Using a Simultaneous Factor Optimization Approach for the Detection of Volatiles in Milk by Dynamic Headspace Gas Chromatographic Analysis

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Optimum conditions for dynamic headspace capillary gas chromatography were investigated to obtain maximum volatile recovery from pasteurized milk. The simultaneous factor optimization approach used for establishing purge and trap conditions resulted in a method of high sensitivity and repeatability. Factors optimized were purging time, sample temperature, and sample volume. Simultaneous factor optimization not only allowed maximum recovery of volatiles but also allowed detection of factor combinations that resulted in trap breakthrough. A Tenax TA trap for volatile concentration was preferred to other combination traps. A midpolarity column coated with cyanopropyl phenyl poly(dimethylsiloxane) was found to provide better resolution than other stationary phases. Several volatiles consisting mainly of aldehydes, ketones, and alcohols were identified in good-quality milk. Poor-quality milk showed not only the presence of new volatiles including esters but also increased amounts of those already present.

INTRODUCTION

Volatile components are responsible for the aroma and much of the flavors developing in pasteurized milk during storage. Off-flavors in milk are caused by the action of native milk enzymes or bacterial enzymes and chemical changes catalyzed by light or heavy metals. This produces a broad spectrum of possible off-flavors, but the main cause of off-flavor in products of short shelf life such as pasteurized milk is likely to be microbial, with possibly detectable levels of lipolytic rancidity (Burton, 1983). Because the deterioration in flavor of milk at the end of storage is due to combined effects of compounds present at low concentrations, sensory evaluation remains the most useful means of assessing milk flavor quality (Baker, 1983). However, there is need for an objective and predictive test for assessing milk flavor quality. Dynamic headspace gas chromatography (DH-GC) has been recognized as one of the most popular methodologies used in the isolation and separation of flavor volatiles. However, few papers on the use of dynamic analysis on milk are available in the literature. One of the reasons for this may be that instrumentation for dynamic headspace analysis is still evolving and milk is cumbersome to analyze by this technique.

Milk sampling presents two problems for purge and trap concentration; it generates large amounts of water vapor in heated work and foams during purging. Previous researchers isolated spoilage volatiles in milk by using DH-GC (Badings et al., 1985; Wellnitz-Ruen et al., 1982; Urbach and Milne, 1988). However, these studies were of preliminary nature since experimental conditions were not optimized for maximum volatile recovery and lacked the sensitivity required to detect volatiles early in refrigerated storage. The onset of spoilage might be due to a composite effect of volatiles found in trace levels rather than the increase of a specific compound. Thus, a highly sensitive technique may be required.

Maximum sensitivity in dynamic headspace sampling can be obtained by finding the best factor combinations of sample temperature, purge volume, and sample size. In addition to these sampling conditions that are subject to optimization, sensitivity is also greatly enhanced by the inclusion of an external cryotrap used to refocus the sample prior to injection. An alternative to the use of an external cryotrap in DH capillary GC is the use of a split capillary system that allows the use of higher flows through the trap. However, most of the sample is discarded through the split vent, resulting in decreased sensitivity.

Optimization of purge and trap conditions in milk (Leland, 1986) and milk-based nutritional products (Park and Goins, 1992) was carried out by the one-factor-at-a-time approach. However, factors interact with each other, and the one-factor-at-a-time approach will not always result in an optimum set of conditions. On the other hand, simultaneous factor optimization techniques can predict the effects of changing more than two factors simultaneously, especially when factors are interacting. Among such techniques are response surface methodology (Vuataz, 1986) and simplex (Morgan and Deming, 1974) and random-centroid optimization (Nakai, 1990; Dou et al., 1993). Response surface methodology belongs to the group of methods based on curve fitting. Simplex and random-centroid optimizations are sequential methods that belong to the group of evolutionary operation techniques (Nakai, 1990; Dou et al., 1993). Talou et al. (1987) optimized purge and trap conditions for the extraction of volatiles in black truffles by using response methodology. Yet techniques such as response surface methodology based on quadratic curve fitting assumed a smooth response surface. On the contrary, in simplex or random-centroid optimization, no previous knowledge of the mathematical relationship among variables is required (Nakai, 1990; Dou et al., 1993). An advantage of the random-centroid optimization (RCO) of Nakai (1990) over the iterative process of sequential simplex optimization (SO) is that RCO has a better chance to home-in on the global optimum rather than being trapped in a local optimum.

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0021-8561/93/1441-2378$04.00/0

The objectives of the present study were to establish optimum conditions for DH-GC to attain maximum recovery or sensitivity and adequate separation of pasteurized milk volatiles by using the random-centroid technique as a simultaneous factor optimization approach and to identify volatiles present in good- and poor-quality milk samples. In this study a cryotrap or capillary interface was used so that there was no need for splitting the injection.

