

## Chemometric Characterization of the Cypriot Spirit “Zivania”

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In 42 alcoholic beverages produced in Cyprus and other countries, 26 chemical and physical-chemical variables were determined by HPLC and GC chromatography,  $^1\text{H}$  NMR and ICP spectroscopy, and other techniques. Data were processed using multivariate chemometric techniques, involving principal component analysis, cluster analysis, regularized discriminant analysis, and classification and regression trees. Zivania can be differentiated from beverages from other countries. Using 2- and 3-methyl-butanol, 2-methyl-propanol, furfural, methanol, and the alcoholic grade and the chemical shift of  $-\text{CH}_3$  in  $^1\text{H}$  NMR spectra as features, a nearly correct classification for zivania was achieved. The reasons for diversions are given.

**KEYWORDS:** Zivania; alcoholic beverage; authenticity; classification; discriminant analysis; trace elements; sugars; volatile compounds;  $^1\text{H}$  NMR spectroscopy

### INTRODUCTION

Zivania is a traditional Cypriot spirit, which is still produced today, using essentially the same methods and recipes as those passed down from many years ago by tradition (1, 2). Until recently, zivania was produced by vineyard growers for their own use and also by industry exclusively for export. Recently, however, laws prohibiting the commercial manufacture of the product for local use have been repealed, opening the opportunity for the local wine producers to exploit its appeal. Zivania has played an important role in the everyday life of Cypriots (3). Both archaeological evidence and written sources including the reports of foreign pilgrims and other visitors to Cyprus are the main sources of information which enlightens us on the traditional production of zivania. This apparently started very early, ever since the distillation technology was developed, at least since the Hellenistic era. Depending on the time of year, it was used either as a hot beverage or as a refreshment and depending on the time of day, it occupied the place that drinking of wine or coffee holds in modern living (4). It is currently the most widely consumed spirit on the island of Cyprus, while it is gaining in popularity elsewhere in Europe.

Zivania is exclusively produced by the distillation of grape marc from local varieties of grapes. Grape varieties used are typically “Xynisteri” (white grapes), “Ofthalmon” (black grapes) and “Maratheftikon” (black grapes). Cyprus legislation permits the use of wine as the distillate, but this is rarely used and is not the traditional recipe. Samples used here came from marc. Differences from other similar spirits appear to stem from this fact. The name zivania in Cyprus comes from the word “zivana”, the term for grape marc in the Greek dialect spoken in Cyprus. There are similar products from other countries, which derive

their names from the term used in each country or region to describe the grape marc (1, 5, 6).

In a previous publication (2), we reported on the use of the concentration of trace elements to differentiate zivania from other alcoholic spirits. We believe that this basis for the differentiation of zivania is related to the variety of grapes produced in Cyprus and used in its preparation, the methods of production and distillation, and the geological and climatic conditions existing on Cyprus (7–9). The distinct aroma and taste of zivania is due to its chemical composition and it is ensured by the volatile compounds that are transferred from the grape marc during distillation. Climate has an influence, presumably, on the quality of both grapes and zivania. Cyprus has typical Mediterranean climate, with hot, dry summers and short spring and autumn seasons. Relative humidity and evaporation affect both the quantity and quality of grapes and zivania. The amounts of nutrients that are available to the vine depend on the soil’s mineralogy, the acidity of the soil, and the amount and nature of organic matter and are deemed to be characteristic (2, 4).

In this paper, we report the evaluation of spectroscopic, chromatographic, and other data by multiple discriminant analysis, with the aim of differentiating between the different groups of spirits (see below). In various publications (see, for example (10)), pattern recognition methods have been used in the characterization and classification of wines and other foodstuff, according to origin, quality, variety, or other features: Spanish wines by metal ions (11), Italian wines by inorganic and classical determinations plus aroma compounds (12), Portuguese wines by free amino acid profiles (13), French red wines by elements, amino acids, and aromatic alcohols (14), and German wines by proton and carbon-13 spectra (15). Multivariate chemometric techniques were used to classify alcoholic distillates and develop a “typification” for Spanish liquors, on the basis of data from chromatographic analyses (16).

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Table 1. Identification and Provenance of Samples Used

