Keeping-Quality Assessment of Pasteurized Milk by Multivariate Analysis of Dynamic Headspace Gas Chromatographic Data. 2. Flavor Classification by Linear Discriminant Analysis

Belinda Vallejo-Cordoba† and Shuryo Nakai*

Department of Food Science, Faculty of Agricultural Sciences, 6650 North West Marine Drive, Vancouver, British Columbia, Canada V6T 1Z4

The flavor quality of pasteurized milk was classified by applying linear discriminant analysis (LDA) to volatiles as determined by dynamic headspace capillary gas chromatography (DH-GC). LDA was able to classify milk into good, marginal, and poor quality or into fruity, rancid, and normal flavor groups at a greater than 80% success rate. The zone of good or normal flavor groups could be visualized in two-dimensional canonical plots; thus, any sample deviating from this zone could be considered a defective product. In addition, LDA of gas chromatograhic data allowed the association of milk volatiles that contributed most significantly to the discrimination among flavor quality groups. Spoilage-associated volatiles were identified by using DH-GC-MS. The analytical system established has potential for application in the dairy industry for early detection of spoilage or for tracing defects associated with consumer complaints.

INTRODUCTION

Flavor (the complex sensation in which aroma and taste are combined) is the most important factor governing our appreciation of the foods we eat. On various dairy products score cards, flavor is always assigned the greatest emphasis of any of the quality categories. Since odor properties contribute so markedly to flavor, the sense of smell plays a particularly important role in the evaluation of dairy products (Bodyfelt et al., 1988). Volatile constituents of food, which determine its aroma, make a strong contribution to flavor. This is evident when the elimination of our sense of smell (e.g., by a common cold) makes food taste bland and unattractive (Badings and Neeter, 1980). In fact, generally, the olfactory dimension of a food or beverage contributes more to the overall flavor perception than the taste dimensions does (Bodyfelt et al., 1988). As reported by Badings and Neeter (1980), it is the delicate balance between many aroma compounds, most of which are present in subthreshold concentrations, that produces a desirable milk flavor. If this balance is upset, off-flavors may occur. Aroma compounds present in fresh milk belong to many different classes, such as carbonyl compounds, alkanols, free fatty acids, lactones, esters, sulfur compounds, nitrogenous compounds, and aliphatic and aromatic hydrocarbons. It is generally recognized that while analytical data can provide very useful information, sensory data are required for the importance of analytical measurements in flavor research to be established. Indeed, what the researcher wants to know in the correlation of analytical and sensory data is the cause-effect relationship between a group of components and sensory qualities. Once the chemical constituents that are most related to sensory evaluation have been detected by pattern recognition methods, these key features only need to be identified by techniques such as GC-MS (Aishima and Nakai, 1991). Sometimes researchers try to predict sensory qualities on the basis of a single chemical measurement. Indeed, in some instances a single measurement is all that is required to establish a good correlation. For instance, Greig and Manning (1983) found a good correlation between acetaldehyde concentration and consumer acceptability in pasteurized milk. In other instances, attempts to correlate a single component to a sensory response have not been successful. Urbach and Milne (1988) could not find a good correlation between ethanol content in pasteurized milk and palatability, in spite of an observed increase of ethanol with increasing storage time or temperature. Although simple correlations can sometimes be established, it is generally accepted that flavor differences often relate to a complex balance between volatiles rather than to a major change in one or two constituents (Powers, 1981). Therefore, more complex correlation techniques are frequently necessary for extracting useful information from complex gas chromatographic profiles and relating it to sensory responses. When many components may be affecting a sensory response, a multivariate approach must be used to handle the analytical data.

The objective of this research was to apply a highly sensitive analytical technique to detect and identify volatiles naturally developing in milk during refrigerated storage that would allow discrimination of subtle changes in flavor quality, thus allowing the establishment of flavor classification functions which may be used for classifying milk samples in a quality control situation.

MATERIALS AND METHODS

Milk sampling, volatile detection, sensory evaluation, and data manipulation were conducted as described in the preceding paper (Vallejo-Cordoba and Nakai, 1994). Milk spoilage volatiles were identified as described by Vallejo-Cordoba and Nakai (1993).