MATERIALS AND METHODS

Materials. Samples were commercially pasteurized and homogenized whole milk packaged in 250-mL cartons coming from a crate consisting of 36 units and corresponding to consecutive packages from the same production line. Samples were frozen at −40 °C until used for testing the different DH-GC conditions. For the identification of volatiles, milk from a 4-L jug was aseptically dispensed into sterile 250-mL delong culture flasks and was refrigerated at 4 ± 1 °C until the end of shelf life as determined by sensory evaluation. To enhance volatile production, milk was incubated at 25 °C for 18 h before DH-GC.

Analytical grade standards were Polyscience esters and alcohol kits 62CX, 68C, and 11C (Polyscience Corp., Niles, IL) and aldehyde and ketone Theta kit TK-150 (Theta Corp., Newton Square, PA). To diminish foaming, tetradecanol (Sigma, St. Louis, MO) was added to milk during purging. Ten microliters of a 2.5 ppm solution of 4-methyl-2-pentanone in boiled distilled water was used as the internal standard. The standard solution was kept under refrigeration in vials (10 mL) provided with Mininert valves (Supelco, Inc., Bellefonte, PA).

Sensory Evaluation. Sensory testing was carried out as described by Vallejo-Cordoba (1992) following the method of Bishop et al. (1984). Samples were presented to five trained sensory judges. The method used for training and testing was scoring with the ADSA score card for milk on a 10-point scale. The scorecards contain a list of defects which have been encountered in milk, and a numerical score is used to rate the overall flavor quality. A score of 10 is given to a sample that has a "typical fresh" taste and is free of undesirable flavors. Samples having off-flavors are scored lower. Shelf life was ended whenever a score of 5 or lower was recorded by three of the judges, and the day before was considered the end of shelf life. Good-quality milk was a sample with a typical fresh and clean flavor. Poor-quality milk was a sample with detectable off-flavors.

Dynamic Headspace Gas Chromatographic Analysis. A semiautomated purge and trap concentrator (LSC-3, Tekmar, Cincinnati, OH) coupled to a gas chromatograph (GC-9A, Shimadzu, Columbia, MD) was used for determination of milk volatiles. The gas chromatograph was equipped with a flame ionization detector (FID). Milk samples containing 10 mg of tetradecanol were preheated for 5 min with a sample heater (4100, Tekmar) and purged in a glass test tube sampler provided with a needle sparger. The milk sample was purged with nitrogen at a flow rate of 40 mL/min after a 5-min preheating.

Three different traps (12 in. × 1/8 in.) were preliminarily tested for their performance during volatile collection to select one for further work. One of the traps was packed Tenax TA (60/80 mesh, Tekmar). The other two traps were filled with Tenax TA/charcoal (Tekmar) and a Carbopack B/Carbosieve S-III (Supelco). The traps were dry purged for 10 min after the milk was purged, to eliminate water condensed in the tubing. Volatiles trapped on the adsorbents were thermally desorbed at 185 °C for 5 min and transferred onto the capillary column through an external cryotrap or capillary interface (1000, Tekmar). Volatiles were cryofocused in an uncoated fused silica precolumn (0.5 mm i.d.) held at −150 °C with liquid nitrogen. The precolumn was then flash heated at 50 °C/s for 10 s to vaporize and inject the sample.

Three fused silica capillary columns (30 m, 0.32 mm i.d., J&W Scientific, Rancho Cordova, CA) representing a broad polarity range of stationary phases were preselected. DB-Wax (0.5-μm film thickness), a higly polar column, was coated with bonded poly(ethylene glycol), and DB-624 (1.8-μm film thickness), a midpolarity column, was coated with cyanopropyl phenyl poly(dimethylsiloxane). DB-5 (1-μm film thickness), a relatively nonpolar column, was coated with bonded mixed 5% phenyl–95% methyl polysiloxane.

The temperature program of the gas chromatographic oven was as follows: 5 °C for 6 min, raised to 20 °C at 3 °C/min, followed by 5-min holding, raised to 23 °C at 3 °C/min, followed by 5-min holding, raised to 200 °C at 3 °C/min and followed by 30-min holding. The inlet and FID temperatures were maintained at 240 °C. At the end of the day, the DB-624 capillary column was heated at 260 °C (maximum temperature limit of the column) for 1 h to eliminate accumulated water vapor while the FID was maintained at 280 °C. Helium was used as the carrier gas with a flow rates of 2 and 7 mL/min for the 0.32 and 0.50 mm i.d. columns, respectively.

Random-Centroid Optimization of Purge and Trap Conditions. Purge and trap conditions were optimized by using the random-centroid optimization (RCO) computer program of Nakai (1990), written for a personal computer. RCO starts with a first cycle of test conditions consisting of nine experiments ($3k$, where k is the number of factors) which are randomly chosen to formulate a random design. Testing conditions in a random design are computed by using a random generating program so as to scatter within the set search space. Subsequently, a centroid search consisting of two experiments ($k - 1$) is carried out which is constructed from a simplex consisting of the best $k + 1$ test conditions. The random computation is repeated until certain deterministic rules are met. The response data from the random and centroid runs are combined and mapped to select a new search space for the next cycle, which should be narrower in area than that of the previous cycle, locating nearer the optimum. As a final search in a very narrow space simultaneous shift is carried out.

