Gas Chromatographic Organic Acid Profiling Analysis of Brandies and Whiskeys for Pattern Recognition Analysis

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An efficient gas chromatographic profiling and pattern recognition method is described for brandy and whiskey samples according to their organic acid contents. It involves solid-phase extraction of organic acids using Chromosorb P with subsequent conversion to stable *tert*-butyldimethylsilyl derivatives for the direct analysis by capillary column gas chromatography and gas chromatography−mass spectrometry. A total of 12 organic acids were reproducibly identified in liquor samples (1 mL). When the GC profiles were simplified to their retention index spectra, characteristic patterns were obtained for each liquor sample as well as for each group average. Stepwise discriminant analysis provided star symbols characteristic for each liquor sample and group average. As expected, canonical discriminant analysis correctly classified 23 liquor samples studied into two groups of either brandy or whiskey.

Keywords: *Brandy; whiskey; organic acids; pattern recognition; gas chromatography*

INTRODUCTION

Whiskey and brandy, the most widely consumed liquors among the major matured distilled liquors, are produced from unique combinations of different materials and maturing processes, which endow them their characteristic flavors. It is getting to be more important to chemically determine the composition of the characteristic flavors for quality control.

In the literature, discriminant and classification analysis of the volatile phenols as the aroma components was used for the distinction of rum, brandy, and whiskey (Lehtonen, 1983). Cluster analysis and KNN and PLS methods were applied to 9 components including volatile and less volatile aldehydes, esters, and alcohols for the identification of genuine Galician spirit (Ortiz et al., 1993). The discrimination of three different distilled liquors (Hida et al., 1995) was achieved by simple comparison of correlation coefficients based on the contents of ethyl caprylate, ethyl myristate, ethyl laurate, capric acid, and caprylic acid. As the major contributors to the taste and physical stability of alcoholic beverages (Calull et al., 1991; Kim et al., 1994; Kupina et al., 1991; Marce et al., 1991; Rouseff et al., 1992; Shinohara, 1985), organic acids are known to be important indicators of a wide variety of fermentation processes and thus are used to control the vinification process (Kupina et al., 1991). However, attempts were not extensively made to correlate the organic acid profiles to distilled liquors (Kim et al., 1994; Ortiz et al., 1993).

In a previous report (Kim et al., 1994), a simpler pattern recognition method combined with our routine organic acid profiling analysis was found to be useful for the comparative analysis of organic acid profiles among the four different alcoholic beverages (white wine, red wine, brandy, and beer). The procedure involves solid-phase extraction (SPE) of organic acids using Chromosorb P as the solid-phase sorbent in normal-phase partition mode, with subsequent single-step conversion to *tert*-butyldimethylsilyl (TBDMS) derivatives (Kim et al., 1989, 1990) followed by direct gas chromatographic (GC) analysis on dual-capillary columns of different polarity (Kim et al., 1990, 1993). Each organic acid was then identified through computer library matching based on the two retention index (*I*) sets and area ratio comparison. The GC profiles were simplified to their corresponding organic acid *I* spectra of bar graphical form for the visual comparison between liquor samples.

This work was undertaken to examine the usefulness of our previous organic acid profiling analysis combined with *I* spectral and star graphical methods as the simple pattern recognition methods in the characterization of brandy and whiskey. The canonical discriminant analysis was investigated for the correct classification of distilled liquor samples into two groups of either brandy or whiskey.

