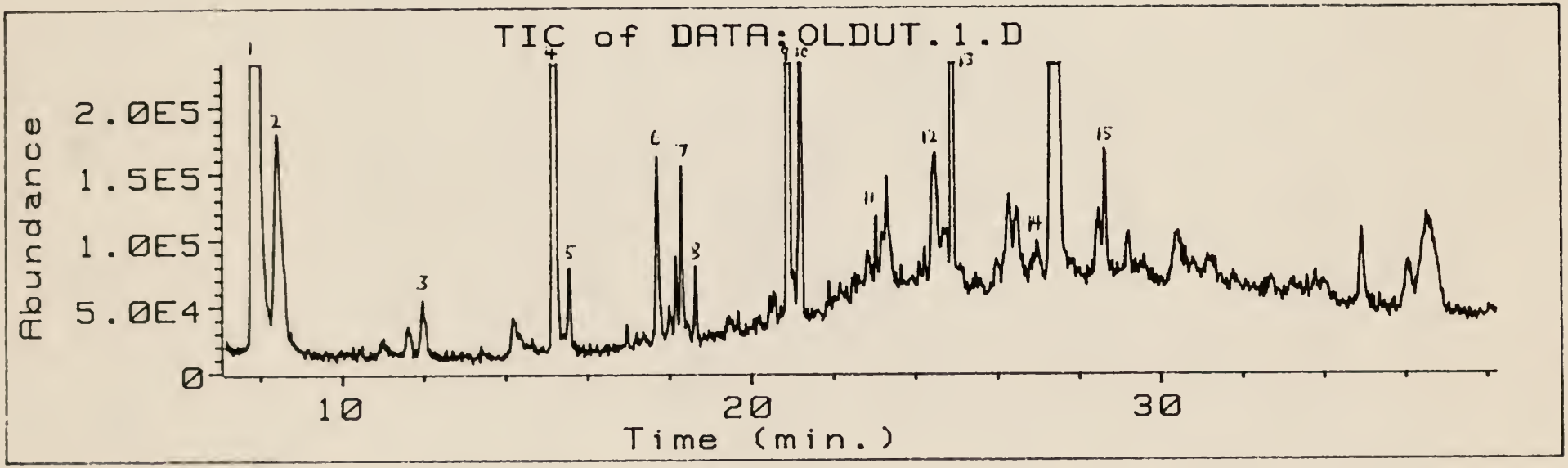
1 = 2-heptanone
2 = 2-nonanone
3 = octanoic acid
4 = decanoic acid
5 = dodecanoic acid

Figure 42. Chromatogram for ethyl-ether elution of Sep-Paks for 7 mo stale UHT sample