Three factors were optimized for maximum recovery of volatiles from pasteurized milk, namely, purging time, sample temperature, and sample volume. On the basis of trial and error experiments, the boundary values of each factor were established. Thus, factor ranges were as follows: temperature, 30–50 °C; sample volume, 5–15 mL; and purging time, 10–30 min.

During gas chromatography, most volatiles in the first 15 min of the run were present at high concentrations, whereas volatiles in the remaining portion of the run were present at relatively lower concentrations. Therefore, to balance the effects of these two portions of the chromatogram on the response, a higher weight (0.7) was given to the latter part of the chromatogram to increase the influence of the most strongly retained compounds. On the contrary, a lower weight (0.3) was given to the first part of the chromatogram to diminish the influence on the response of the most volatile and abundant compounds. Values of 0.3 and 0.7 assigned to W_1 and W_2 were arbitrarily decided upon observation of nine chromatograms which corresponded to the first nine experimental conditions in the optimization. The response value entered in the computer program was

$$\text{response} = (W_1)(A_1) + (W_2)(A_2)$$

where A_1 is the total volatile area from start to retention time 15 min and A_2 is the total volatile area from retention time 15.1 min to the end.

A response of zero was assigned whenever the breakthrough volume was detected by a large upswing of the FID signal connected to the trap vent. The Tenax trap and the DB-624 (0.32 mm i.d.) capillary column were used during optimization experiments.

Identification of Milk Volatiles. Milk volatiles of a typical off-flavored milk of poor quality and of a fresh milk of good quality were tentatively identified by using a gas chromatographic/mass spectrometric system (Hewlett-Packard 5985B, Palo Alto, CA). Dynamic headspace gas chromatographic conditions and instrumentation were as described above. Conditions for purge and trap concentration were those optimized by random-centroid optimization. Milk (9.1 mL) was purged for 12.5 min at 44 °C, and volatiles were adsorbed onto the Tenax trap and chromatographed on the DB-624 (0.32 mm i.d.) capillary column. Mass spectra were acquired with the ionization energy at 70 eV and within the mass energy 33–350 m/z. Tentative identification was based only on matching an unknown mass

spectrum with a spectrum in the EPA–NIH mass spectral database library. Confirmation of peak identity was carried out by matching of retention times with those of analytical grade standards.

Sensitivity and precision of the optimized method were assessed by spiking fresh milk with a mixture of ethyl esters (Polyscience kit 62CX), at a concentration of 0.2 ppb of each individual ester. During chromatographic analysis a reject area of less than 500 units was imposed (signal/noise = 10). Limits of detection were determined by calculating ester concentrations corresponding to areas of 500 units. Approximate volatile concentrations were calculated by using 4-methyl-2-pentanone as an internal standard. Ten microliters of a 2.5 ppm solution of 4-methyl-2-pentanone prepared in boiled distilled water was added to 9.1 mL of milk to make a final concentration in the sample of 2.74 ppb. The standard solution was kept under refrigeration in vials (10 mL) provided with Mininert valves (Supelco). Volatile concentrations were approximate values based on the assumption that response factors as well as correction factors for difference in purgeability were equal to those of the internal standard.

RESULTS AND DISCUSSION

Dynamic Headspace Gas Chromatography. Available literature suggested that dynamic headspace sampling was the most suitable technique for trace analysis of volatile flavor compounds in foods. However, as with any other technique, some shortcomings had to be resolved. First, due to the nature of the sample matrix, the purge and trap concentration technique had to be modified and optimized for the analysis of milk. Two specific problems encountered during milk purging were foaming and generated water vapor. Foaming was effectively controlled by using a sampler with a fritless configuration and 1-tetradecanol. In the fritless configuration, a stream of purge gas bubbled through the milk by means of a needle sparger. Tetradecanol was the only suitable antifoaming agent since it did not generate volatiles of its own. Silicone-based antifoams, on the other hand, were found to contribute many contaminants. One of the most critical parts of dynamic headspace sampling is the type of adsorbent used in the trap. Ideally the adsorbent will efficiently retain sample components during purging and release them during desorption. In addition, it is very important that an ideal adsorbent be so hydrophobic that it will not adsorb water vapor during purging and thus interfere with chromatography. In this research, the three traps tested were hydrophobic. Since Tenax TA does not retain highly volatile components, two other combination traps containing stronger adsorbents and known to retain these volatiles were tested. However, both Tenax/charcoal and Carbopack/Carbosieve traps were found to be unsatisfactory. These traps appeared to retain large amounts of water vapor, generated during purging, which extinguished the detector flame during chromatography. Thus, a Tenax trap was found to be acceptable. This trap was assumed to retain the least amount of water since the FID flame was not extinguished during chromatography. However, a 10–15-min dry purge was always required. Although all of the adsorbents were highly hydrophobic, water vapor possibly condensed in the tubing. The Carbopack/Carbosieve trap had only 8.8 cm of its 30-cm length packed with adsorbent; therefore, there was more room for water condensation.