S/n	sample label	provenance	group	S/n of grouping	S/n	sample label	provenance	group	S/n of grouping
1	S1	Kykkotiki	zivania	1	22	S23	Vodka Russian	various distillings	3
2	S2	Kyk. Cabernet	zivania	1	23	S6	Grappa	various distillings	3
3	S8	Pachna	zivania	1	24	S12	Tsuica	various distillings	3
4	S19	Kaminaria	zivania	1	25	S26	Rakea	various distillings	3
5	S20	Kellaki 1	zivania	1	26	S56	Barberino	various distillings	3
6	S21	Chrysoroyiatissa	zivania	1	27	S57	Segrel	Various distillings	3
7	S22	Omodos	zivania	1	28	S58	Becherovka	various distillings	3
8	S31	Ag. Varvara	zivania	1	29	S15	ARAK al bustan	various distillings	3
9	S33	Lofou	zivania	1	30	S90	Gantous & Abou Raad	various distillings	3
10	S45	LOEL	zivania	1	31	S7	Tsipouro Agioritiko	Greek distillings	4
11	S46	SODAP	zivania	1	32	S16	Tsipouro Makedoniko 1	Greek distillings	4
12	S51	Hjpvavlou	zivania	1	33	S25	Tsipouro Timavou	Greek distillings	4
13	S59	Yeni Raki	zivania	1	34	S9	Tsikoudia Varvaki	Greek distillings	4
14	S66	KEO	zivania	1	35	S52	Tsipouro Makedoniko 2	Greek distillings	4
15	S3	Kykkotiki dry	zivania red	2	36	S97	Tsipouro AMVYX	Greek distillings	4
16	S4	Kykkotiki sweet	zivania red	2	37	S11	Vat4 (6 years)	eau de vie	5
17	S93	Kykkotiki dry	zivania red	2	38	S18	Vat17 (1 year)	eau de vie	5
18	S94	Kykkotiki sweet	zivania red	2	39	S98	EDV ETKO	eau de vie	5
19	S95	Kykkotiki dry	zivania red	2	40	S99	EDV SODAP	eau de vie	5
20	S96	Kykkotiki sweet	zivania red	2	41	S100	EDV LOEL1	eau de vie	5
21	S5	Vodka Absolute	various distillings	3	42	S101	EDV LOEL2	eau de vie	5

## MATERIALS AND METHODS

**Samples Analyzed.** Forty-two samples of different alcoholic beverages were analyzed to compare the composition of 26 cypriot samples zivania (both commercial and home produced), including 6 of the so-called red zivania (17, 18) variant with that of 16 alcoholic beverages from other countries, such as tsipouro, grappa, vodka, tsuica, and rakea, and six samples of eau de vie. Grape marc is used as raw material for the production of zivania, tsipouro, grappa, tsuica, and rakea, while potatoes are used in vodka or corn and wheat. Eau de vie is a wine distillate and has been chosen because it is often used as a cheap substitute for zivania. The samples and their provenances are presented in Table 1. Twenty-six parameters and components were chosen to obtain a general picture of their physical-chemical and chemical characters.

**Analytical Determinations.** Samples were analyzed to determine the following parameters (abbreviations and measurement units are given in parentheses): pH, brix, refractive index (RI), alcoholic grade (alc. grad., % vol.), NMR chemical shift of methyl, methylene, and hydroxyl groups of ethanol ( $-\text{CH}_3$ ,  $-\text{CH}_2-$ ,  $-\text{OH}$ , ppm), and the concentration of the following components: acetaldehyde, methanol, propanol, ethyl acetate, 2-methyl propanol, 2-methyl butanol, 3-methyl butanol, ethyl lactate, furfural, and butanol-1 (all alcohols in units of mg/100 mL abs alc.), iron (Fe, mg/L), copper (Cu, mg/L), magnesium (Mg, mg/L), calcium (Ca, mg/L), potassium (K, mg/L), zinc (Zn, mg/L), fructose (mg/L), glucose (mg/L), and sucrose (mg/L). All determinations were made twice and the mean was retained.

**Preparation of Samples.** All the samples, both for analysis by NMR and ICP, were concentrated using a Christ, Alpha 1–2 freeze-drier (1, 2, 19). The condenser temperature was 233 K and the final pressure in the drying chamber was 3 mPa. The freeze-drying procedure required 24 h to be completed and the residue was used for NMR measurements. For ICP analysis, the residue was dissolved in 5 mL  $\text{HNO}_3$  (BDH–Spectrosol 10% v/v) and was filtered through folded filter paper and stored in polypropylene bottles (1).

**Analytical Procedures.** The measurements of pH, brix, refractive index (RI), and alcoholic grade were carried out by classical procedures.

**NMR Spectroscopy.**  $^1\text{H}$  NMR spectra were recorded on a BRUKER 300 MHz NMR spectrometer, using benzene as the internal standard and the following experimental parameters: number of scans 32, pulse duration 3  $\mu\text{s}$ , digital resolution 0.2 Hz/pt, spectrum width 3125 Hz, relaxation delay 1 s, temperature 300 K, and acquisition time 5.24 s (1). The data were processed using ACD Full, version 3.3.3.3.

**Inductively Coupled Plasma Spectroscopy (ICP).** The determination of Fe, Cu, Mg, Ca, K, and Zn was carried out with a Shimadzu ICP-7500. The sample solution was introduced into the core of inductively

coupled argon plasma at a temperature of approximately 8270 K. The advantage of using ICP spectroscopy as compared to classical atomic absorption spectroscopy (AAS) was the determination and simultaneous detection of the six metals in a single sample analysis step (2).

