Analysis of the Fruity Off-Flavor in Milk Using Headspace Concentration Capillary Column Gas Chromatography

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Ethyl butyrate and ethyl hexanoate, compounds considered to be primarily responsible for the fruity off-flavor in milk, were measured by using an automated headspace concentration system coupled to a capillary column gas chromatograph. Sampling conditions were optimized for recovery of the esters in whole milk. Concentrations as low as 0.001 ppm could be detected and quantified. The coefficients of variation for ethyl butyrate and ethyl hexanoate at this concentration were 61.7% and 53.2% while relative errors were 55.6% and 23.5%, respectively. Sensory analysis determined a fruity flavor threshold of 0.01 ppm (0.005 ppm of ethyl butyrate and 0.005 ppm of ethyl hexanoate) in whole milk. At this level, the headspace concentration system quantified these esters with 29.9–36.4% relative error and 25.3–30.7% coefficient of variation. Commercial milks judged to be fruity by sensory analysis were found to contain ethyl butyrate and ethyl hexanoate concentrations of 0.026–0.152 and 0.20–0.268 ppm, respectively.

Growth of psychrotrophic organism *Pseudomonas fragi* can cause the development of the fruity off-flavor in milk (Witter, 1961; Morgan, 1970) and presents a major problem in milk flavor quality. The compounds primarily responsible for this defect are ethyl butyrate (B) and ethyl hexanoate (H). Action of two *P. fragi* enzymes, a lipase and an esterase, causes cleavage of butyric and caproic acids from milk fat and esterification of these fatty acids with ethanol (Reddy et al., 1968; Morgan, 1976). Sensory thresholds for B and H have been reported to be in the range of 0.015–0.025 and 0.021–0.075 ppm, respectively (Honkanen et al., 1964; Reddy et al., 1969a).

Analysis of the fruity esters in milk has involved gas chromatography combined with precolumns, cold traps, and headspace samplings to trap and conconcentrate the volatile esters (Reddy et al., 1968; Hosono et al., 1974; Pierami and Stevenson, 1976). A commercially available purge and trap analysis system (PTAS) combined headspace sampling with the use of a precolumn to trap and conconcentrate volatiles and offers potential as a method for the analysis of milk off-flavors (Snyder, 1978).

This paper examines the feasibility of using the PTAS for analyzing fruity esters added to milk systems and for analyzing milks which have developed fruity aromas after extended refrigerated storage. In addition, the PTAS analytical threshold is compared with human sensitivity to fruitiness in milk by correlating sensory threshold determinations with PTAS data.

MATERIALS AND METHODS

Sampling Technique. All chromatographic analyses were performed by using a Hewlett-Packard Model 5840A gas chromatograph (GC) equipped with a flame ionization detector, a 18335A glass capillary inlet system, and a 7675A purge and trap sampler. All quantification was based on peak areas as determined by the GC integrator. The column was a 60 m × 0.25 mm i.d. WCOT glass capillary column coated with Carbowax 20M (J & W Scientific). Helium was used as both carrier and purge gas.

A 10- or 20-mL milk sample was placed in a 50-mL test tube which was then screwed into the head of the purge and trap apparatus. Helium was bubbled through the sample at room temperature, carrying volatile constituents onto a 12.5 cm × 4 mm i.d. precolumn where they were adsorbed and concentrated. The precolumn was held at room temperature and was packed with Tenax-GC. Following the appropriate purge time, the precolumn was heated, and the desorbed volatiles were swept onto the capillary column for analysis.

The sampling conditions used were as follows: 40-min purge time, 250 mL/min purge gas flow rate, 7-min desorb time, 200 °C desorb temperature, 25 mL/min desorb gas flow rate, and 30:1 capillary column inlet split ratio.