From the three capillary columns tested, a midpolarity column (DB-624) was found to provide the best resolution. Adequate resolution of the low-boiling components present in milk was not only due to the higher selectivity of the stationary phase but also due to the higher capacity of the column. This column had the highest capacity of the three capillary columns tested since it possessed the thickest stationary phase (1.8 μm). Increasing the film thickness of the stationary phase increases solute retention; therefore, when highly volatile compounds are analyzed, a thick film column is better than a thin film column. DB-5, a relatively nonpolar column, showed poor resolution of the most volatile components. Poorer resolution of these volatiles, which are the most abundant components in milk, might have been due to the lower capacity of this column (1-μm film thickness) coupled with the low selectivity of this phase. DB-Wax, the most polar column, was found to provide the worst chromatography. The poor performance of this column was probably the result of its having the lowest capacity of the three columns tested. But since polar columns with stationary phases thicker than 0.5 μm were not available in "true" capillary columns (0.32 mm i.d. or less), their performance could not be tested. In addition, water vapor generated during purging may have promoted oxidation and stripped the liquid phase, thus resulting in a rising baseline.

Table I. Summary Data for Random-Centroid Optimization of Purge and Trap Conditions

vertex	temp (°C)	purging time (min)	volume (mL)	response[a]
1	41.0	29.0	6.1	392
2	32.5	13.0	10.2	603
3	47.0	14.0	5.4	680
4	31.0	25.0	13.0	0
5	32.5	13.0	8.6	503
6	43.0	10.0	12.4	612
7	45.0	13.0	14.4	0
8	48.0	29.0	8.8	0
9	37.0	25.0	7.1	475
10	41.0	13.0	9.3	556
11	40.0	13.0	8.3	645
12	44.0	12.5	9.1	652
13	33.5	19.5	9.4	478
14	42.0	17.0	8.0	542
15	36.0	12.0	10.5	429
16	37.0	20.0	9.0	444
17	39.0	16.0	9.3	574
18	41.0	10.0	8.2	480

[a] Response = 0, assigned whenever breakthrough volume was detected by a large upswing of the FID signal connected to the trap vent.

Random-Centroid Optimization of Purge and Trap Conditions. The capillary column and the adsorbent trap having been selected, the next step toward establishing the methodology was the optimization of purge and trap conditions for the maximum recovery of volatiles. Boundaries for the factors used in optimization were based on trial and error experimentation and other limiting factors. For example, the minimum reliable sample temperature in the heated module was above room temperature; thus, the lowest temperature in the range was 30 °C. The highest temperature in the range was 50 °C since higher temperatures caused excessive water vapor generation and condensation in the system. Problems associated with water vapor were a rising baseline in the chromatogram and reduced sensitivity. Since baking of the column at 260 °C alleviated this loss in sensitivity, it is presumed that the water was causing plugging of the column. Trial and error experiments were carried out, and it was found that the FID flame was extinguished when excessive water was transferred to the column. The sample volume range was also limited by the capacity of the glassware. Purging times of longer than 30 min would prolong analysis time; thus, it was imposed as the upper boundary.

Summary data of the 18 experiments carried out are shown in Table I. Vertices with responses of zero represent the conditions that exceeded the maximum retention

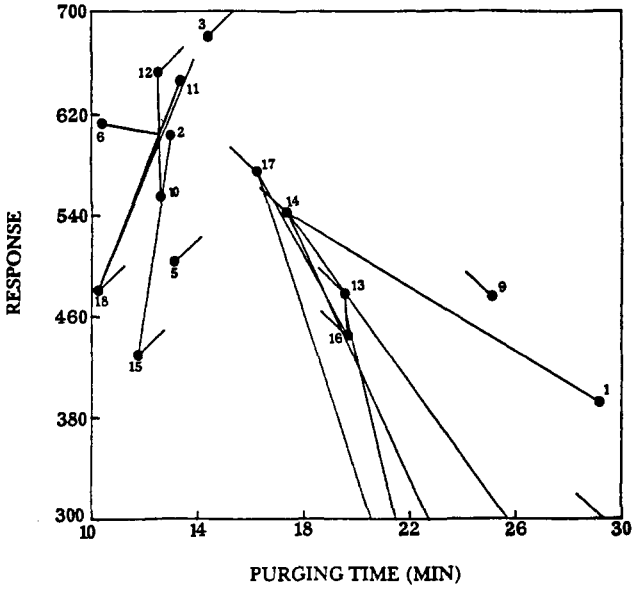

Figure 1. Response surface pattern as a function of purging time after 18 experiments during random-centroid optimization. The number at each point corresponds to the vertex (optimum condition = vertex 12). Short 45° lines attached to data points demonstrate the direction toward the optimum.