Statistical Analysis. A Lotus spreadsheet consisting of gas chromatographic data with milk samples assigned to the different flavor quality groups as determined by sensory evaluation was imported to a Systat statistical software package (version 5.0, Systat, Inc., Evanston, IL, 1990). A log transformation of the data was used to improve the fit to multivariate normality. The Systat statistical procedures used were discriminant analysis (MGLH), analysis of variance (MGLH), Tukey's HSD multiple comparison test (MGLH), and principal component analysis (FACTOR).

Linear discriminant analysis (LDA) works by deriving linear

† Health Protection Branch, Health and Welfare Canada, 3155 Willingdon Green, Burnaby, BC, Canada V5G 4P2.

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combinations of the independent variables, for example, GC peaks that will discriminate between a priori defined groups, for example, good or poor quality milk, then subsequently allocates new observations to one of the groups. LDA was performed on either principal components or the log transformation of standardized peak areas (referred to as peaks, ratios of peak area to the area of the internal standard). The general form of the ith discriminant function is

$$Z_i = a_{i1}X_1 + a_{12}X_2 + ... + a_{ip}X_p \quad (1)$$

where Z_i is a weighed linear composite score, $X_1...X_p$ are either the PCs or peaks and, $a_{i1}...a_{ip}$ are the coefficients of the discriminant function.

Milk samples of different shelf lives (as determined by sensory evaluation) were assigned to three quality groups, namely, good (shelf life of 15 days or more, $n = 60$), marginal (shelf life of 8–14 days, $n = 42$), and poor (shelf life of 1–7 days, $n = 32$). Discriminant analysis was performed either on the log transforms of selected peaks or on the first 30 principal components (PC). Selected peaks were either 32 peaks of higher loading (larger than 0.5) on the first PC or 37 significant peaks ($P < 0.05$) as determined by univariate analysis. In addition, samples were reclassified into two quality groups, poor (shelf life of 1–9 days, $n = 44$) and good quality (shelf life of more than 10 days, $n = 90$). A factor that was considered for optimum classification was prior probabilities of group membership since the class size for the quality groups was different. If one class contains a larger population than another, the prior probabilities should be weighed accordingly. The classification ability of the discriminant functions was verified by using cross-validation. Twenty-three cases were held for validation, and 111 cases were used for building the model.

Tests to assess compliance with the formal discriminant analysis assumptions of equal covariance and multivariate normality were performed. Variance across groups for each individual variable used in the model were examined by using the heuristic rule from Johnson and Wichern (1982) instead of an overall covariance test. Accordingly, the ratio of the maximum within-group variance to its minimum within-group variance was examined for each predictor variable. Corrective action was recommended only when this ratio was greater than four; otherwise, the variance was considered to be relatively homogeneous. Compliance with the assumption of multivariate normality was assessed by plotting Mahalanobis distances against chi-square by using a two-dimensional plot generated by Sygraph.

To gain some insight into the nature of the off-flavors developing in milk during storage, samples were assigned to three flavor groups, namely, fruity ($n = 48$), rancid ($n = 26$), and normal ($n = 17$) flavors. Samples were assigned to the corresponding off-flavor groups by recognition of the flavor defect at the end of shelf life as detected by the sensory judges. Samples with1 week or less of remaining shelf life were also assigned to the corresponding off-flavor groups, detected at the end of storage. Samples were assigned in this manner so that the model would show some predictive ability since no off-flavors were detected by the sensory judges at this point. The normal flavor group was formed by samples collected within 36 h of production with no off-flavors detected by the sensory panel.

Discriminant analysis was performed either on the first 30 principal components or on the log transforms of 27 significant peaks ($P < 0.05$) as determined by univariate analysis. Assumptions in discriminant analysis were tested for the two models as described before. Classification ability of the discriminant functions was tested by using cross-validation. Sixteen cases were held out for validation, and 75 cases were used for building the model.