MATERIALS AND METHODS

Materials. Nine brandies and 14 whiskeys were studied. One bottle of each brand was purchased locally and refrigerated until being used. Isooctane was obtained from Junsei (Tokyo, Japan) and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) of silylation grade from Pierce (Rockford, IL). All other chemicals were of analytical grade and used as received. Chromosorb P (acid-washed, 80−100

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Table 1. Organic Acids Found in Liquor Samples from 9 Brandy Brands

		normalized peak area ratio (%)[a]										
no.	acid	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	mean[b]	median[c]
1	lactic[d]	60.54	58.25	54.28	59.75	44.02	53.81	39.34	48.10	58.16	52.92	54.28
2	glycolic	5.46	11.36	9.41	7.01	13.45	10.81	16.04	10.57	8.83	10.33	10.57
3	oxalic	9.16	12.28	13.63	11.50	21.17	14.79	20.94	18.28	5.95	14.19	13.63
4	malonic	0.38	0.67	1.33	1.02	2.81	0.60	1.16	2.01	0.74	1.19	1.02
5	capric	10.45	2.67	4.50	8.74	7.83	1.89	3.94	7.58	9.78	6.38	7.58
6	succinic	5.25	4.20	4.84	3.41	4.53	4.92	4.48	2.83	4.96	4.38	4.53
7	lauric	2.03	1.85	2.38	1.95	1.42	0.98	0.97	1.98	2.60	1.80	1.95
8	myristic	0.22	0.37	0.40	0.23	0.30	0.38	0.31	0.24	0.60	0.34	0.31
9	malic	0.90	1.51	1.98	1.52	0.71	1.29	1.66	1.40	0.81	1.31	1.40
10	palmitic	1.04	0.58	0.51	0.55	0.82	1.29	1.00	0.55	2.78	1.01	0.82
11	tartaric	3.14	5.30	5.67	3.79	1.84	7.82	8.54	5.48	2.89	4.94	5.30
12	stearic	1.42	0.96	1.08	0.53	1.09	1.42	1.62	0.97	1.90	1.22	1.09

[a] Mean peak area ratios relative to IS (tridecanoic acid) from triplicate runs of each brand on a DB-5 column were normalized to area ratio sum. [b] Mean normalized peak area ratios of 9 brands. [c] Median normalized peak area ratios of 9 brands. [d] Unresolved trace caprylic acid was included.

Table 2. Organic Acids Found in Liquor Samples from 14 Whiskey Brands

		normalized peak area ratio (%)[a]														
no.	acid	W-1	W-2	W-3	W-4	W-5	W-6	W-7	W-8	W-9	W-10	W-11	W-12	W-13	W-14	mean[b]	median[c]
1	lactic[d]	23.18	29.68	11.83	11.92	18.83	26.54	30.73	23.95	12.55	5.37	8.56	22.61	27.21	4.74	18.41	20.72
2	glycolic	17.80	10.05	10.04	7.88	15.61	9.18	4.62	8.81	8.29	11.42	7.29	6.43	8.59	7.90	9.56	8.70
3	oxalic	18.51	15.37	19.53	20.20	20.35	17.29	11.42	14.07	18.51	18.19	12.61	15.12	16.28	15.34	16.63	16.79
4	malonic	1.08	1.56	1.79	1.41	0.71	1.60	1.70	1.84	3.02	5.09	3.94	1.82	1.11	1.53	2.02	1.65
5	capric	13.28	18.85	18.64	16.57	10.29	10.13	21.88	25.91	9.92	9.53	9.87	14.00	8.42	31.05	15.60	13.64
6	succinic	8.51	7.29	8.42	4.85	6.38	15.65	5.69	5.78	15.38	28.05	18.41	18.72	13.09	8.93	11.80	8.72
7	lauric	7.97	9.17	10.57	11.92	7.50	6.87	13.82	9.33	5.16	2.30	3.22	4.12	1.92	4.41	7.02	7.18
8	myristic	0.97	0.60	1.25	1.62	1.51	0.73	1.04	0.93	1.33	1.04	1.13	1.04	1.28	1.14	1.12	1.09
9	malic	0.74	0.97	0.90	0.81	1.11	2.90	0.95	1.06	2.48	2.84	12.75	1.91	1.61	1.60	2.33	1.35
10	palmitic	2.83	1.65	5.38	6.46	4.60	2.56	2.36	1.57	11.35	3.91	4.65	5.47	8.61	5.26	4.76	4.63
11	tartaric	1.87	2.84	6.09	7.68	7.60	1.28	3.31	4.39	4.43	6.44	14.15	3.91	3.74	9.32	5.50	4.41
12	stearic	3.25	1.98	5.56	8.69	5.50	5.27	2.47	2.35	7.57	5.82	3.42	4.86	8.15	8.78	5.26	5.39