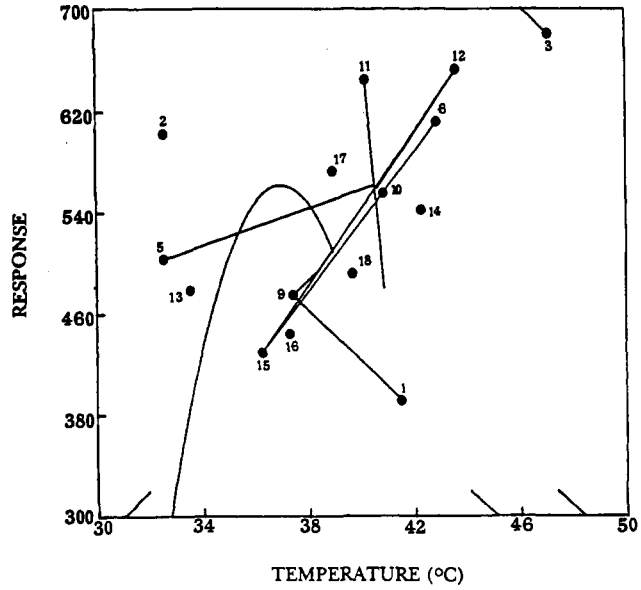

Figure 2. Response surface pattern as a function of sample temperature after 18 experiments during random-centroid optimization. The number at each point corresponds to the vertex (optimum condition = vertex 12).

volume of the trap. This situation occurred mainly in longer purging times with larger sample volumes or higher temperatures. Simultaneous factor optimization is extremely important for the selection of purge and trap conditions, since some factor combinations may exceed the retention volume and result in breakthrough of certain volatiles on the trap. Although increasing the purge volume (longer purging time at constant purge flow rate) may increase the recovery of some of the less volatile components, at high purge volumes, there might be a reduction in recovery caused by breakthrough, on the trap, of certain volatiles. The highest response was obtained in vertex 3; however, this high temperature generated large amounts of water vapor which were transferred to the column as shown by a rising baseline. Therefore, the next best response, corresponding to 9.1 mL of sample, 44 °C, and 12.5 min, was selected as the optimum (vertex 12). Trends of the response surface plotted as a function of purging time, sample volume, or temperature are shown in Figures 1–3. The optimum purging time clearly pointed between 12 and 14 min (Figure 1). Similarly, the optimum temperature region fell between 40 and 46 °C (Figure 2). However, the optimum for sample volume was not as clearly defined, probably because it strongly depended on sample temperature and purging time (Figure 3). Overall, simultaneous factor optimization produced a 66% improvement in volatile recovery from the worst response (vertex 1) to the best response (vertex 12). In addition, this method allowed for the detection and elimination of conditions that exceeded the trap breakthrough volume.

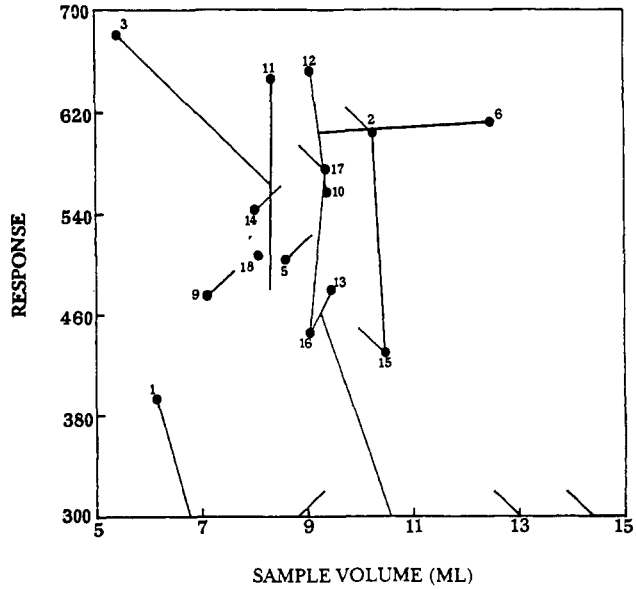

Figure 3. Response surface pattern as a function of sample volume after 18 experiments during random-centroid optimization. The number at each point corresponds to the vertex (optimum condition = vertex 12).

Table II. Precision of Dynamic Headspace Gas Chromatography of Ethyl Esters Added to Milk (n = 5)

	coefficient of variation (%)	
ethyl ester	1[a]	2[b]
acetate	18.18	11.05
ethanoate	9.71	5.73
butanoate	4.83	3.85
pentanoate	6.53	3.52
hexanoate	8.54	4.02

[a] Ratio of ester peak area to area of internal standard (4-methyl-2-pentanone). [b] Ratio of ester peak area to total ester peak area.