RESULTS AND DISCUSSION

Classification According to Potential Shelf Life. The results of milk quality classification as to its potential shelf life are summarized in Table 1. High percent correct classifications for either groups of good or poor quality milk were found. Total percent correct classification higher than 80% was obtained whether discriminant analysis was performed on principal components or on peaks. Yet for marginal quality milk, correct classification lower than 80% was obtained. It has been reported that success in classification based on sensory evaluation alone rarely exceeds 90% and more commonly the success level is approximately 80% (Powers and Ware, 1986). The canonical plot illustrates the three quality groups with marginals sharing groups of overlap on either side of the border (Figure 1). The continuous nature of the shelf-life phenomena is evident in the plot. Fortunately for crude quality estimates, good quality milk is well separated from poor quality milk. Thus, the classification of "unknowns" (samples not used for building the model) into the "risky zone" (marginal) during quality monitoring might be an alert calling for implementation of corrective measures. Although shelf life is a continuous variable and multiple linear regression might be a more appropriate model for shelf-life prediction (Vallejo-Cordoba and Nakai, 1994), LDA provides a graphic illustration in a two-dimensional plot which might be more easily interpreted in a quality control situation.

The classification ability for the three models was tested by using cross-validation. As expected, the total percent correct classification dropped slightly since a major proportion of the sample was not used for the model-building process (Table 2). These results confirmed the strength of the model for classification purposes. Clas-

Table 1. Percent Correct Classification of Milk Samples According to Their Potential Shelf Life for Their Separation into Good, Marginal, and Poor Flavor Quality Groups ($n = 134$)

description of analysis[a]	group[b] g	m	p	total
30 PC	92	67	84	82
32 peaks	92	67	87	83
37 peaks	93	64	94	84

[a] PC, principal components; peaks, log transformation of standardized GC peak areas; 32 peaks, peaks with factor loadings greater than 0.5 in PCA; 37 peaks, significant peaks ($P < 0.05$). [b] g, m, and p, good, marginal, and poor quality.

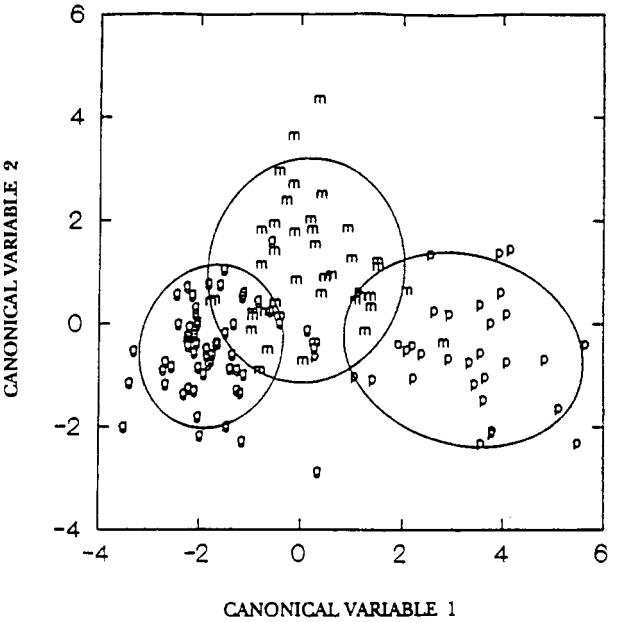

Figure 1. Canonical plot of pasteurized milk samples grouped by flavor quality (model with 30 PC). g, good; m, marginal; and p, poor quality. Isodensity ellipses are at a probability level of 0.80 ($n = 134$).

Table 2. Percent Correct Classification of Milk Samples According to Their Potential Shelf Life in Cross-Validation (n = 111)

description of analysis[a]	group[b] g	m	p	total
30 PC	88	64	84	80
32 peaks	90	55	91	79
37 peaks	90	57	91	80

[a] PC, principal components; peaks, log transformation of standardized GC peak areas; 32 peaks, peaks with factor loadings greater than 0.5 in PCA; 37 peaks, significant peaks ($P < 0.05$). [b] g, m, and p, good, marginal, and poor quality.