Precision and Accuracy of the PTAS. A stock solution containing 5 mL of each ester, 5 mL of *n*-butyl propionate (internal standard), and 85 mL of absolute ethanol was prepared. From this, the 0.5 ppm (microliter per liter of solution) of standard solution was made by adding 1 μL of stock solution to 100 mL of commercially pasteurized whole milk.

The precision of the PTAS was determined by analyzing 10 samples of the 0.5-ppm standard solution with isothermal gas chromatographic conditions (110 °C oven temperature). The precision and accuracy of the PTAS as a function of ester concentration were examined by analyzing whole milk samples containing 0.5-, 0.1-, 0.05-, and 0.01-ppm concentrations of B and H. These concentrations were prepared by using the appropriate dilution of the 0.5-ppm standard, and triplicate runs were analyzed at each concentration. The GC was temperature programmed from 50 to 150 °C at 5 °C/min to obtain quantitation at all concentrations. This temperature program was then used for all further analyses.

Analysis of Commercial Milks with Fruity Aromas. Commercially processed milks which had developed fruity off-flavors after extended refrigerated storage were analyzed for the presence of B and H (identification based on retention times). Whole, 2%, and skim milks with fruity aromas as determined by taste panel were examined in duplicate. Blanks of commercially processed control milks were run in all cases.

Sensory Threshold Determination for Fruitiness. A stock solution containing 5 mL of each ester in 90 mL of absolute ethanol was initially prepared. A 10-μL aliquot from the stock solution was added to 1000 mL of commercially pasteurized, homogenized whole milk to obtain 0.5-ppm ester concentrations. Dilutions in milk were then made to achieve the concentrations needed for the taste panels. Alcohol was added to the control milks at a level equivalent to that added with the esters to eliminate sample selection due to the presence of ethanol.

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The paired comparison test was used. At each testing session, 30 screened judges (16 women; 14 men) were presented with 7 pairs of randomly coded, two-oz milk samples that had been warmed to 18–20 °C. Judges were asked to smell and then taste the pairs of samples. Each pair contained one fruity milk and one control milk. The judges were asked to indicate which sample in each pair was fruitier. Sample pairs were presented in order to increasing concentration. Sample presentation was randomized so that within each pair, half of the judges tasted the fruity milk first and half of the judges tasted the control milk first. The ester concentrations used were 0.001, 0.005, 0.01, 0.025, 0.05, 0.1, and 0.25 ppm. There were three tasting sessions.

Taste panel data were analyzed statistically by using a method similar to that of Vaisey-Genser and Moskowitz (1977) to determine the threshold concentration for fruitiness in milk as simulated by the addition of B and H. The percentage of judges correctly identifying the fruiter samples (without guessing) was calculated for each concentration tested by using the equation

$$P = (T - 100c)/(1 - c)$$

where T = observed percentage of correct answers, c = probability of finding the correct answer by chance (c = $^1/_2$ for the paired comparison test), and P = percentage of judges that are able to find the correct answer without guessing.

The P values were then plotted against concentration, and a curve was fit through the data points. The threshold concentration for fruitiness in milk was that concentration corresponding to P = 50%, i.e., the concentration that was perceptible 50% of the time.

Correlation between PTAS and Sensory Data. Prior to each of the taste sessions, a 100-mL sample of each fruity milk being tasted was removed for GC analysis. An appropriate amount of internal standard was added to each sample, and duplicate runs were made for each concentration.

RESULTS AND DISCUSSION

Precision and Accuracy of the PTAS. Ethyl butyrate and ethyl hexanoate were added to whole milk at a concentration of 0.5 ppm and then analyzed 10 times to evaluate the accuracy and precision of the PTAS. The mean concentration of B was determined as 0.50 ppm for the 0.5-ppm standard, while the mean concentration of H was 0.49 ppm for the 0.5-ppm standard. Corresponding relative errors for B and H were quite low, 0.9% and 0.5%, respectively. The coefficient of variation (cv) for 10 consecutive runs was 1.4% for B and 5.1% for H.