Sensitivity and Precision of Method. Precision of the method was very good. The coefficients of variation (CV) for five replicates of a mixture of ethyl esters spiked into milk are summarized in Table II. Coefficients of variation of less than 10% for all ethyl esters except for acetate were calculated at 0.2 ppb. It has been reported that for most compounds for which recovery is greater than 40% the coefficients of variation would be in the order of 2–8%, and as the recovery drops below 40%, the reproducibility rapidly deteriorates (Westerndorf, 1985). For small molecular weight compounds such as ethyl acetate, the purge efficiency may be very high, but trapping efficiency may become a factor. Thus, a higher CV for ethyl acetate may be indicative of some breakthrough occurring. Lower coefficients of variation were obtained when ratios of ester peak area to total ester peak area were used in the calculation than when ratios of ester peak area to area of internal standard were used (Table II). This

may indicate that the addition of the internal standard itself has added some variability. Nevertheless, since the final aim of developing this DH-GC was to use it as an analytical tool in milk shelf-life studies carried out over a relatively extended period, there was still need for the inclusion of an internal standard to account for instrumental variability. In addition, since 4-methyl-2-pentanone (IS) eluted in the middle part of the chromatogram, lower recovery of volatiles caused by small leaks or water vapor condensation was easily noticed by a decreased peak area of the internal standard or by no response at all in spite of appreciable recovery of low-boiling-point compounds in the initial part of the chromatogram.

Sensitivity of the method was very high, with the limit of detection ranging from 4 to 7 parts per trillion for the same ethyl esters. Ethyl esters in the range of C_3 to C_5 appear to purge efficiently from aqueous solutions at room temperature since recoveries of higher than 80% have been reported (Leahy and Reineccius, 1984). High sensitivity for these ethyl esters may be attributed to their high purge efficiency resulting in high recoveries since volatiles were purged at 44 °C. Elevating the temperature during purging is the most efficient method of increasing sensitivity. In addition, interfacing the purge and trap concentrator to the capillary column by cryofocusing on a precolumn compensated for the slow transfer from the trap to the GC while retaining the high sensitivity of splitless capillary GC analysis.

Identification of Milk Volatiles. Compounds found in pasteurized milk of poor and good quality are listed in Table III. In addition, quantitative data for typical gas chromatographic profiles (Figures 4 and 5) representing good- and poor-quality pasteurized milk are included for comparative purposes. From 60 good (shelf life of 15 days or longer), 42 marginal (shelf life of 8–14 days), and 32 poor (shelf life of less than 8 days), the most common GC profiles of good and poor milk samples were selected. It is important to note that the values presented in Table III are approximate concentrations. For the purpose of this study only approximate quantitative data were required since the ultimate goal was to obtain chemical fingerprints of milk flavor quality which would be used in shelf-life prediction studies. Quantitation in DH-GC requires the calculation of correction factors accounting not only for detector response but also for difference in volatile purgibility (Park and Goins, 1992).

Although the characteristic flavor of fresh milk is very subtle in character and low in intensity, several volatiles consisting mainly of aldehydes, ketones, alcohols, and esters were identified. A typical GC profile for good quality milk (shelf life > 9 days) is illustrated in Figure 4. A combination of volatiles contributed to the aroma of good-quality fresh milk. A GC profile of poor-quality milk showed the presence of not only many more newly identified volatiles but also increased amounts of some of those already present (Figure 5). Besides the groups of compounds present in fresh milk, esters were also present in spoiled milk. Concentrations of ethanol, acetone, and 2-propanol (Table III) approximately double in poor-quality milk. These volatiles were reported to be produced by psychrotrophic bacteria and to be associated with milk quality deterioration (Urbach and Milne, 1988). Volatiles present in high concentrations only in poor-quality milk were 2,3-butanedione, 2-methylbutanal, 3- and 2-methyl-1-butanol, and ethyl butanoate. Of these volatiles, 2-methylbutanal and 3- and 2-methyl-1-butanol were reported to cause malty off-flavors and to be produced by