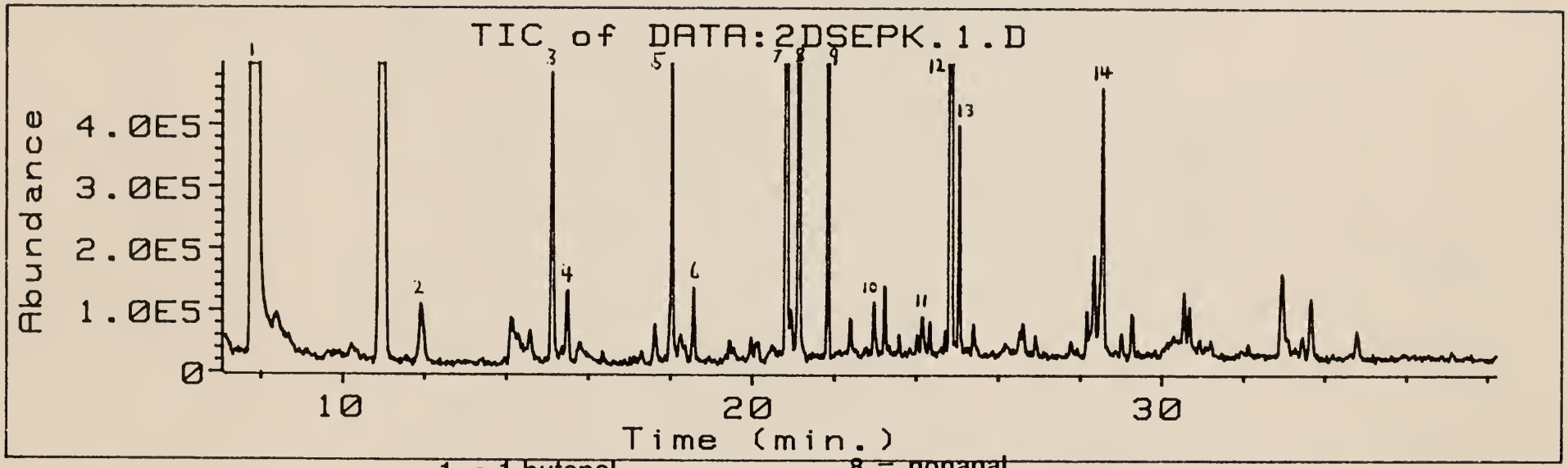
1 = 2-heptanone 2 = 2-nonanone

Figure 43. Chromatrogram of 20 mo stale UHT sample



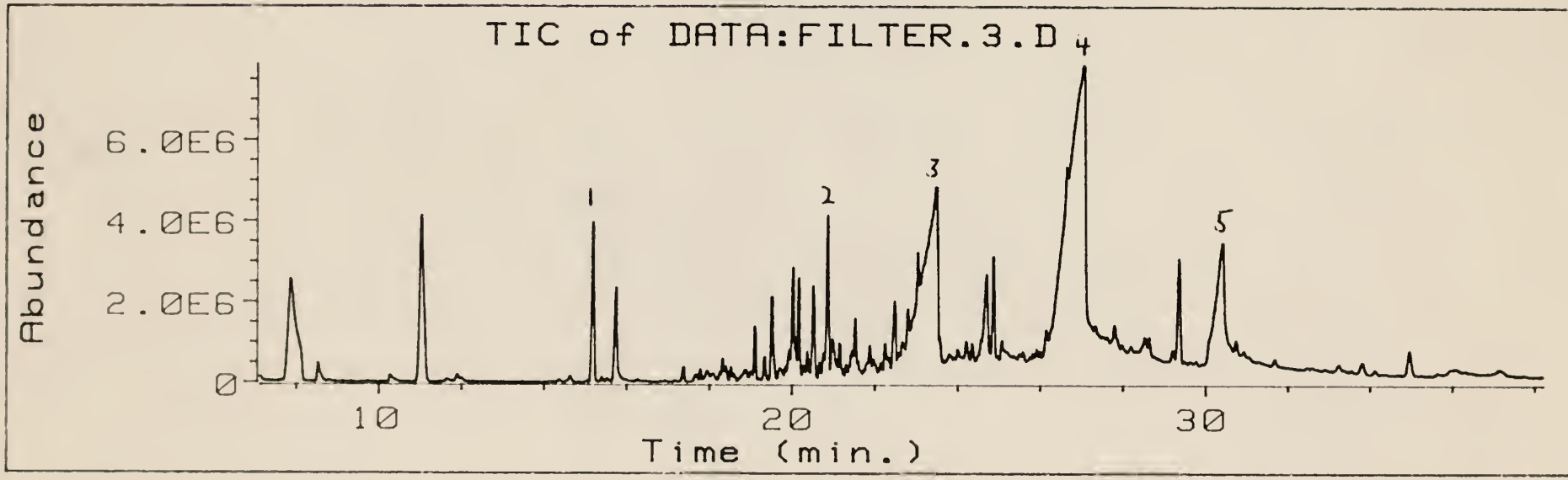
1 = 1-butanol
2 = 2-butanone
3 = hexanol
4 = heptanone
5 = heptanal
6 = benzaldehyde
7 = 2-heptanone 6-methyl
8 = octanal
9 = 2-nonanone
10 = nonanal
11 = decanone
12 = cyclobutanone 2-methyl
13 = undecanone
14 = decanoic acid
15 = tridecanone

Figure 44. Chromatograpm of 20 mo. stale UHT filtered through Sep-Paks



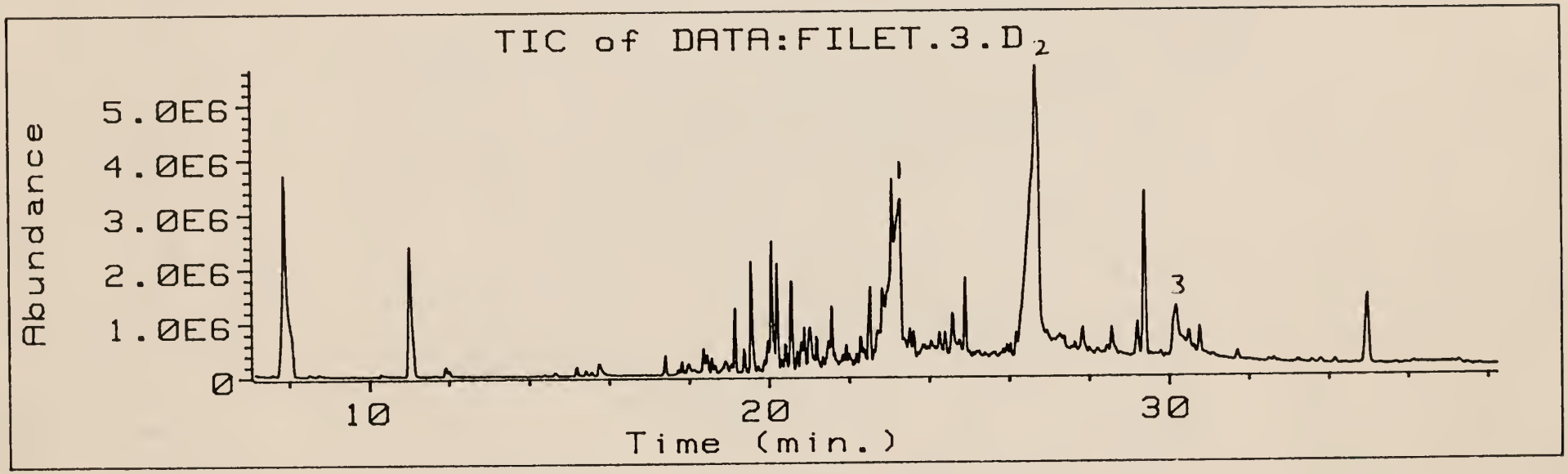
1 = 1-butanol
2 = heptanone
3 = hexanal
4 = heptanal
5 = cyclotetra siloxane
6 = octanal
7 = 2-nonanone
8 = nonanal
9 = cyclopenta siloxane
10 = 2-decanone
11 = benzothiazole
12 = undecanone
13 = cyclohexasiloxane
14 = tridecanone