Table 3. Coefficients of Discriminant Functions from Principal Components for the Separation of Milk Samples into Good, Marginal, and Poor Flavor Quality Groups (n = 134)

variable	good	marginal	poor
constant	−2.766	−1.809	−6.325
PC_1	−2.475	0.060	3.484
PC_2	−1.663	−0.524	3.253
PC_3	−1.729	−0.492	2.884
PC_4	1.467	0.040	−2.194
PC_5	−0.096	0.029	−0.281
PC_6	−0.618	−0.468	1.042
PC_7	−0.008	−0.428	−0.024
PC_8	0.144	0.151	−0.748
PC_9	0.825	−0.251	−1.259
PC_{10}	0.433	−0.202	−0.467
PC_{11}	−0.066	0.293	−0.338
PC_{12}	−0.768	0.138	0.716
PC_{13}	0.055	−0.043	−0.083
PC_{14}	−0.989	0.110	1.852
PC_{15}	−0.420	−0.412	0.585
PC_{16}	0.797	−0.286	−1.283
PC_{17}	−0.475	0.215	0.421
PC_{18}	0.040	0.271	−0.685
PC_{19}	−0.423	0.206	0.251
PC_{20}	0.119	−0.166	−0.278
PC_{21}	−0.639	0.098	0.682
PC_{22}	0.034	0.056	0.394
PC_{23}	−0.013	0.015	−0.005
PC_{24}	−0.274	−0.071	0.007
PC_{25}	0.062	0.577	−0.074
PC_{26}	0.201	0.128	−0.405
PC_{27}	−0.583	0.295	0.672
PC_{28}	−0.291	0.256	0.110
PC_{29}	−0.439	−0.085	0.738
PC_{30}	−0.288	0.384	0.270

sification errors were again due to marginals being misclassified as either poor or good quality. A much higher percent correct classification was obtained when samples were reclassified into either poor or good quality groups. A total of 95% correct classification was obtained; misclassified samples again were at the border of the two classes. An important aspect of model building is checking that the data set meets the assumptions made by the statistical model chosen. Compliance with the rules of equal covariance and multivariate normality were observed for all models. Coefficients of the discriminant functions for the model using principal components are presented in Table 3, which typifies the computer printout generated for every model. Thus, a discriminant function can be derived for each flavor quality group, and these can be used to classify unknown samples. The general form of the function for each flavor quality group was obtained by using eq 1, where $PC_1 ... PC_{30}$ are principal component scores and, $a_1 ... a_{30}$ are the coefficients of the discriminant functions in Table 3. Thus, the actual discriminant functions for the three flavor groups would be

$$\text{good} \quad 2.475(PC_1) - 1.663(PC_2) + \ldots - 0.288(PC_{30}) - 2.766$$

$$\text{marginal} \quad 0.060(PC_1) - 0.524(PC_2) + \ldots + 0.384(PC_{30}) - 1.809$$

$$\text{poor} \quad 3.484(PC_1) + 3.253(PC_2) + \ldots + 0.270(PC_{30}) - 6.325$$

An unknown milk could be classified by substituting values for the principal components in each equation for the three quality groups. The unknown sample would be allocated to the flavor quality group with the highest value of the discriminant function. Similarly, discriminant functions can be derived for the other models using "peaks" instead of principal components. Univariate and multivariate statistics for the model using principal components are summarized in Table 4 and typify the computer printout obtained for each model. Only six of the principal components were significant at or beyond the 5% level for univariate statistics, yet multivariate statistics were all highly significant ($P < 0.001$). Elimination of nonsignificant principal components for the model building resulted in poorer percent correct classification rates, thus indicating that all 30 principal components contributed somewhat to the discrimination among groups. These results were expected since although the first four principal components already accounted for 56.7% of the total variation, the rest of the 26 principal components accounted for the rest of the variation to a total of 96.4% (Vallejo-Cordoba and Nakai, 1994). Therefore, PC_5–PC_{30} may still contain useful information for classification. The two canonical variables were highly significant ($P < 0.001$), indicating that both canonical variates contributed to the discrimination among groups.

Milk volatiles that were highly significant ($P < 0.01$) in developing the discriminant functions for flavor quality classification are listed in descending order of importance in Table 5. A composite of these volatiles strongly influenced the discrimination by the models among flavor quality groups and ultimately the potential shelf life of milk. Some of these compounds (those in bold letters) have been reported to be associated with psychrotrophic bacteria in milk (Bassette et al., 1986). Carrying out discriminant analysis on principal components avoids including peaks or variables that are highly correlated and may be redundant if included in classification. However, to find the contribution of peaks to the discriminant functions specifically, principal components need to be converted back to the original variables. Thus, interpretation of results is not as straightforward as when discriminant analysis is carried out on the original variables.