**Gas Chromatography (GC).** Acetaldehyde, methanol, propanol, ethyl acetate, 2-methyl propanol, 2-methyl butanol, 3-methyl butanol, ethyl lactate, furfural, and butanol-1 were determined by GC SHIMADZU 9000. Five microliters of each sample was injected (splitless 1:25, 200 °C) onto a 30 m  $\times$  0.32 mm i.d. (5.0- $\mu\text{m}$  film thickness) capillary GC column Econo-Cap EC Wax. The initial temperature was 60 °C (held for 6 min) and increased at 30 °C/min to 200 °C. The method used helium as the carrier gas with flame-ionization detection (FID). The identification of the volatile compounds was confirmed by comparison of retention times with the standard (NEOCHEMA) and by using spikes (1, 20, 21).

**High-Performance Liquid Chromatography (HPLC).** The samples were filtered through 0.45- $\mu\text{m}$  Millex filter and 10  $\mu\text{L}$  was injected directly onto a  $\mu$ -Bondapak-NH<sub>2</sub> 300  $\times$  3.9 mm i.d. HPLC column. The mobile phase ( $\text{CH}_3\text{CN}/\text{H}_2\text{O} = 830/170$ ) was run at 1.5 mL/min and the column was kept at ambient temperature in a column block heater. A WATERS refractive index detector was used to record the chromatograms; fructose, glucose, and sucrose were eluted as well-separated peaks after 12 min (1, 22). The identification of sugars was confirmed by comparison of retention times with standards and using spikes.

**Data Analysis.** Each sample (object) was considered as an assembly of seven variables represented by the chemical data. These variables, called “features”, formed a “data vector” which represented a spirit sample in the way used by other authors (11, 23). Data vectors belonging to the same group, as defined geographic origin, were analyzed. The group was then termed a “category”. Pattern recognition tools used in this work were as follows.

**Principal Component Analysis (PCA).** This procedure was used mainly to achieve a reduction of dimensionality to permit a primary evaluation of the between-category similarity (24).

**Cluster Analysis (CA).** This technique comprises an unsupervised classification procedure that involves a measurement of either the distance or the similarity between objects to be clustered. Objects are grouped in clusters in terms of their nearness or similarity. The initial assumption is that the nearness of objects in the p-space, defined by the variables, reflects the similarity of their properties (25).

**Classification and Regression Trees (CART).** This technique builds classification and regression trees for predicting continuous depended variables (regression) and categorical predictor variables (classification). It is a nonparametric technique that can select the variables and their

**Table 2.** Mean (Standard Deviation) and Minimum–Maximum Values of Chemical and Physical-Chemical Variables, by Type of Distills