Figure 45. Chromatogram for methylene chloride elution of Sep-Paks of 20 mo. stale UHT sample



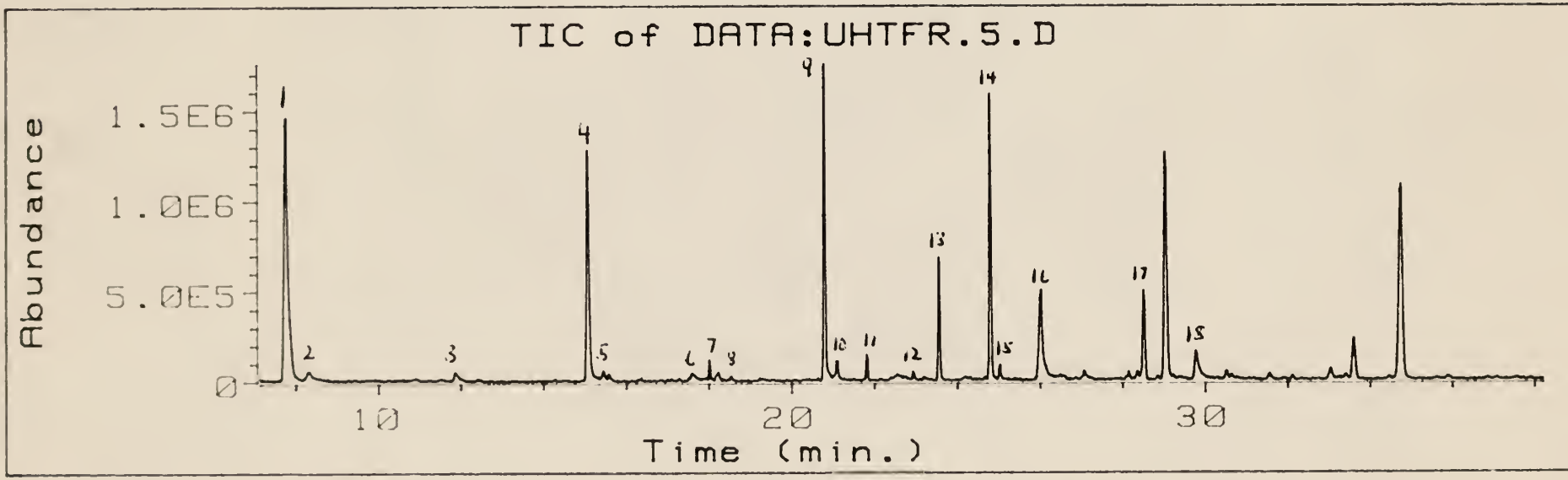
1 = 2-heptanone
2 = 2-nonanone
3 = octanoic acid
4 = decanoic acid
5 = dodecanoic acid

Figure 46. Chromatogram for ethyl-ether elution of Sep-Paks of 20 mo. stale UHT sample



1 = octanoic acid 2 = decanoic acid
3 = dodecanoic acid

Figure 47. Chromatogram of fresh UHT sample



1 = 1-butanol
2 = pentanone
3 = hexanal
4 = 2-heptanone
5 = heptanol
6 = benzaldehyde
7 = cyclopentasiloxane
8 = octanal
9 = 2-nonanone
10 = nonanal
11 = cyclopentasiloxane
12 = decanone
13 = octanoic acid
14 = undecanone
15 = cyclohexasiloxane
16 = decanoic acid
17 = tridecanone
18 = dodecanoic acid