[a] Mean peak area ratios relative to IS (tridecanoic acid) from triplicate runs of each brand on a DB-5 column were normalized to area ratio sum. [b] Mean normalized peak area ratios of 14 whiskey brands. [c] Median normalized peak area ratios of 14 whiskeys. [d] Unresolved trace caprylic acid was included.

mesh) was purchased from Supelco (Bellefonte, PA). A luer-tipped glass tube (5 mm i.d.) packed with Chromosorb P (2.0 g) was washed successively with 0.1 M sulfuric acid, methanol, acetone, dichloromethane, and diethyl ether, followed by activation under vacuum (150 °C, 3 h) prior to being used as a tube for SPE in normal-phase partition mode.

Sample Preparation. All liquor samples were individually processed in triplicate for organic acid analysis as follows. After addition of tridecanoic acid as an internal standard (IS) at 15 ppm, an aliquot (2 mL) of each liquor sample was adjusted to about pH 8.5 with sodium hydroxide solution, followed by washing with diethyl ether (2 mL × 3). The aqueous layer (1 mL) was acidified (pH < 2) with concentrated sulfuric acid and saturated with sodium chloride. The mixture was then loaded onto a Chromosorb P tube, followed by elution with diethyl ether (2 mL × 3) using a solid-phase extractor (Supelco Inc., Bellefonte, PA). The eluate was evaporated under a gentle stream of nitrogen, followed by silylation with MTBSTFA (20 μL) in the presence of isooctane (20 μL) at 60 °C for 2 h for direct analysis by GC and GC-MS.

Gas Chromatography and Gas Chromatography–Mass Spectrometry. GC analyses were performed with a Varian Star 3400cx gas chromatograph equipped with one split/splitless capillary inlet system and one flame ionization detector (FID) and interfaced to a Varian 4400 Integrator (Varian Associates Inc., Walnut Creek, CA). A DB-5 (SE-54 bonded phase) fused-silica capillary column (J&W Scientific, Rancho Corodova, CA; dimensions of 30 m × 0.25 mm i.d., 0.25 mm film thickness) was used for this study. The inlet pressure of helium as the carrier gas was set to 34.5 kPa. Samples (0.5 μL) were injected in the splitless injection mode (purge delay time of 0.7 min) with the hot needle fast (15 s) injection technique. The oven temperature was held at 60 °C for 2 min and then programmed to 280 °C at a rate of 4 °C/min. The injector and detector temperatures were 260 and 300 °C, respectively. The peak area ratio of each identified organic acid with respect to IS was calculated for the subsequent pattern recognition analysis.

Peak identification was done on a Hewlett-Packard HP 6890 series gas chromatograph interfaced to a HP 5972A MSD (70 eV, electron impact mode) which was on-line to a HP G1701AA MSD ChemStation (Hewlett-Packard, Avondale, PA). A HP-5MS (SE-54 bonded phase) capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness) was used in split injection mode (10:1). The oven temperature was initially at 100 °C for 2 min and then raised to 290 °C at a rate of 5 °C/min. The injector and interface temperatures were 270 and 290 °C, respectively. Each peak was identified by library matching from our MS library file containing organic acid standards as their TBDMS derivatives. Temperature-programmed *I* values of the organic acids identified by GC-MS were adopted from the compiled *I* reference library of our previous work (Kim et al., 1990). The peak area ratio of each identified organic acid with respect to IS was calculated using DB-5 chromatographic data for the subsequent pattern recognition analysis.