As the ester concentration decreased, both the relative error and the cv increased (Table I). This trend is as expected, since the ability of the PTAS to detect and quantify the esters should decrease as the ester concentration approaches the limits of the sampling system [1 μg/L in 5 mL of water (Snyder, 1978)]. It is of interest that the cv and relative error were generally smaller for H than B. This may be due to a better or more stable base line by the time H elutes or the fact that quantification generally is more variable when the compound elutes close to the solvent front. Since this work was completed, we have made a modification to the gas chromatograph which has substantially improved chromatography of the trapped milk flavors. We have switched to fused silica columns which permits immersion of the first 20 cm of the capillary column in liquid nitrogen during desorption of the Tenax trap (Jennings, 1981). This cooling of the capillary column results in condensation of the milk volatiles on the head of the column. This effectively eliminates the need for splitting in the inlet system (enhances sensitivity) and vastly improves column resolution. Peaks are nearly as sharp as making a direct injection.

Table I. Analysis of Ethyl Butyrate and Ethyl Hexanoate in Milk Using the PTAS

individual ester concn,[a] ppm	amount of ester added,[b] μL/10 mL of milk	mean amount by PTAS,[c] μL/10 mL of milk	coeff of variation (PTAS),[c] %	rel errors (PTAS),[c] %
		Ethyl Butyrate		
0.25	2.195	2.184	2.5	0.5
0.1	0.878	0.866	4.6	1.4
0.05	0.439	0.454	7.0	4.6
0.025	0.220	0.251	7.5	14.1
0.01	0.088	0.096	15.7	9.1
0.005	0.044	0.060	30.7	36.4
0.001	0.009	0.014	61.7	55.6
		Ethyl Hexanoate		
0.25	2.180	2.215	3.9	1.6
0.1	0.872	0.921	4.1	5.6
0.05	0.436	0.467	7.9	7.1
0.025	0.218	0.232	10.9	6.4
0.01	0.088	0.090	16.6	2.9
0.005	0.044	0.057	25.3	29.9
0.001	0.009	0.011	53.2 1	23.5

[a] Intended concentration of each ester (microliters per liter of solution). [b] Actual amount of each ester added to milk. [c] Average of six analyses.

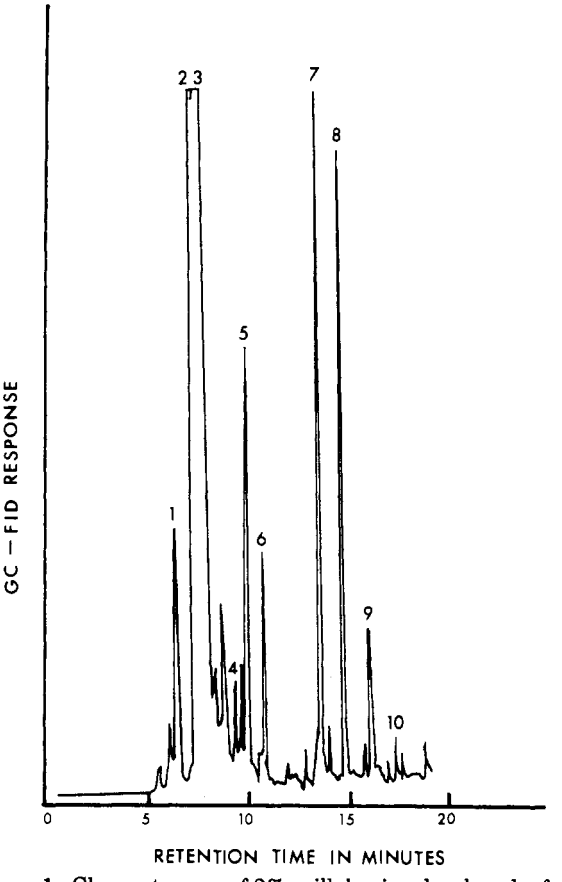

Figure 1. Chromatogram of 2% milk having developed a fruity aroma after refrigerated storage.