Figure 48. Chromatogram of compounds eluted from Sep-Pak with methylene chloride

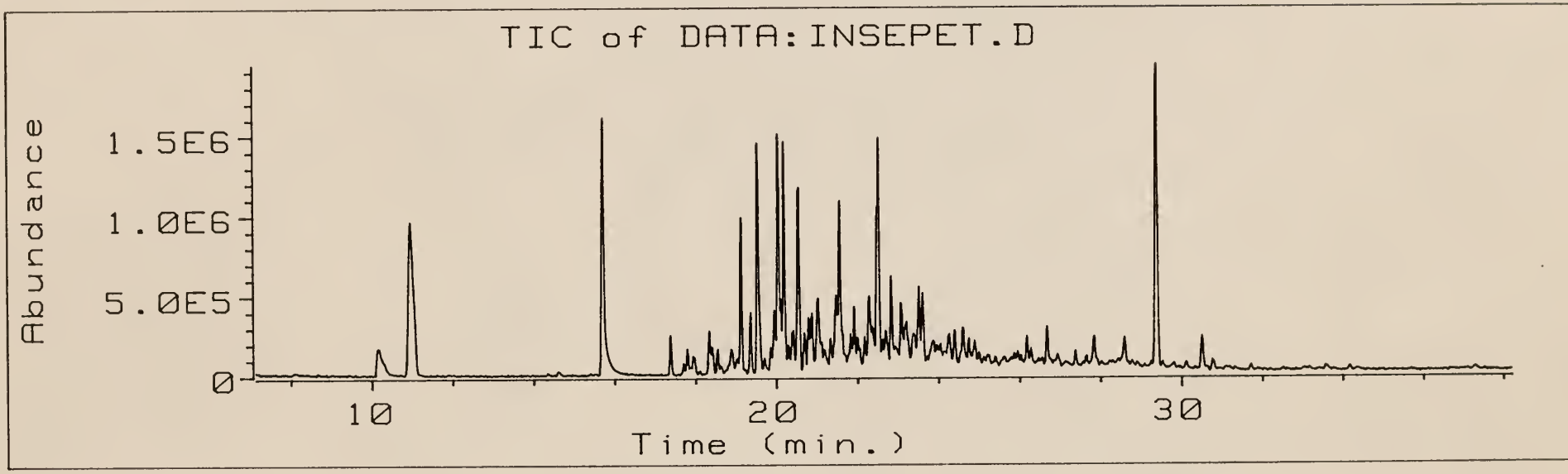
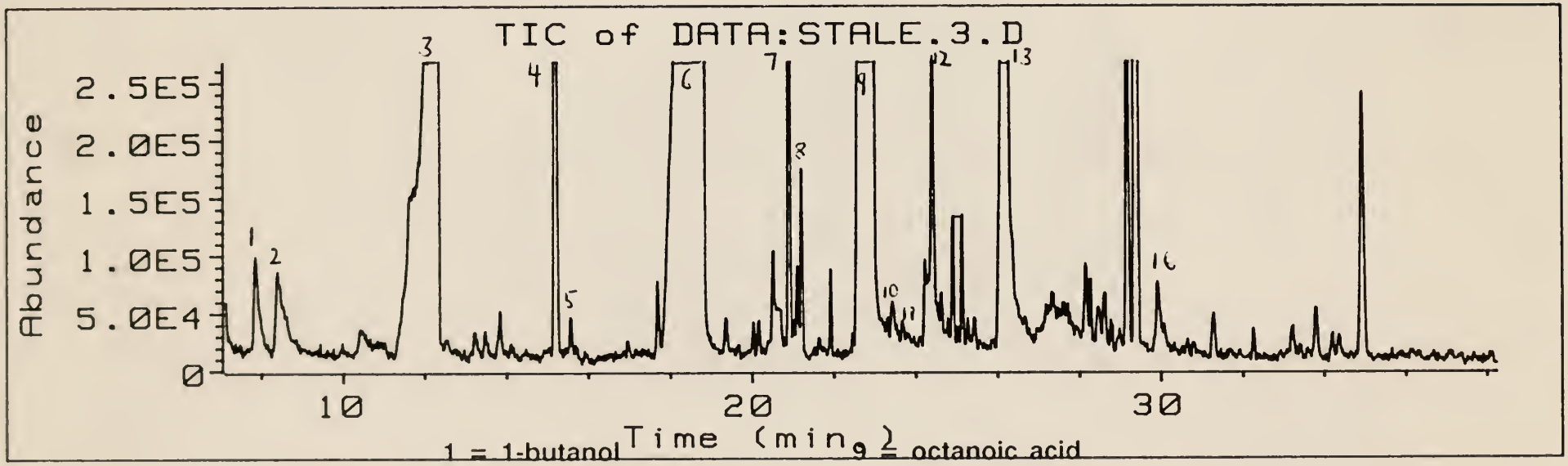