Classification According to Specific Flavors Detected by Sensory Test. During this study, the sensory panel reported the development of lack of freshness in the initial stages of refrigerated storage. Lack of freshness was generally recognized by the sensory panel as creamier and richer flavor. These notes were not necessarily unpleasant and were generally followed by the appearance of rancid and/or fruity flavors. In some cases, malty and unclean flavors were also recognized.

According to Cousin (1982), slight biochemical changes occur in the early growth phase of psychrotrophs, resulting in a lack of freshness or a stale state. Upon subsequent cold storage a variety of defects become apparent. Development of these off-flavors is usually a result of

Table 4. Univariate and Multivariate Statistics of Discriminant Analysis for Milk Classification Grouped by Flavor Quality (Model Using 30 PC)

	variable	df	F^a
univariate	PC_1	2, 131	19.40***
	PC_2	2, 131	14.46***
	PC_3	2, 131	7.94***
	PC_4	2, 131	6.12***
	PC_5	2, 131	1.22
	PC_6	2, 131	1.44
	PC_7	2, 131	2.18
	PC_8	2, 131	1.30
	PC_9	2, 131	2.33
	PC_{10}	2, 131	1.33
	PC_{11}	2, 131	1.12
	PC_{12}	2, 131	1.93
	PC_{13}	2, 131	0.04
	PC_{14}	2, 131	6.65**
	PC_{15}	2, 131	1.08
	PC_{16}	2, 131	3.12*
	PC_{17}	2, 131	1.51
	PC_{18}	2, 131	1.52
	PC_{19}	2, 131	2.22
	PC_{20}	2, 131	0.46
	PC_{21}	2, 131	0.94
	PC_{22}	2, 131	1.41
	PC_{23}	2, 131	0.09
	PC_{24}	2, 131	0.00
	PC_{25}	2, 131	2.46
	PC_{26}	2, 131	0.38
	PC_{27}	2, 131	0.72
	PC_{28}	2, 131	0.75
	PC_{29}	2, 131	0.36
	PC_{30}	2, 131	1.04
multivariate			
Wilk's lambda = 0.13		60, 204	5.85***
Pillai trace = 1.13		60, 206	4.44***
Hotteling–Lawley trace = 4.46		60, 202	7.51***

[a] *, significant difference ($P < 0.05$); **, significant difference ($P < 0.01$); ***, significant difference ($P < 0.001$).

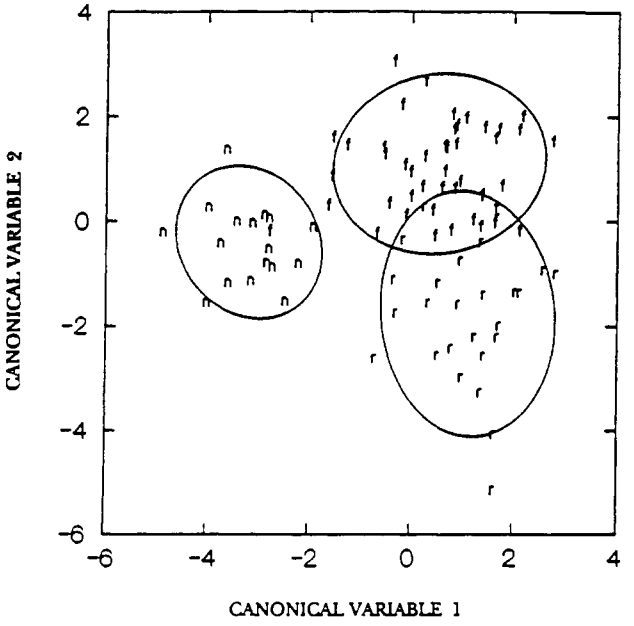

Figure 2. Canonical plot of pasteurized milk samples grouped by flavor type (model with 30 PC). n, normal; f, fruity; and r, rancid flavor. Isodensity ellipses are at a probability level of 0.80 ($n = 91$).

proteolysis and/or lipolysis by psychrotrophs. These two degradative reactions in milk are of great concern to dairy processors.