Pattern Recognition Analysis. The mean peak area ratios (relative to IS) of organic acids identified in each liquor sample were normalized to the largest peak as the base peak. Using Sigma Plot 3.01 for Windows, the percent normalized area ratios were plotted against *I* values in bar graphical form to obtain organic acid *I* spectrum of each sample as described earlier (Kim et al., 1994). For *I* spectra of the group averages, normalized median area ratios were used.

Stepwise discriminant analysis for the selection of the most discriminant variables was performed on the peak area ratios by means of the statistical software package SAS. Using MS Excel, the normalized mean peak area ratio of each discriminant variable selected was plotted as a line radiating from a common central point and the far ends of the lines were joined together to produce a star symbol plot of each brandy and

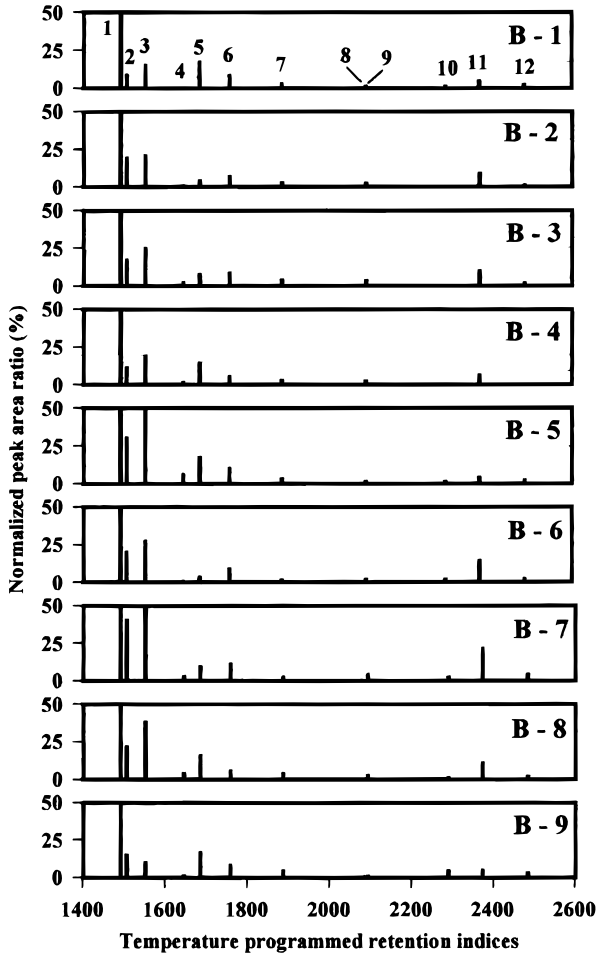

Figure 1. Retention index spectra of organic acids from 9 brandies as TBDMS derivatives separated on a DB-5 (30 m × 0.25 mm i.d. and 0.25 mm film thickness) capillary column. GC conditions are described in the text. Peaks: 1 = lactic acid; 2 = glycolic acid; 3 = oxalic acid; 4 = malonic acid; 5 = capric acid; 6 = succinic acid; 7 = lauric acid; 8 = myristic acid; 9 = malic acid; 10 = palmitic acid; 11 = tartaric acid; 12 = stearic acid.

whiskey. The variables in decreasing order of discriminating power were assigned to rays of the star clockwise. The normalized median area ratios were used for the star plots of the group averages. Canonical discriminant analysis was performed on the peak area ratios of each sample as the data vectors using SAS, followed by plotting the first canonical function (CAN1) against the second canonical discriminant function (CAN2) for each sample to produce a canonical plot.