Figure 49. Chromatogram for 3 mo stale UHT sample with HCL added



1 = 1-butanol
2 = pentanone
3 = butanoic acid
4 = 2-heptanone
5 = benzaldehyde
6 = hexanoic acid
7 = nonanone
8 = nonanal
9 = octanoic acid
10 = benzothiazole
11 = nonanoic acid
12 = undecanone
13 = decanoic acid
14 = phenol, 2-methoxy-6 (1-propenyl)
15 = phenol, 2, 6-bis, 1-dimethylethy
16 = dodecanoic acid

V. Stale Flavor Replication

As reported earlier in the study of free fatty acids and neutral volatile compounds, octanoic acid (C_8), decanoic acid (C_{10}), n-hexanal, 2-heptanone, n-heptanal, 2-nonanone, n-nonanal, 2-butanone, 2-decanone, 2-undecanone, and 2-tridecanone showed increases in concentration for the stale UHT sample when compared to the fresh UHT sample. The differences in chemical concentration between the two samples were added directly to fresh UHT milk to test the replicability of stale flavor. Results from the organoleptic evaluation of these samples are listed in Table 9.

Table 9. Means of sensory flavor scores for stale flavor replication.

	Sample				
Defect	**C8**	**C10**	**C8 & C10**	**Volatiles**	**C8 & C10 & Volatiles**
			Scores[1]		
Cooked Aroma Intensity	2.3	2	2.2	3	2.3
Stale Aroma Intensity	1.7	1.5	2.2	2	2.2
Cooked Flavor Intensity	2.9	2.3	2.3	2.6	2.3
Stale Flavor Intensity	2.1	3.6	3.7	1.9	3.9

[1] Scores: 1 = none to 5 = extremely intense

Cooked aroma and flavor intensity is graphically represented in Figures 50 and 51. In all the samples, a slight to moderate level of cooked aroma and flavor were detected. Differences among any of the samples were negligible. This result

Fig. 50 Summary of Organoleptic Results

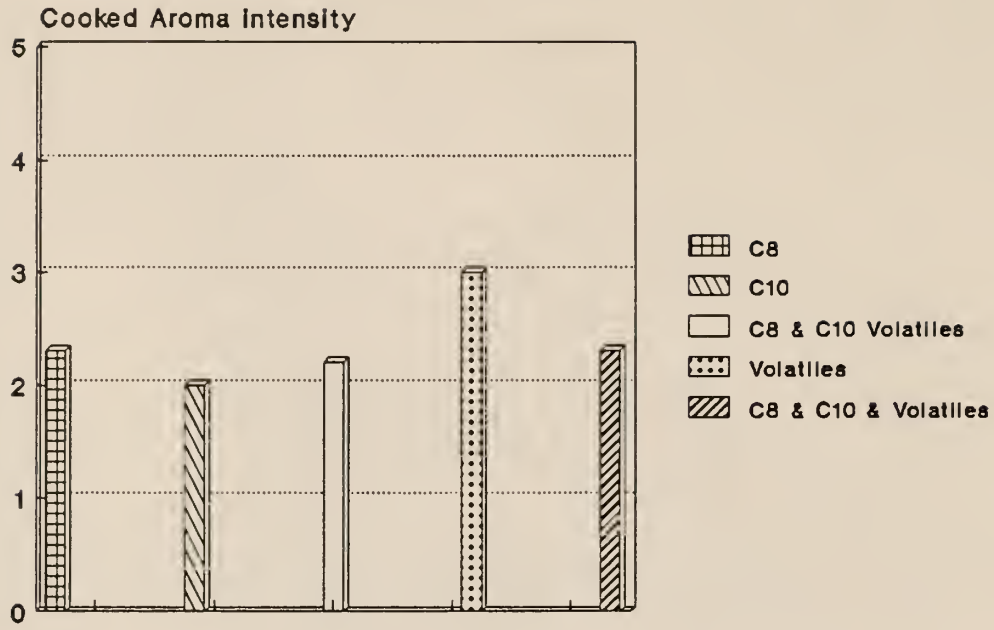
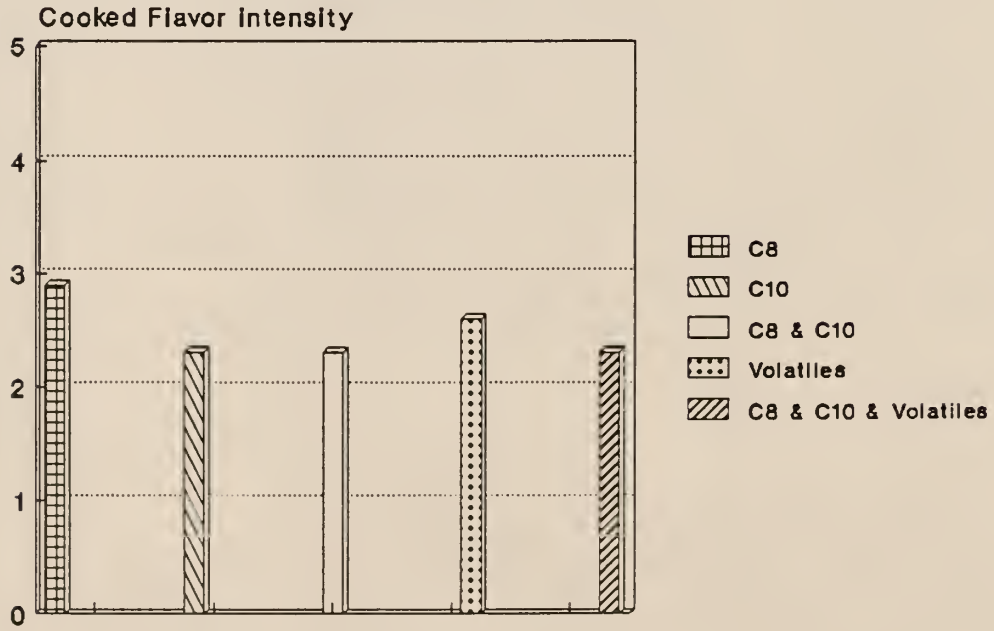