S/n	variable	units	group 1 Zivania (14 samples)	group 2 Zivania red (6 samples)	group 3 various distillates (10 samples)	group 4 Greek distillates (6 samples)	group 5 eau de vie (6 samples)
1	pH		4.43 (1.01) 3.20–7.41	3.95 (0.09) 3.85–4.05	6.07 (1.91) 3.41–8.15	6.44 (1.83) 3.16–8.74	3.36 (0.44) 2.80–3.82
2	brix		10.47 (5.87) 0.3–16.0	22.38 (5.90) 16.7–27.8	13.26 (5.25) 2.7–22.6	12.21 (5.70) 3.0–18.7	15.83 (2.74) 13.2–18.4
3	refractive index		1.349 (0.008) 1.333–1.357	1.368 (0.008) 1.358–1.378	1.353 (0.009) 1.336–1.368	1.351 (0.009) 1.337–1.361	1.356 (0.004) 1.352–1.361
4	alcoholic grade	% vol.	48.1 (3.95) 44.6–58.9	40.3 (0.24) 40.1–40.8	42.6 (4.16) 38.9–50.2	42.3 (3.79) 38.0–48.6	69.2 (0.66) 68.2–70.0
5	–CH <sub>3</sub>	ppm	1.34 (0.05) 1.29–1.47	1.34 (0.03) 1.31–1.37	1.32 (0.05) 1.23–1.39	1.32 (0.03) 1.28–1.36	1.48 (0.03) 1.45–1.52
6	–CH <sub>2</sub> –	ppm	3.79 (0.05) 3.75–3.92	3.79 (0.02) 3.77–3.82	3.78 (0.04) 3.70–3.84	3.79 (0.02) 3.75–3.82	3.95 (0.04) 3.89–3.99
7	–OH	ppm	5.05 (0.04) 5.00–5.17	5.04 (0.02) 5.02–5.07	5.00 (0.04) 4.90–5.04	5.00 (0.02) 4.96–5.02	5.18 (0.04) 5.14–5.22
8	acetaldehyde	mg/100 mL abs alc.	37.5 (57.9) <0.1–152.9	13.1 (14.4) <0.1–26.7	18.1 (38.2) <0.1–91.8	<0.1	<0.1
9	methanol	mg/100 mL abs alc.	127.1 (32.6) 51.4–164	127.9 (6.4) 122.0–134.3	201.3 (262) <0.1–768	198.4 (52.6) 97.4–242	141.9 (17.6) 120–164.6
10	propanol	mg/100 mL abs alc.	39.9 (6.08) 23.2–50.3	44.8 (4.1) 40.8–49.0	44.4 (40) <0.1–95.5	54.6 (15.4) 36.5–74.1	35.2 (6.7) 27.1–44.4
11	ethyl-acetate	mg/100 mL abs alc.	74.4 (25.8) 21.4–103	10.5 (0) 10.5–10.5	70.3 (64.8) <0.1–158	70.2 (80) 16.1–223.5	165.6 (84) 78.1–276.5
12	2m-propanol	mg/100 mL abs alc.	44.9 (6.6) 36.6–60.1	43.0 (2.0) 40.9–45.5	58.4 (58.2) <0.1–150.1	47.6 (8.8) 29.9–53.4	51.2 (11.9) 32.6–63.5
13	2m-butanol	mg/100 mL abs alc.	12.5 (1.17) 10.6–14.5	14.3 (0.3) 13.9–14.7	10.8 (10.1) <0.1–24.8	13.2 (1.7) 10.5–15.0	9.5 (0.6) 8.8–10.1
14	3m-butanol	mg/100 mL abs alc.	154.1 (23.4) 122–202.5	184.1 (1.2) 182.5–185.6	112.5 (108) <0.1–268.9	172.4 (17.6) 147.1–194.7	121.4 (11.6) 109.2–135.9
15	ethyl-lactate	mg/100 mL abs alc.	49.4 (40) <0.1–116.4	19.4 (21.3) <0.1–39.1	7.7 (17.1) <0.1–50.2	4.8 (11.7) <0.1–28.6	50.0 (16.9) 30.0–73.7
16	furfural	mg/100 mL abs alc.	<0.1	84.0 (70.7) 19.2–150.1	1.56 (4.9) <0.1–15.6	<0.1	16.3 (18.1) <0.1–38
17	butanol-1	mg/100 mL abs alc.	4.31 (8.9) <0.1–27.5	143.2 (111) 41.8–245	1.22 (3.8) <0.1–12.2	<0.1	14.6 (4.5) 9.24–20.2
18	Fe	mg/L	0.070 (0.06) <0.001–0.236	0.069 (0.05) 0.04–0.162	0.041 (0.06) <0.001–0.189	0.150 (0.14) 0.005–0.323	0.022 (0.01) 0.016–0.034
19	Cu	mg/L	2.87 (3.1) <0.001–12.91	0.23 (0.34) 0.05–0.895	1.545 (2.4) 0.001–5.938	0.527 (0.76) <0.001–1.539	0.531 (0.04) 0.510–0.620
20	Mg	mg/L	0.66 (0.25) 0.29–1.25	9.6 (1.8) 7.9–12.6	0.95 (0.76) 0.72–1.86	1.54 (0.9) 0.25–2.32	0.167 (0.04) 0.118–0.220
21	Ca	mg/L	2.25 (1.1) 1.09–5.02	24.7 (3.6) 20.5–31.2	3.71 (3.6) 0.12–11.8	5.94 (4.6) 1.29–11.0	0.44 (0.06) 0.39–0.54
22	K	mg/L	4.48 (14.2) <0.001–53.7	25.5 (5.4) 18.2–32.8	1.64 (2.5) <0.001–7.08	2.67 (1.8) <0.001–4.78	0.61 (0.17) 0.36–0.82
23	Zn	mg/L	0.19 (0.25) 0.08–0.95	0.056 (0.04) <0.001–0.095	0.35 (0.31) 0.08–0.91	0.101 (0.04) 0.07–0.165	0.19 (0.07) 0.10–0.26
24	fructose	g/100 mL	0.007 (0.014) <0.001–0.048	0.43 (0.4) <0.001–0.912	0.177 (0.4) <0.001–1.291	0.006 (0.04) <0.001–0.098	<0.001
25	glucose	g/100 mL	0.034 (0.13) <0.001–0.477	0.429 (0.47) <0.001–0.880	0.089 (0.13) <0.001–0.332	0.388 (0.24) <0.001–0.65	<0.001
26	sucrose	g/100 mL	0.027 (0.02) <0.001–0.051	1.022 (0.12) 0.915–1.220	0.273 (0.47) <0.001–1.155	0.160 (0.36) <0.001–0.899	<0.001

interactions that are most important in determining the outcome, among a variable to be explained among a large number of variables (26, 27).