To gain some insight into the nature of these flavors, 91 samples were classified according to fruity, rancid, and normal flavors. Since a total of 97% of the total variance was explained by the first 30 principal components, they were used for developing classification functions (data not shown). Results of dicriminant analysis based on either 30 principal components or 27 significant ($P < 0.05$) peaks are summarized in Table 6. One hundred percent correct classification was obtained for normal milk, and higher than 80% correct classification was obtained for rancid or fruity flavors. The canonical plot indicated that misclassified samples were due to some overlapping of the off-flavor groups (Figure 2). This is not unexpected since more than one off-flavor may be present, with one predominating over the other. In fact, the sensory panel was able to perceive both fruity and rancid off-flavors for one misclassified sample and unclean flavors in the other five misclassified samples. Cross-validation resulted in a total of 88 and 90% total correct classification for the models using principal components and peaks, respectively, confirming the validity of the models for classification of the unknown samples.

For the model using principal components, 5 of the 30 principal components were significant at or beyond the 5% level by univariate statistics (Table 7), yet multivariate statistics were all highly significant ($P < 0.001$). Elimination of the nonsignificant principal components for model building resulted in lower classification rates, thus indicating that all 30 principal components contributed somewhat to the discrimination among groups. On the other hand, the model using 27 peaks included those

Table 5. Highly Significant ($P < 0.01$) Volatiles Identified in the Discrimination of Milk into Good, Marginal, and Poor Flavor Quality Groups (Model with 37 Peaks)

identified compound[a]	F value
2-methylbutanal/3-methylbutanal[b]	**64.93**
2-heptanone[b]	54.34
2-propanol[b]	**49.98**
2-methyl-1-butanol/3-methyl-1-butanol[b]	**30.90**
1-hexanol[b]	30.07
2-pentanone/pentanal[b]	20.61
ethyl hexanoate[b]	**18.97**
ethylbenzene[c]	16.46
nonane[b]	12.81
2-octen-1-ol[c]	11.88
ethyl octanoate[b]/2,4-dimethylhexanoate[c]	11.60
m-xylene/*p*-xylene[c]	10.74
6-methylheptanol[c]	10.42
2-nonanone/nonanal[c]	9.82
ethyl butanoate[b]	**8.63**
1-propanol/ethyl acetate[b]	**7.87**
benzaldehyde[c]	7.62
2-octanone/octanal[b]	7.45
2-butanone[b]/2,3-butanedione[c]	7.12
4-methylhexanal[c]	6.61
2-methyl-1-propanol[b]	**6.20**
ethyl propanoate[b]	5.85
methyl butanoate[b]	5.26
1-butanol[b]	**4.85**

[a] Compounds in boldface have been reported to be associated with psychrotrophic bacteria in milk (Bassette et al., 1986). [b] Positively identified by GC–MS and retention time of an authentic analytical grade standard. [c] Tentatively identified by GC–MS.

Table 6. Percent Correct Classification of Milk Samples into Flavor Groups ($n = 91$)

	group[b]			
description of analysis[a]	f	r	n	total
30 PC	94	85	100	93
27 peaks	97	81	100	93

[a] PC, principal components; peaks, log transformation of standardized GC peak areas; 27 peaks, significant peaks ($P < 0.05$). [b] f, r, and n, fruity, rancid, and normal flavors.

Table 7. Univariate and Multivariate Statistics of Discriminant Analysis for Milk Classification Grouped by Flavor Type (Model Using 30 PC)

	variable	df	F^a
univariate	PC_1	2, 88	15.37***
	PC_2	2, 88	14.46***
	PC_3	2, 88	4.18*
	PC_4	2, 88	6.67**
	PC_5	2, 88	6.83**
	PC_6	2, 88	2.54
	PC_7	2, 88	2.49
	PC_8	2, 88	0.48
	PC_9	2, 88	0.53
	PC_{10}	2, 88	0.56
	PC_{11}	2, 88	1.88
	PC_{12}	2, 88	0.26
	PC_{13}	2, 88	0.14
	PC_{14}	2, 88	0.39
	PC_{15}	2, 88	0.68
	PC_{16}	2, 88	1.75
	PC_{17}	2, 88	0.22
	PC_{18}	2, 88	0.07
	PC_{19}	2, 88	0.62
	PC_{20}	2, 88	0.86
	PC_{21}	2, 88	1.54
	PC_{22}	2, 88	2.99
	PC_{23}	2, 88	0.41
	PC_{24}	2, 88	0.41
	PC_{25}	2, 88	0.28
	PC_{26}	2, 88	1.86
	PC_{27}	2, 88	0.65
	PC_{28}	2, 88	0.49
	PC_{29}	2, 88	0.53
	PC_{30}	2, 88	2.06
multivariate			
Wilk's lambda = 0.11		60, 118	3.94***
Pillai trace = 1.32		60, 120	3.95***
Hotteling–Lawley trace = 4.07		60, 116	4.07***