Figure 51 Summary of Organoleptic Results



is consistent with the expected outcome. Since fresh UHT milk was used to prepare all the samples, the high heat used in UHT processing resulted in the cooked flavor due to the denaturation of the serum proteins and subsequent release of free -SH groups (Ashton, 1965; Hostettler, 1972; Clarke, 1967; Burton, 1988).

Stale aroma and flavor intensity are represented graphically in Figures 52 and 53, respectively. The detection of stale aroma was slight for all of the samples and the differences were negligible. The outcome for stale flavor intensity, however, did not follow the same pattern. Neither the addition of octanoic acid (C_8) or the volatile compounds to UHT milk appeared to significantly affect the stale flavor development. On the other hand, the addition of decanoic acid (C10) resulted in a significant increase of stale flavor intensity, far greater than for any other single factor. However, the combination of C_8 and C_{10} added to the sample resulted in a slightly higher stale flavor intensity than C_{10} alone. An even greater stale flavor intensity was achieved by adding C_8, C_{10}, and the volatiles to the UHT sample.

This organoleptic data suggests that decanoic acid (C_{10}) plays a major role in stale flavor development and that octanoic acid (C_8) and the volatiles play only minor roles. The combination of C_8, C_{10}, and the volatiles together in the correct concentrations appear to blend together to form the characteristic stale flavor of UHT milk.