**Regularized Discriminant Analysis (RDA).** The purpose of the regularization is to reduce the variance related to the sample-based estimates and to perform well even if the dimensionality of the sample space is small compared to the dimension of the measurement space. It uses a complex biasing scheme to get better class covariance matrix estimates. The biasing is controlled by two parameters: lambda and gamma, and their values can be chosen by cross-validation (28, 29).

The data analysis was performed in the following steps:

(1) Preliminary data analysis by principal component analysis and cluster analysis using the complete data set.

(2) Classification techniques RDA and CART were applied to the complete data set with a category arrangement: category 1, training set of 14 zivania, category 2 comprising 6 samples of red zivania (with color, taste, and flavor due to addition of cinnamon), category 3 consisting of 10 other alcoholic beverages, category 4 comprising 6 Greek distillates, and category 5 consisting of 6 samples of eau de vie.

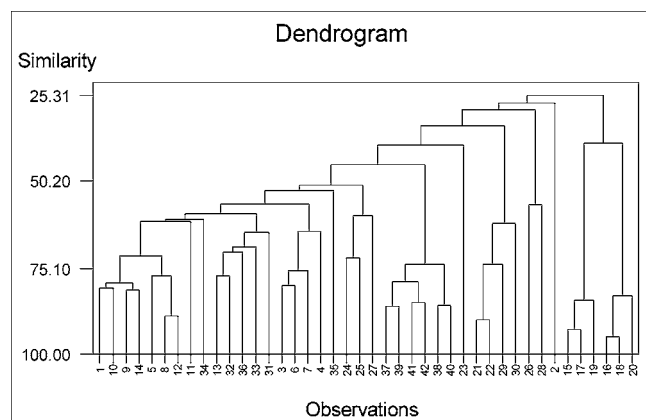
(3) For practical reasons, it is important to know the minimum number of features needed to obtain a correct classification. This could be achieved by choosing features that contained the most discriminant information for the classification. The criterion used for selection was Mg, Ca, 2- and 3-methyl-butanol, and ethyl-lactate.

(4) The reliability of the classification obtained previously was checked. The 42 objects were randomly divided between training set and prediction set.

Pattern recognition analyses were performed by means of the statistical software package SCAN (Software for Chemometric Analysis) (30).

## RESULTS AND DISCUSSION

We present a summary of the analytical data obtained in **Table 2**. The search for natural groupings in the samples is one of the main ways to study the data structure (31–34).



**Figure 1.** Dendrogram of cluster analysis of alcoholic beverages.

Hierarchical cluster analysis describes the nearness between samples of alcoholic drinks (objects), using the squared Euclidean distance between one object from the rest. Clusters are defined by an agglomerative algorithm. We start with each object in its own cluster. In the first step, the two objects closest together are joined. In the next step, either a third object joins the first two, or two other objects join together into a different cluster. Each step results lowering the number of clusters to one less than the step before, until at the end, all objects are in one cluster.

**Figure 1** shows the so-called dendrogram that displays the amalgamation of clusters in the form of a binary tree, exhibiting the clustering of the alcoholic samples grouped on the basis of the control parameters. The tree can be cut at any similarity level. The cut determines a certain set of clusters. At a similarity level of 0.5, six clusters were found, which can be identified as follows: The first cluster was composed of 13 zivania samples, 6 Greek samples, and 3 samples one each from Romania (serial number 24, S12 in **Table 1**), Bulgaria (serial number 25, S26), and Spain (serial number 27, S57); the second cluster was formed by the six samples of eau de vie; the third cluster was composed of four samples from Russia (serial number 21, S5), Lebanon (serial number 29, S15), and Jordan (serial number 30, S90); the fourth cluster contained only two samples from Austria (serial number 26, S56) and the Czech Republic (serial number 28, S57); the fifth cluster was formed by the three samples of red sweet zivania; and the sixth cluster was formed by the three samples of red dry zivania. Finally, one sample of zivania (serial number 2, S2, a special type “Cabernet”) and one sample of Italian “Grappa” (serial number 23, S6) were not assigned to any group. At this level of similarity, the classification appears to be approximately according to provenance and also according to the raw material.

Examination of the dendrogram at a similarity level of 0.75 provided eight clusters. The first and the third clusters were composed of 7 and 3, respectively, samples of zivania; the second and sixth clusters were formed by 4 and 3 samples, respectively, from various countries; the fourth cluster contained only two samples from Romania (serial number 24, S12 in **Table 1**) and Bulgaria (serial number 25, S26); the fifth cluster was formed by the six samples of eau de vie; the seventh cluster was formed by the three samples of red dry zivania, and the eighth cluster was formed by the three samples of red sweet zivania. Three samples of zivania were not assigned to any group, one of them was zivania Cabernet (serial number 2, S2 in **Table 1**) and the second one was double-distilled (serial number 4, S19). The distillate “Yeni Raki” (serial number 13, S59) from the north part of Cyprus was grouped in a cluster

**Table 3.** Principal Component Analysis: Cumulative Proportion of Total Variation (%), Calculated from Correlation Matrix by SVD

component	1	2	3	4	5	6	7	8	9	10
cumulative	25.0	42.5	56.3	64.1	71.0	77.2	82.4	86.7	89.4	91.9

with a high level of similarity with a nonzivania sample (serial number 32, S16). Three samples from Greece, Tsipouro from Macedonia (serial number 35, S52) and Tsikoudia from Crete (serial number 34, S9), and four samples from different European countries were not assigned to a group.