[a] *, significant difference ($P < 0.05$); **, significant difference ($P < 0.01$); ***, significant difference ($P < 0.001$).

variables that were significant to at least the 5% level by univariate statistics; therefore, they were all important for classification (data not shown). Multivariate statistics were all highly significant ($P < 0.001$), thus indicating between-flavor-group differences involving all of the predictor variables. The two canonical variables were highly significant ($P < 0.001$), thus indicating that both canonical variates contributed to the discrimination among groups by using either of the two models. Discriminant functions can be derived for each flavor group by using the general form of the function (eq 1), and these can be used to classify unknown samples. Since mild malty off-flavors were also detected in some of the fruity samples, they were reclassified as malty and the two-dimensional canonical plot was reexamined (Figure 3). Again, clear distinction of the off-flavor milk from normal milk was obtained. Regions of overlap of malty and fruity or fruity and rancid groups suggested that misclassification was due to the presence of more than one off-flavor. The first two canonical variables of the three generated were highly significant ($P < 0.001$), indicating that only these two canonical variates contributed to the discrimination among groups.

Volatiles that were most highly significant ($P < 0.01$) in the discrimination of specific flavor groups are tabulated in descending order of importance (Table 8). Fruity flavors were reported in the literature to be caused by the presence of butanoate and hexanoate produced by *Pseudomonas fragi* as a result of postpasteurization contamination (Reddy et al., 1968). Some strains of *Pseudomonas fluorescens* have been associated with the production of 2-propanol (Urbach and Milne, 1988). Malty flavors were

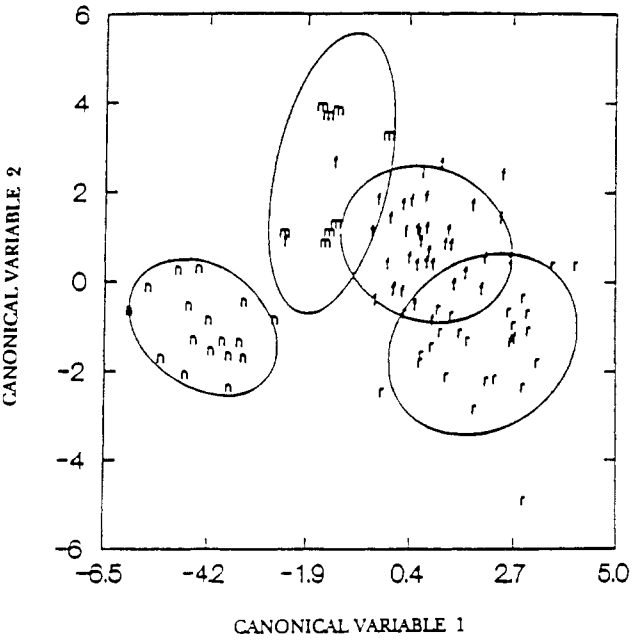

Figure 3. Canonical plot of pasteurized milk samples grouped by flavor type (model with 35 peaks). n, normal; f, fruity; r, rancid; and m, malty flavor. Isodensity ellipses are at a probability level of 0.80 ($n = 91$).