RESULTS AND DISCUSSION

In this study, 9 brandies (B-1 through B-9) and 14 whiskeys (W-1 through W-14) were screened for organic acids. Our profiling method produced good GC profiles with 1 mL of liquor. Among the organic acids positively identified from the 23 liquor samples by GC-MS, volatile acids such as acetic acid, propionic acid, isovaleric acid, and caproic acid were not quantifiable from GC analysis using a DB-5 capillary column due to their incomplete resolution from solvent and derivatization reagent peaks. In most cases, citric acid was detected at trace levels with low precision. Therefore, these 5 acids were excluded in the statistical analysis for the pattern recognition study. The contents of 12 acids reproducibly detected from triplicate runs of each liquor sample on the DB-5 column were expressed as the mean peak area

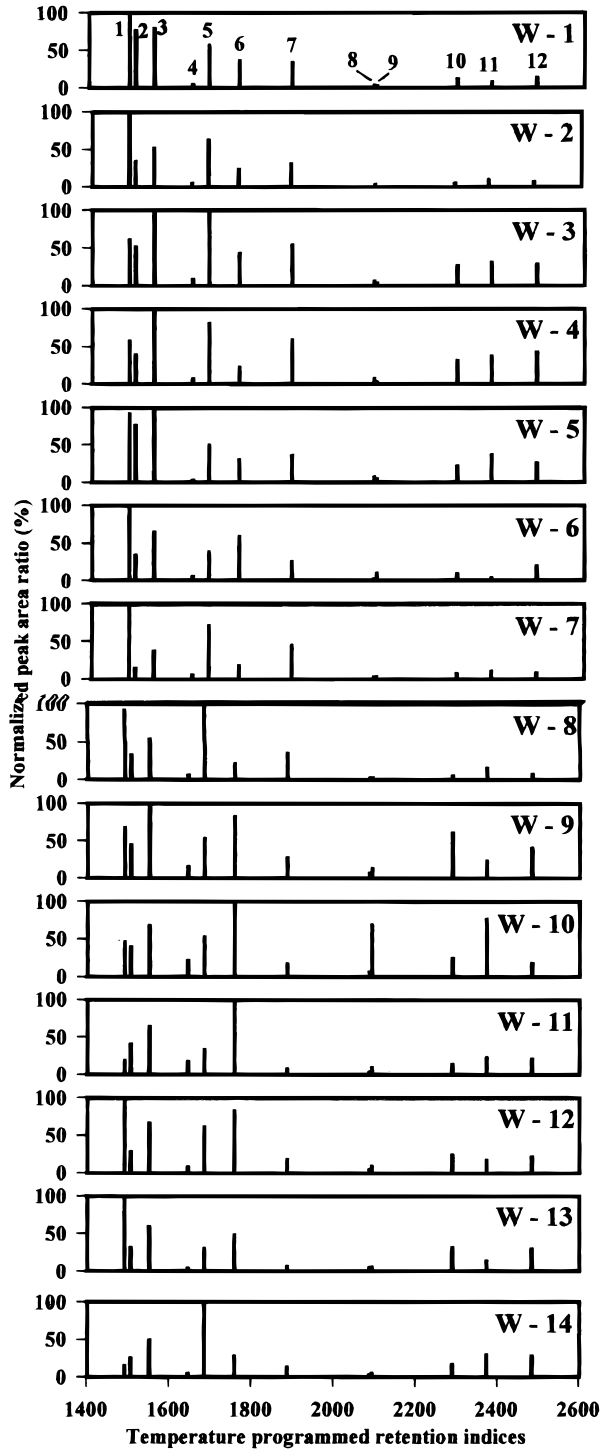

Figure 2. Retention index spectra of organic acids from 14 whiskeys. GC conditions are described in the text, and peak numbers correspond to those in Figure 1.

ratios normalized to the area sum (Tables 1 and 2). Most liquor samples studied contained a high level of lactic acid, and thus, the adjacent minor caprylic acid was unresolvable. The presence of trace caprylic acid could be detected only in the selected ion chromatograms of GC-MS runs. Thus, its trace amount was included in the lactic acid amount.