Principal component analysis was performed using the program SCAN (30). The singular value decomposition (SVD) algorithm calculates all components together, and so the computer space and time it requires both increase with an increase in the number of variables. Each principal component is orthogonal and is a linear combination of the original variables. **Table 3** reports the cumulative percentage of the total variance provided by the first 10 principal components obtained from the whole data set.

With regard to the overall PCA, the first three components, which account for 56.3%, explain only a low fraction of the total variance, but the 71.0% of the total variance is explained by the first five components. This finding can be explained by the wide scatter of the so-called “various distillates” as can be seen from the scatter plot (**Figure 2**).

The first principal component (PC) contrasts beverages with high alcoholic grade versus those with high quantity of calcium, magnesium, butanol-1, and 2- and 3-methyl-butanol. The second PC contrasts drinks with high brix, refractive index, sucrose, and furfural versus those with high propanol. In the third, pH is contrasted with a high chemical shift of  $-\text{OH}$ ,  $-\text{CH}_2-$ , and  $-\text{CH}_3$  and alcoholic grade. In the fourth, butanol and ethyl-lactate are contrasted with pH and refractive index, while factor V is a function of copper versus high pH.

In **Figure 2**, the distillate scores are plotted for principal components I and II in a scatter plot, showing a clear separation of red zivania (two groups: sweet and dry), eau de vie samples, and Greek distillates. Zivania are well-defined except for the sample from northern Cyprus (Yeni Raki: serial number 13, S59 in **Table 1**) and the double-distilled sample (from Kaminaria: serial number 4, S19 in **Table 1**). The variables characterizing this separation are mainly ethyl acetate and ethyl-lactate. The samples with provenance outside Cyprus and Greece could not be discriminated from each other using this analysis method. This was not unexpected, given the wide provenance of these samples. The PCA result is consistent to a large extent with the conclusions obtained by cluster analysis.

Two classification methods, RDA (26, 27) and CART (28, 29), were applied to the complete data set after autoscaling to eliminate the effect of different size variables (14).

Regularized discriminant analysis (RDA) was applied to an initial matrix containing the 42 objects and the 26 variables. The misclassification matrix calculated without and with -one out-cross validation shows how well the classes are separated (see **Table 4** and **Figure 4** for the graphical presentation). The rows correspond to the true classes (i.e., those suggested by RDA); the columns correspond to the assigned classes (provided by the user). Diagonal values are numbers of correct distillate classifications and shaded off diagonal values are numbers of misclassified distillings (in a perfect classification all the off diagonal numbers are zero) (30). The recognition ability for zivania, red zivania, Greek distillates, and eau de vie was highly satisfactory; all samples were correctly classified. One

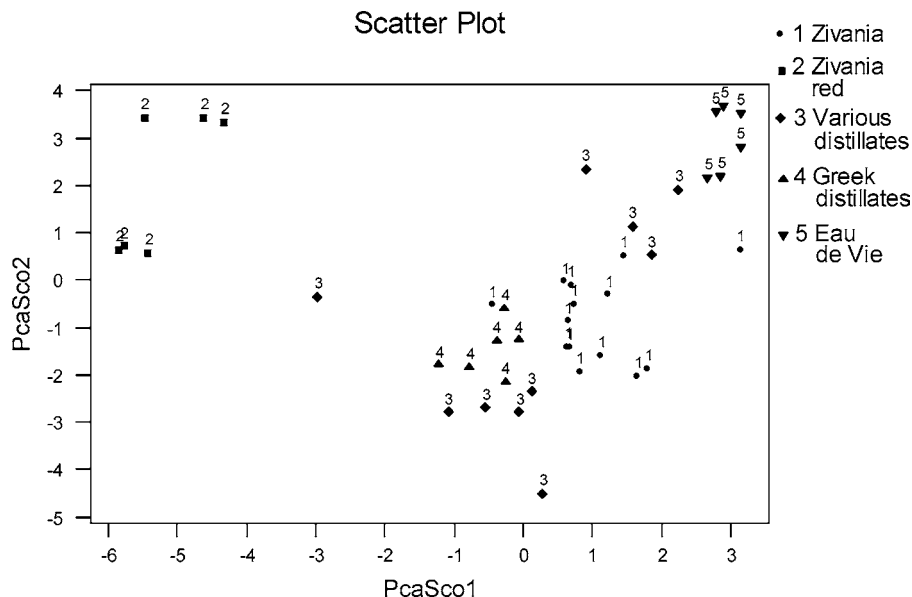


Figure 2. PCA Distribution of the distillates in the plane defined by the first two principal components.