Figure 52 Summary of Organoleptic Results

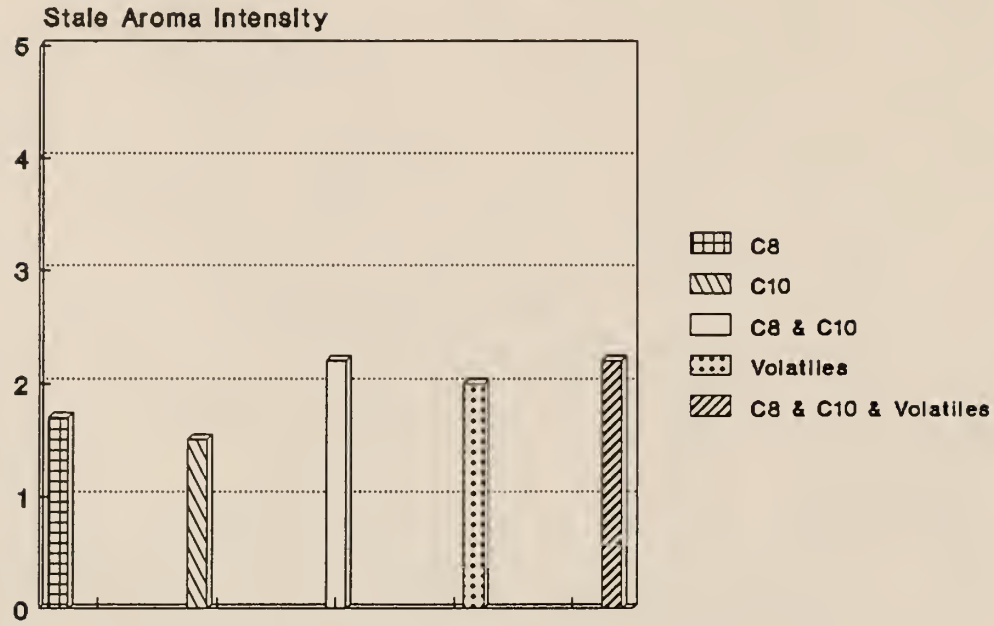
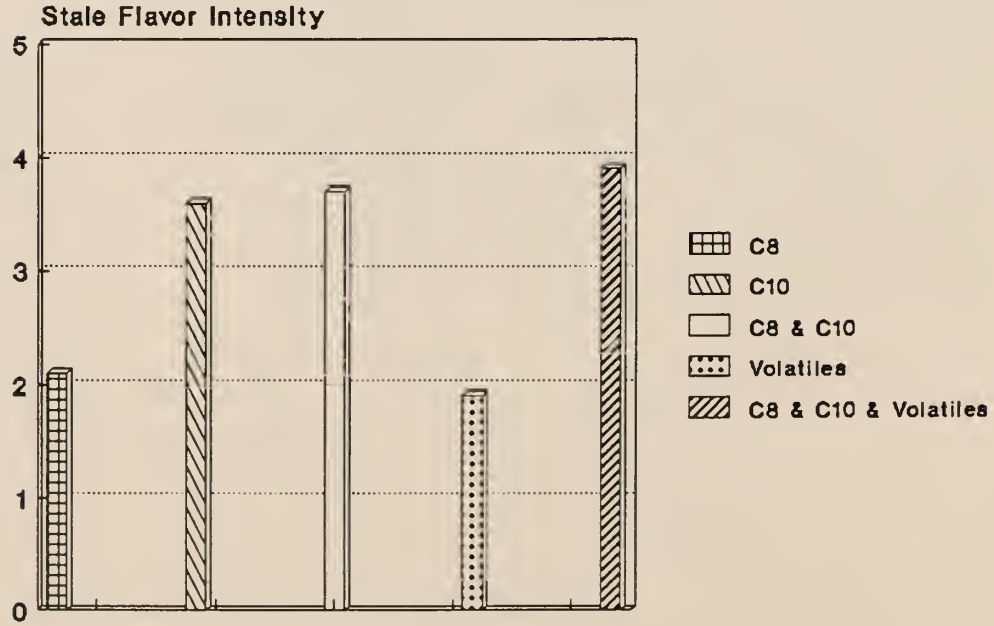


Figure 53. Summary of Organoleptic Results



CONCLUSIONS

The following results were obtained from this study:

1. The use of C_{18} Sep-Pak cartridges reduce the stale flavor intensity of stale UHT milk.

2. The concentrations of all the free fatty acids in milk increase over time.

3. The compounds entrapped in the C_{18} Sep-Pak are: 2-heptanone, 2-nonanone, octanoic acid (C_8), decanoic acid (C_{10}), and dodecanoic acid (C_{12}).

4. When stale UHT milk is passed through the Sep-Paks, most of the C_8 and C_{10} are removed while leaving the other free fatty acid concentrations unchanged.

5. Increased concentrations of decanoic acid (C_{10}) play a dominant role in stale flavor development but the influence of other compounds (octanoic acid C_8 and volatiles) contribute towards the characteristic stale flavor in UHT milk.

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SOLID PHASE EXTRACTION OF STALE FLAVOR COMPONENTS FROM ULTRA-HIGH-TEMPERATURE PROCESSED MILK

by

FRANK TAKACS, JR.

B.B.A., Roanoke College, 1977

AN ABSTRACT OF A THESIS

Submitted in partial fulfillment of the

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MASTER OF SCIENCE

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ABSTRACT

Various solid phase filters were tested to determine their effect in removing the stale flavor components from ultra-high-temperature (UHT) processed milk. Organoleptic studies indicate that passing stale UHT milk through C_{18} Sep-Pak cartridges (solid phase) substantially reduced the stale flavor intensity of milk. Samples from fresh UHT, stale UHT, and stale UHT milk filtered through C_{18} Sep-Pak cartridges were then analyzed for the presence of free fatty acids and neutral volatile compounds using a gas chromatograph and gas chromatograph - mass spectrometer. Based on a comparison of the chromatographic results, selected compounds were added directly to fresh UHT milk to test replicabilty of stale flavor.