Table III. Quantitative Comparison of Volatiles in Good- and Poor-Quality Milk

peak	component	ID	concentration (ppb) good	poor
1	acetone	a	1287	26.98
2	ethanol	a	0.60	1.47
3	2-propanol	a	2.74	5.58
4	2-butanone	a	17.56	22.0
5	2,3-butanedione	b	ND[d]	102.91
6	1-propanol	a	ND	(–)[e]
7	ethyl acetate	a	ND	(–)
8	chloroform	b	0.27	ND
9	2-butanol	a	ND	(–)
10	benzene	b	0.19	(–)
11	3-methylbutanal	a	ND	5.83
12	2-methylbutanal	a	ND	98.14
13	2-methyl-1-propanol	a	ND	ND
14	2-pentanone	a	1.23	6.93
15	pentanal	a	ND	2.63
16	1-butanol	a	ND	3.64
17	ethyl propanoate	a	ND	(–)
18	methyl butanoate	a	ND	(–)
19	toluene	b	ND	2.98
20	4-methyl-2-pentanone	a, c	2.74	2.74
21	3- and 2-methyl-1-butanol	a	ND	181.66
22	ethyl butanoate	a	ND	64.49
23	2-hexanone	a	ND	(–)
24	hexanal	a	2.52	0.93
25	ethyl benzene	b	ND	29.45
26	*m*- or *p*-xylene	b	1.04	ND
27	nonane	a	ND	(–)
28	1-hexanol	a	ND	1.61
29	2-heptanone	a	0.27	4.19
30	4-methylhexanal	b	0.87	ND
31	benzaldehyde	b	ND	(–)
32	ethyl hexanoate	a	ND	8.13
33	2-octanone	a	0.65	1.39
34	octanal	a	ND	(–)
35	2-ethyl-1-hexanol	b	0.63	ND
36	6-methyl-1-heptanol	b	ND	(–)
37	ethyl heptanoate	a	ND	0.82
38	8-nonen-2-one	b	ND	(–)
39	2-nonanone	b	0.41	1.53
40	nonanal	b	4.05	5.97
41	2-octen-1-ol	b	ND	(–)
42	methyl 2,4-dimethylhexanoate	b	ND	(–)
43	ethyl octanoate	a	ND	0.41
44	decanal	b	1.86	ND
45	tridecane	b	1.61	6.71

[a] Positively identified by DH-GC/MS and retention time of authentic analytical grade standards. [b] Tentatively identified by DH-GC/MS. [c] Added internal standard. [d] ND, not detected in typical GC profiles of Figures 4 and 5. [e] (–) not detected in GC profiles of Figures 4 and 5 but detected in other poor-quality milk.

Streptococcus lactis var. *maltigenes* (Morgan et al., 1966). Fruity flavors were associated with the presence of ethyl butanoate and hexanoate produced by *Pseudomonas fragi* as a result of postpasteurization contamination (Reddy et al., 1968).

Volatile fatty acids (VFA), which are associated with rancid or lipolyzed flavor of milk, were not detected in this study. This finding agrees with previous work (Bassette et al., 1963). These researchers argued that these acids were not volatile enough to be detected in the headspace. Also, polarity of the fatty acids prevented them from being drawn into the headspace vapor. Although the quantitative data of Table III give some indication of the volatile composition associated with milk quality deterioration during refrigerated storage, their relationship with flavor quality requires the analysis of a large number of samples. The cause–effect relationships could not be recognized without the application of multivariate analysis to sensory and gas chromatographic data. These statistical studies will be the subject of future papers.

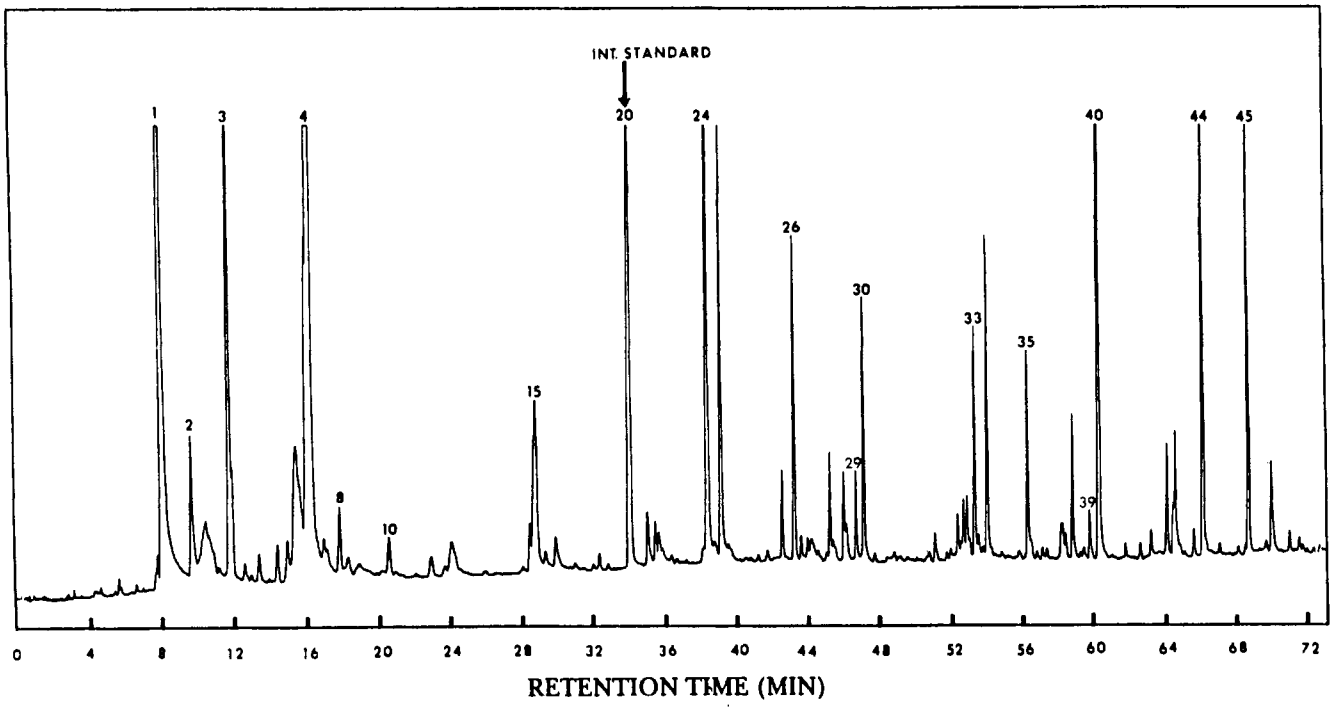

Figure 4. Typical GC profile of good-quality milk (shelf life > 9 days). The peak numbers correspond to compounds in Table III.