Table 8. Highly Significant ($P < 0.01$) Volatiles Identified in the Discrimination of Milk into Normal, Rancid, and Fruity Flavor Groups (Model with 27 Peaks)

identified compound[a]	F value
1-hexanol[b]	35.79
2-methylbutanal/3-methylbutanal[b]	**31.75**
2-propanol[b]	**30.66**
2-heptanone[b]	17.73
2-pentanone/pentanal[b]	17.14
m-xylene/*p*-xylene[c]	17.03
ethyl hexanoate[b]	**14.43**
2-methyl-1-butanol/3-methyl-1-butanol[b]	13.65
ethylbenzene[c]	13.61
ethyl octanoate[b]/methyl 2,4-dimethyl hexanoate[c]	13.20
6-methylheptanol[c]	12.74
benzaldehyde[c]	11.07
ethyl butanoate[b]	**11.06**
1-propanol/ethyl acetate[b]	9.68
tridecane[c]	7.95
2-octanone[b]/octanal[b]	**6.33**
2-nonanone/nonanal[c]	5.44
2-ethyl-1-hexanol[c]	5.37
2-butanol[b]	**4.92**

[a] Compounds in boldface have been reported to be associated with psychrotrophic bacteria in milk (Bassette et al., 1986). [b] Positively identified by GC–MS and retention time of an authentic analytical grade standard. [c] Tentatively identified by GC–MS.

reported to be caused by the presence of 2- and 3-methylbutanal produced by *Streptococcus lactis* var. *maltigenes*. The corresponding alcohols are also associated with this organism (Morgan et al., 1966). In general, the off-flavors and some volatiles detected in this research were previously reported to be caused by postpasteurization contamination by different psychrotrophic bacteria. Among other substances, ethanol has been reported to be a psychrotrophic metabolite associated with milk quality deterioration (Urbach and Milne, 1988). Although ethanol was also detected in this research, it was not found to be significant in the discrimination of flavor quality groups. Since ethanol was reported to be weakly retained by Tenax (Westendorf, 1985), results might be inaccurate and no valid conclusion can be drawn concerning its effect on milk quality deterioration. The use of a combination trap

of Tenax with other adsorbents may be necessary to perform quantitative work for weakly retained compounds such as ethanol. Although ethanol is produced by bacteria during refrigerated storage of milk, it is also utilized to produce other metabolites. It is, therefore, possible that ethanol may not be directly related to the keeping quality of milk.

Although most volatiles listed in Table 8 were present in both rancid and fruity samples, means for 1-propanol/ethyl acetate, butanoate, and hexanoate for rancid samples were significantly ($P < 0.01$) larger than means for fruity samples. On the other hand, means for 2-butanone and 2,3-butanedione were significantly ($P < 0.05$) larger in fruity samples than in rancid ones. Discrimination based on the rest of the identified volatiles was due to differences between the rancid and fruity and normal milk groups. Identification of volatiles other than hexanoate and butanoate in fruity samples, as reported in this work, may be useful in the preparation of synthetic standards for fruity flavor simulation. These cause–effect relationships could not be recognized without the application of multivariate analysis of gas chromatographic data. Cormier et al. (1991) have recognized the difficulty in trying to simulate fruitiness by mixing only ethyl butanoate, 3-methylbutanoate, and ethyl hexanoate. A mixture of only these compounds resulted in an artificial candylike aroma rather than in a fruity off-flavor. They concluded that the complexity, richness, and natural qualities of the aroma depend on the harmony and balance of many aroma compounds. Cormier et al. (1991) used purge and trap isolation followed by GC sniffing analysis to identify the odor-active components of a complex mixture of volatiles produced by *P. fragi* after inoculation of skim milk. Although GC sniffing analysis is useful for the recognition of single odor-active components, what the flavor researcher really wants to know is the cause–result relationships between a group of components and sensory qualities.

Results in this research suggest that although DH-GC could not detect volatile fatty acids (VFA), which are the compounds responsible for the rancid flavor of milk, other volatiles present in rancid milk may be useful rancidity indicators. It appears that the simulation of rancid flavor notes in milk may require not only the addition of VFA but also the addition of other contributing volatiles. This would be an important improvement in the methodology used in the preparation of standards with simulated rancid flavor. Previous attempts to duplicate rancid or lipolized flavor in milk by the addition of blends of VFA to milk were unsuccessful (Shipe, 1980).

Conclusions. An objective and rapid analytical system was established for detecting the flavor quality of milk and identifying spoilage volatiles responsible for off-flavors at the end of shelf life. Although other instrumental techniques have been proposed for assessing milk quality, dynamic headspace gas chromatography coupled to mass spectrometry (DH-GC–MS) in conjuction with multivariate statistics is the only system that allows the chemical identification of spoilage volatiles in pasteurized milk, which are ultimately the cause of off-flavors and termination of shelf life.

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