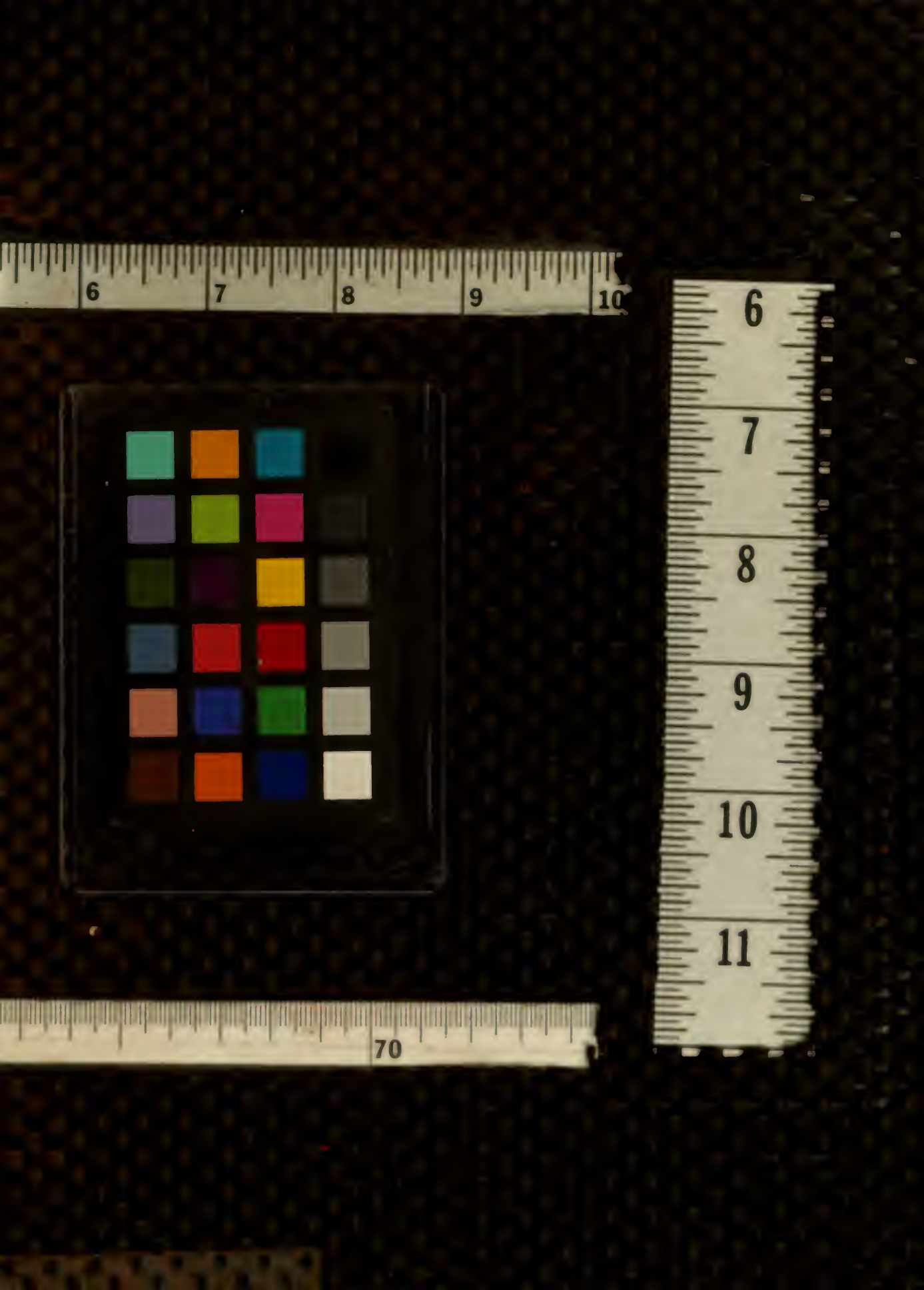
By comparing the free fatty acid profiles of stale with filtered stale milk, the C_{18} Sep-Pak cartridges were effective in removing most of the C_8 and C_{10} free fatty acids while leaving the other fatty acids relatively unchanged. The C_8 and C_{10} free fatty acid concentrations for the stale filtered UHT milk were similar to the levels found in fresh UHT milk. A fresh UHT sample evaluated at 30, 60, and 90 day intervals showed a substantial increase in all of the free fatty acids monitored over the period (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$).

Steam distillation along with organic solvent extraction of volatile compounds from fresh, stale, and filtered UHT milk were analyzed using a Gas Chromatograph-Mass Spectrometer. Numerous volatile compounds were identified and

concentrations of n-hexanal, 2-heptanone, n-heptanal, 2-nonanone, n-nonanal, 2-butanone, 2-decanone, 2-undecanone, and 2-tridecanone showed an increase for the stale samples. Organic solvent elution of the Sep-Pak cartridges and GC-MS analysis revealed that large concentrations of C_8 and C_{10} free fatty acids were entrapped in the filters.

Organoleptic data suggests that C_{10} free fatty acid has a greater effect on stale flavor development than any other single compound and that individually, C_8 and the volatile compounds exert minimal influence. However, when C_8, C_{10}, and the volatile compounds were combined, the intensity of stale flavor increased.

The data in this research implicates increased concentrations of C_{10} free fatty acids as playing a major role in the stale flavor development of UHT milk. Although C_{10} appears to play a dominant role, the influence of other compounds (C_8 and volatiles) contribute towards the characteristic stale flavor in UHT milk.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