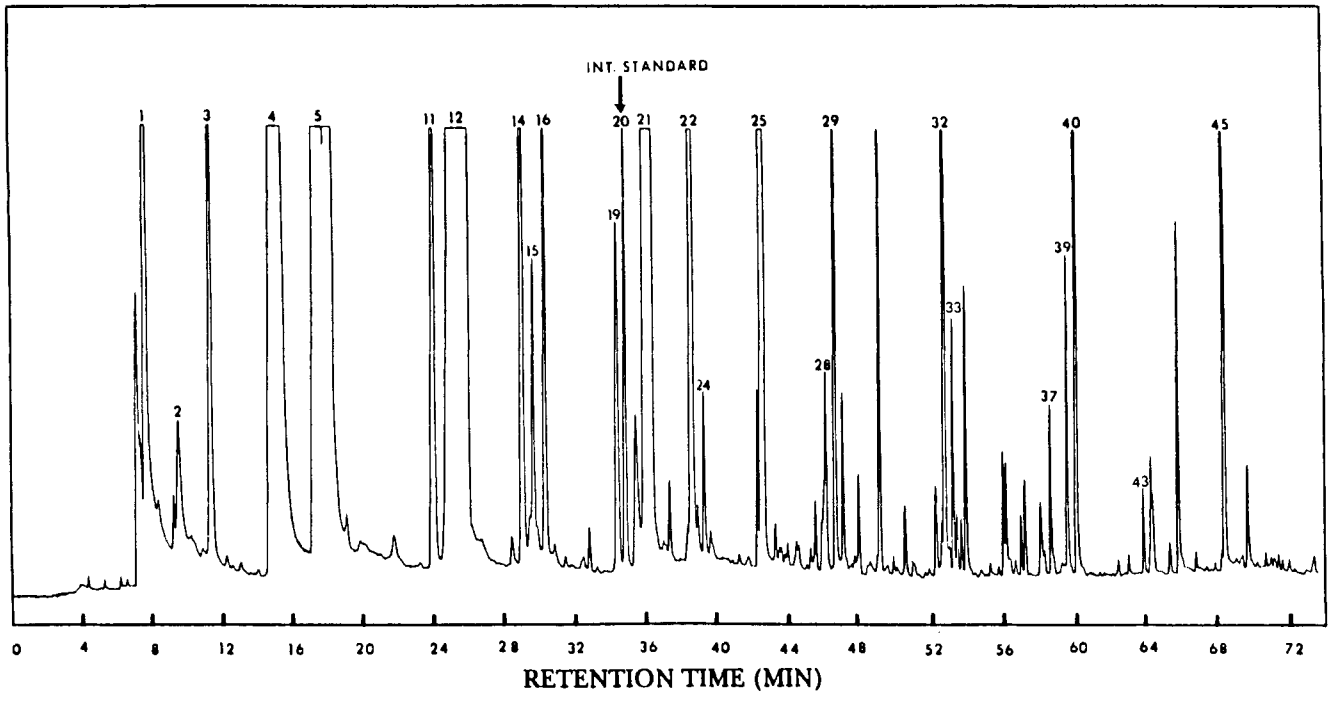

Figure 5. Typical GC profile of poor-quality milk (shelf life < 9 days). The peak numbers correspond to compounds in Table III.

CONCLUSIONS

Optimization of DH-GC conditions for the detection of milk volatiles resulted in a method of high sensitivity and repeatability. Simultaneous factor optimization of purge and trap conditions not only allowed maximum recovery of volatiles but also allowed detection of factor combinations that resulted in trap breakthrough. Without simultaneous factor optimization, lower recovery of certain volatiles and poor repeatability could have resulted. Volatile isolation and separation by purge and trap capillary gas chromatography proved to be a useful technique for the detection of compounds developing during refrigerated storage of pasteurized milk.

ACKNOWLEDGMENT

The financial support of the Natural Sciences and Engineering Research Council of Canada and of the Consejo Nacional de Ciencia y Tecnologia (Mexico) is gratefully acknowledged. This study was partly presented at the 1991 National Meeting of the American Chemical Society, Agricultural and Food Chemistry Division, Atlanta, GA, April 14–16.

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Received for review April 26, 1993. Revised manuscript received August 30, 1993. Accepted September 16, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1993.