Table 4. Classification with RDA (All Features)

	true class	total number	assigned classes					% correct recognition
			1	2	3	4	5	
zivania	1	14	14	0	0	0	0	100
zivania red	2	6	0	6	0	0	0	100
various distillates	3	10	0	0	9	1	0	90
Greek distillates	4	6	0	0	0	6	0	100
eau de vie	5	6	0	0	0	0	6	100
% error rate: 2.4								
cross-validation	true class	total number	1	2	3	4	5	% correct prediction
zivania	1	14	13	0	0	1	0	92.8
zivania red	2	6	0	6	0	0	0	100
various distillates	3	10	0	0	9	1	0	90
Greek distillates	4	6	1	0	1	4	0	66.6
eau de vie	5	6	1	0	0	0	5	83.3
% error rate: 11.9								

sample out of 10 various distillates was misclassified. The total recognition ability was 97.6%. The cross-validated error is a measure of the correct prediction, which was 92.8% for zivania and 100% for red zivania. The misclassified sample was the only one from the north part of Cyprus (serial number 13, S59 in Table 1). For Greek distillates, the percentage of correct classification was less successful (only 66.6%). The total prediction ability was satisfactorily good, 88.1% (37 of 42 samples were correctly classified).

The class centroid profile plot in Figure 4 explains the good performance for zivania and other samples and how they are separated on each coordinate. 3-Methyl butanol (serial number 14 in Table 2) was the most important analytical variable for this classification, and it was the feature that contained the most discriminatory information for the classification of the zivania samples.

Classification and regression trees (CART) allow the user to detect a logical grouping of variables and an incipient characterization of the beverages studied. Gini splitting criterion was studied to select a limited number of six variables that allowed an accurate classification of the samples. For model selection, the cross-validated  $F_k$  was used with leave-one-out method. The

selection table lists six potential trees and the optimal is the one with the smallest cross-validated risk, with an  $F_k$  value between 0.0 and 0.02, risk = 0.02, and cross-validated risk = 26.2%. The structure of the classification tree is seen graphically as a dendrogram in Figure 5, and it contains seven nonterminal nodes first and then eight terminal nodes. The variables that were well suited to detect group structures for the alcoholic beverages were the alcoholic grade, the content of furfural, 2-methyl popanol, 2-methyl butanol, and methanol, and the chemical shift of the  $-\text{CH}_3$  group. The chemical shift of  $-\text{CH}_3$  and the content of 2-methyl-butanol were the discriminator variables for zivania.

The recognition ability for zivania, red zivania, various distillates, and eau de vie was highly satisfactory; all samples were correctly classified. One sample out of six Greek distillates was misclassified. The total recognition ability was 97.6%, and it is consistent with the results of RDA. The cross-validated error (the correct prediction) was 71.4% for zivania and 100% for red zivania. One of the misclassified samples was the one from northern Cyprus (serial number 13, S59 in Table 1) and the second one was the double-distilled (serial number 4, S19). For various distillates, the percentage of correct classification was less successful (70%) and for Greek distillates it was poor (only 33.3%). The prediction for the eau de vie samples was 100% (all samples were correctly classified). The total prediction ability was 73.8%.

In conclusion, the study reported here shows that the combination of chemical and chemometric analysis was able to differentiate the Cypriot traditional spirit zivania from other alcoholic drinks from various countries or cheaper spirits. The chemical and physical-chemical variables (classical determinations, trace elements, sugars, and many volatile and aroma components) were used to provide a scientific characterization of the drinks. The variables were well suited to detect and to single out good multivariate group structures for the samples of zivania. The reason that two samples of zivania were misclassified is well understood: the sample from the north part of Cyprus turned out on inquiry to be "raki", a distillate of Turkish origin (S59) which is produced using a different process to zivania, whereas the Kaminaria (S19) sample was double-distilled and thus prepared using a nonstandard method.

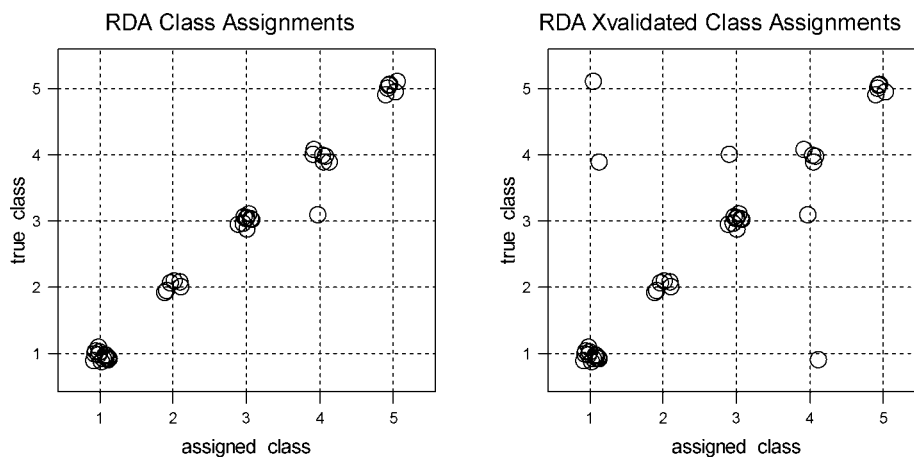


Figure 3. Class assignment plot.