Analysis of Commercial Milks with Fruity Aromas. In all cases, B and H in the fruity milks were tentatively identified based on retention times. No positive identifications were made. Of the milks examined, the 2% milk had the highest concentrations of B (0.152 ppm) and H (0.268 ppm). The whole milk contained 0.051 ppm of B

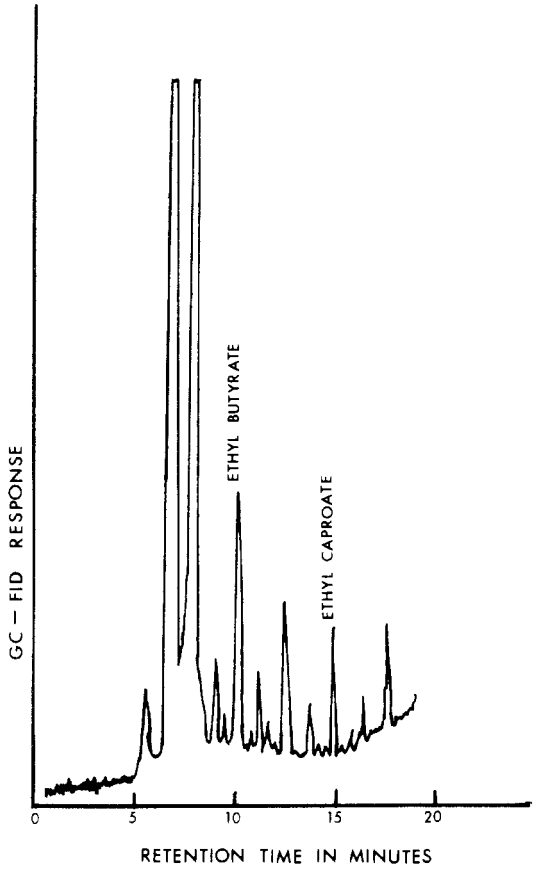

Figure 2. Chromatogram of whole milk containing 0.005 ppm of ethyl butyrate and ethyl hexanoate.

and 0.095 ppm of H, while the skim milk contained 0.101 ppm of B and 0.020 ppm H.

Figure 1 shows a chromatogram of 2% milk having developed a fruity aroma. Peaks 5 and 8 were tentatively identified as B and H, respectively. Peaks 1 and 3 were present in all control and fruity milks analyzed. Peaks 2 and 6 cochromagtographed with ethyl acetate and ethyl isovalerate, respectively, and could be these esters, since both ethyl acetate and ethyl isovalerate have been identified in fruity milk (Reddy et al., 1968). Peaks 4, 7, 9, and 10 were present in chromatograms of whole, 2%, and skim milks having fruity aromas. They may be related to fruity off-flavor development, or they may merely be indicative of concomitant chemical activity in milk.

Sensory Threshold Determination for Fruitiness. The average flavor threshold determined for fruitiness in whole milk simulated by addition of B and H was 0.01 ppm. This represents B and H concentrations of 0.005 ppm, respectively. The threshold determined in this research is reasonably close to the 0.0163-ppm threshold reported by Reddy et al. (1969b) when B and H were added to milk.

Correlation between PTAS and Sensory Data. The PTAS was capable of detecting and quantifying ester concentrations as low as 0.001 ppm (Table I). The sensitivity of the PTAS is, therefore, better than that of the human sensory system (0.005 ppm). A typical analysis of whole milk which had been spiked with B and H at sensory threshold concentrations (0.005 ppm each) is shown in Figure 2. Although relative error and the coefficient of variation were 30% at these low ester concentrations, this type of objective methjodology is comparable to the sensitivity of the human sensor system. The relative short analysis time (1 h), ease of sample preparation, and reproducibility at higher concentrations makes the PTAS a feasible alternative to sensory detection of fruity milk.

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