In the brandy group, lactic acid was the most abundant for all brands, followed by oxalic acid, glycolic acid, capric acid, and tartaric acid, except for B-1 and B-9 where capric acid was the second most prominent (Table 1). The orders of all the acids in magnitude of both the

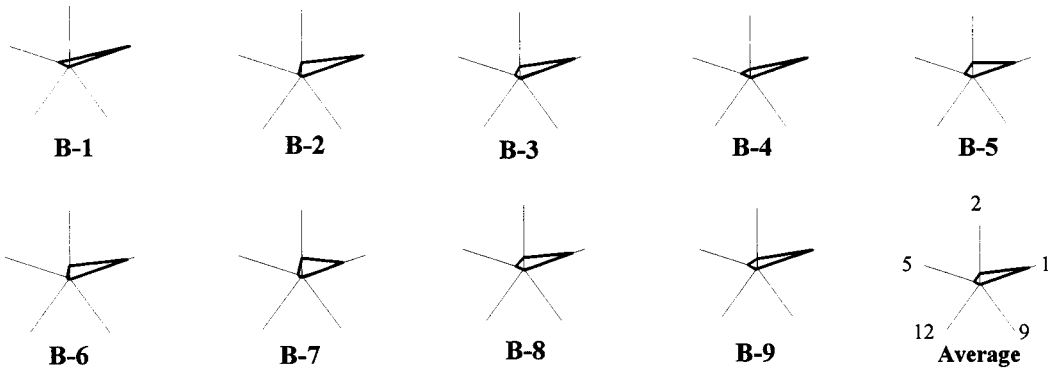

Figure 3. Star symbol plots of 9 brandies and group average drawn based on 5 discriminant variables. Rays: 2 = glycolic acid; 1 = lactic acid; 9 = malic acid; 12 = stearic acid; 5 = capric acid.

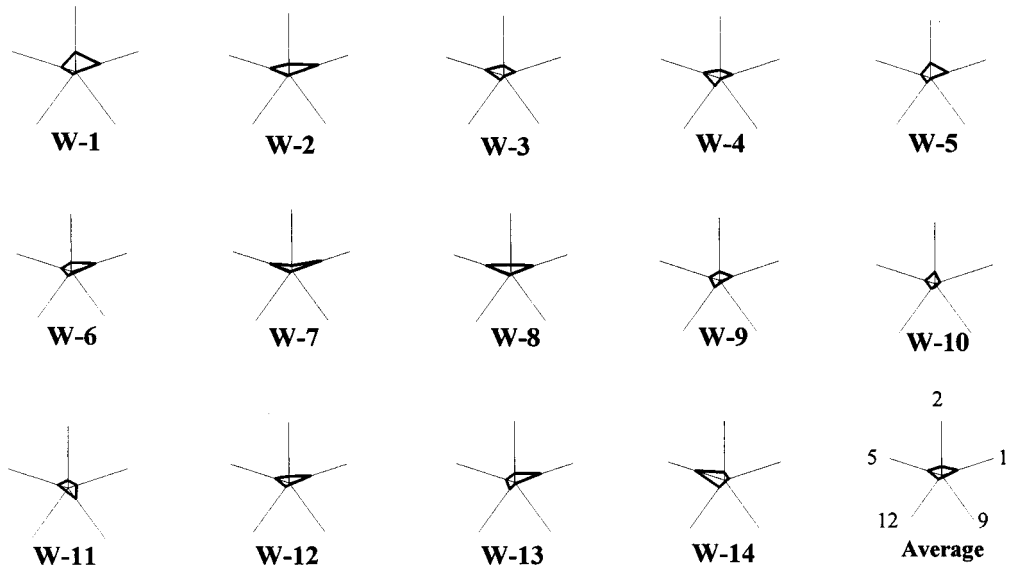

Figure 4. Star symbol plots of 14 whiskeys and group average drawn based on 5 discriminant variables. Rays: 2 = glycolic acid; 1 = lactic acid; 9 = malic acid; 12 = stearic acid; 5 = capric acid.

mean and median area ratios were identical, indicating that the maturing processes of brandy seems to be strictly controlled in its organic acid contents among the 9 brands studied.