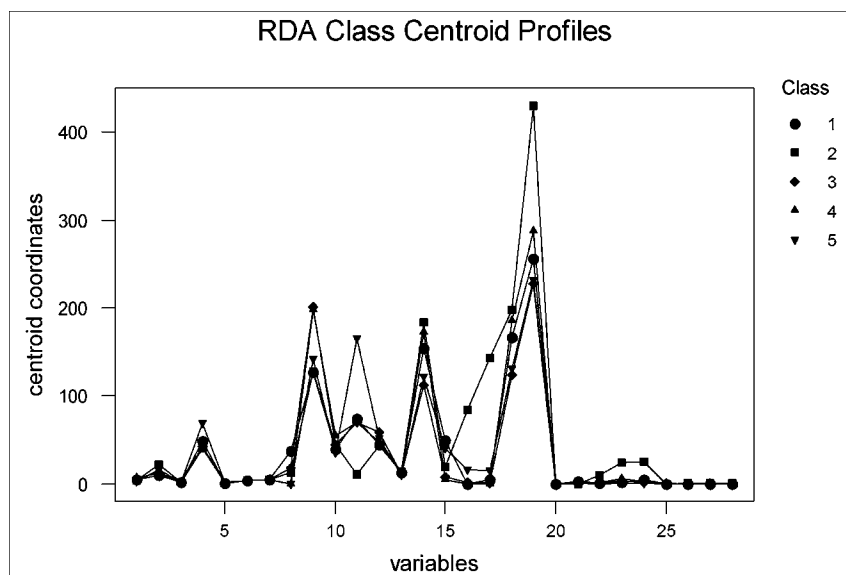


Figure 4. Class centroid profile plot.

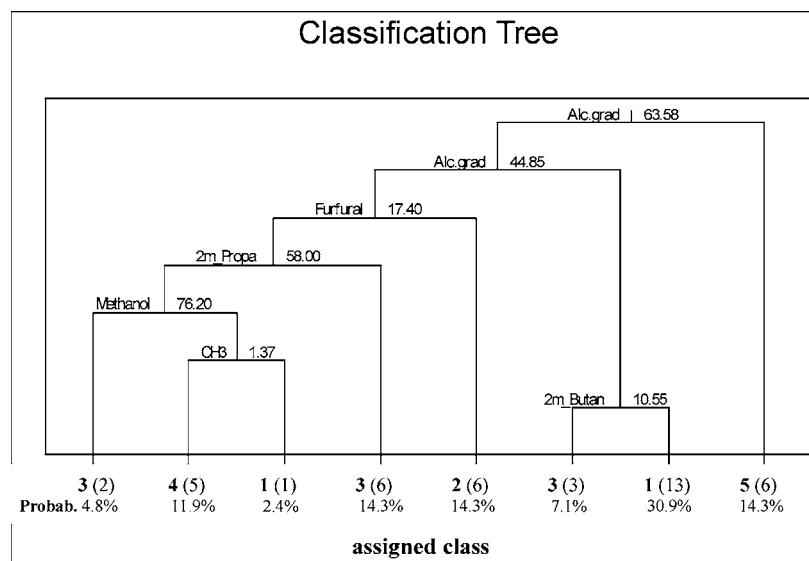


Figure 5. The CART tree: all splits occur on just six variables. For each terminal node, the following are shown: the assigned class associated, the number of objects in brackets, and the probability.

The low methanol content of zivania can be explained by the production method used. Specifically, fractional distillation is used, where the first fraction where most of the methanol would be concentrated is discarded. The higher Ca, Mg, and K

content in the so-called red zivania is due to addition of cinnamon and other extracts during production.

The classification was satisfactorily good, although most of the variables were in all the samples. Only 2- and 3-methyl-

butanol, 2-methyl-propanol, furfural, methanol, and the alcoholic grade and the chemical shift of  $-\text{CH}_3$  were sufficient to predict the origin of zivania.

With regard to the chemometric procedures adopted to analyze the data, best results were obtained using PCA, while CA showed poorer efficiency in highlighting the group structure. The total correct recognition ability was very high with both RDA and CART techniques, but the prediction for zivania was higher using RDA. These results augur well for the use of chemometric techniques in the characterization of spirit samples as well as wines.

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