Unlike the brandy, large variations in the levels of organic acids were observed (Table 2) in the whiskey group. Lactic acid was most abundant only in 6 cases (W-1, W-2, W-6, W-7, W-12, and W-13). Instead, oxalic acid in four brands (W-3, W-4, W-5, and W-9), capric acid in two brands (W-8 and W-14), and succinic acid in two brands (W-10 and W-11) turned out to be the most prominent. The large differences in the organic acid profiles among 14 whiskeys studied indicate that the manufacturing processes of whiskey appear to be less controlled.

When the GC data in tabular form were transformed to their corresponding organic acid *I* spectra in bar graphical form, visual comparison between liquors was much easier, as demonstrated by each characteristic *I* spectrum. As expected, overall patterns of the individual *I* spectra in the brandy group look similar except for B-5 and B-7, which show higher peaks of oxalic acid (Figure 1). Tartaric acid was prominent in B-7 but low in B-5. Large variations in *I* spectral patterns of individual whiskeys are more clearly seen (Figure 2).

It was desired to classify organic acid profiles of 23 liquor samples into two groups of either brandy or whiskey. The GC data of organic acids in the two tables were thus subjected to stepwise discriminant and canonical discriminant analyses. When stepwise discriminant analysis was performed, glycolic acid was selected as the most discriminating variable, followed by lactic acid, malic acid, stearic acid, and capric acid. Star symbol plots drawn based on the area ratios of these acid variables were very useful for the visual pattern recognition between individuals as well as groups. Star patterns of all brandies resemble one another and look similar to their average star pattern (Figure 3). Fourteen whiskeys are individually different but look similar to their average except for W-7, W-13, and W-14 (Figure 4). The average star plots representing the brandy and whiskey groups are distinguishable from one another.

When canonical discriminant analysis was applied to all 12 acids as data vectors, 23 liquors were well-separated into two distinct clusters of brandy and whiskey in the two-dimensional canonical function space (Figure 5). As expected, the liquors were correctly classified into two groups, but individual samples in the whiskey group show more scattered clustering than those of the brandy group.

CONCLUSIONS

The present SPE and silylation with subsequent capillary column GC analysis were suitable for the

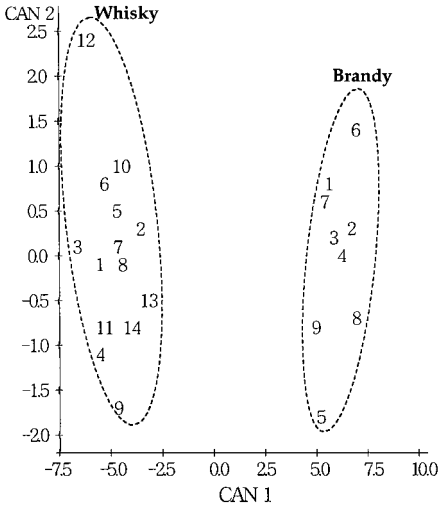

Figure 5. Plot of the first and second canonical functions of the 12 acid variables for the 9 brandies and 14 whiskeys.

profiling and screening for organic acids in matured distilled liquors, brandy, and whiskey. Simplification of GC profiles to their retention index spectra enabled one to detect qualitative and quantitative differences among liquor samples readily. Star symbol plots based on the normalized peak area ratios of glycolic acid, lactic acid, malic acid, stearic acid, and capric acid as the discriminant variables were useful for visual pattern recognition between brandies and whiskeys. A canonical plot based on 12 organic acids correctly grouped 23 liquor samples into two separate clusters of either brandy or whiskey.

From our results, it can be stated that the present method is potentially useful in the manufacturing and quality control of brandy and whiskey.

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