JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Authenticity of the Traditional Cypriot Spirit "Zivania" on the Basis of Metal Content Using a Combination of Coupled Plasma Spectroscopy and Statistical Analysis

REBECCA KOKKINOFTA,[†] PANOS V. PETRAKIS,[§] THOMAS MAVROMOUSTAKOS,[#] AND CHARIS R. THEOCHARIS*,[†]

Porous Solids Group, Department of Chemistry, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus; National Agriculture Research Foundation−Institute of Mediteranean Forest Research−Athens, Laboratory of Entomology, Terma Alkanos, 11528 Ilissia, Athens, Greece; and National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, Vas. Constantinou 48, 11635 Athens, Greece

Sixty-eight alcoholic beverages ranging in alcoholic degree between 40 and 55 from different countries were analyzed for their 16 most abundant metal elements using inductively coupled plasma (ICP) spectroscopy. The results were analyzed statistically using two different types of analytical methods: canonical discriminant analysis and classification binary trees. The aim of this study was to investigate which of the metals analyzed constitute diagnostic parameters that establish authenticity of the traditional Cypriot spirit zivania. The two statistical methods revealed that Mg, Zn, and Cu are promising distinctive parameters capable of differentiating zivania from other spirits similar in alcoholic degree. It is believed that this differentiation in metals between the alcoholic beverages examined is related to the unique geological and climatic conditions existing on the island of Cyprus.

KEYWORDS: Zivania; alcoholic beverage; metals; trace elements; classification

INTRODUCTION

Zivania is a traditional alcoholic drink, which has been produced for centuries in Cyprus by distillation and has found many applications in the daily life of Cypriots. For example, it was widely used as a therapeutic agent for colds, toothache, and loss of consciousness. Zivania is clear in color and is produced as a byproduct of wine production, by the fermentation of grape marc, such as grape skins and stones, stems, etc., followed by a single distillation step. The grape marc is separated from the juice after fermentation is complete by pressing. The grape marc is then placed in copper stills for the distillation. The first distillate has the highest alcohol content. This is controlled in the final product by mixing fractions of different strengths. Similar products are produced in other countries, their appellation depending on the name they give to grape marc (i.e., in Cyprus it is called "zivania", in Greece "tsipoura", on the island of Crete "tsikoudia", and in Italy "grappa"). In addition, in Greece and Slavic countries it is also called "rakea" from the Arabic word "arak", which means spirit (alcohol).

Until recently, zivania was produced in small amounts in wine producers' homes for their own use, and industrially exclusively for export, as a raw material for other spirit-containing products. The sale of zivania within Cyprus was prohibited. However, a change in the Cyprus legislation in 1998 has legalized the commercial sale of zivania. This change in the law has meant that zivania became available to the private consumer both in Cyprus and internationally, without, however, any attempt by the producers to standardize and register the production process. Zivania is currently a very popular drink with healthy sales, increasing the importance of the drink to the Cypriot economy. One exception to this was the patenting and protecting of the manufacture of the so-called red zivania, a product of the Kykkos Holy Monastery Winery (*1*, *2*). The major characteristics of this product are its red color, taste, and flavor due to the addition of cinnamon, an additive absent from the traditional product.

Given the proven commercial value of the product, it is necessary to establish zivania as a traditional spirit with a unique chemical composition. The definition of geographical appellation of zivania will protect its trade within the European Union and abroad. As part of a wider study (*3*) we report here the use of metal content as measured by inductively coupled plasma (ICP) spectroscopy as a means of distinguishing zivania from spirits produced in other countries. The aim of this work is to distinguish the geographical production area of zivania from that of other similar products. Chemometric methods were employed in analyzing the data, because 16 metals were measured in 68 alcoholic beverages. The application of chemometric methods for characterizing or classifying products

* Author to whom correspondence should be addressed (fax 35 72 23 39 063; e-mail charis@ucy.ac.cy).
[†] University of Cyprus.
[§] National Agriculture Research Foundation.
[#] National Hellenic Research Foundation.

10.1021/jf034476o CCC: $25.00
Published on Web 09/16/2003

according to origin, quality, variety, type, or other features has already attracted considerable attention from researchers (*4*−*6*). Numerous applications of chemometrics in wine classification were reported (*7*−*10*), in which several classes of chemical variables are considered and different statistical methods were used.

In various publications, ^{1}H and ^{13}C NMR, as well as ^{2}H SNIF (site natural isotopic fractionation) NMR, are mentioned as the most applicable methodologies to characterize the geographic origin and authenticity of wine products and are considered to be powerful tools for the authentication of beverages (*11*−*13*). Therefore, NMR spectroscopic techniques may be useful in establishing the authenticity of zivania; these results will be presented elsewhere. SNIF NMR is often combined with isotope ratio mass spectroscopy (IRMS), which was shown to be a rapid and convenient technique for evaluating the authenticity of wine components (*13*).

In addition, other techniques have also been applied to the study of the authenticity and geographic origin of spirits with some promising results. Coupled plasma mass spectrometry using a double-focusing sector field for the simultaneous determination of a number of elements in alcoholic beverages was investigated and was found to be useful in authenticity evaluations (*14*). Chromatographic techniques such as HPLC in combination with UV, plasma spectroscopy, and principal component analysis along with sensory analysis was used to classify 33 Greek wines from various regions (*15*). Gas chromatography on a chiral stationary phase coupled with selected ion monitoring mass spectrometry was used to examine the presence of amino acid enantiomers (D-Aas) in different beers and vinegar (*16*). Solid phase microextraction (SPME) in combination with gas chromatography analysis is used in the analysis of wines (*17*). Flame atomic absorption spectrophotometry was applied to differentiate sweet wines between the Canary Islands Lanzarote and La Palma of Spain and between the Canary Islands and Malaga. Stepwise discriminant analysis showed that two variables, Mg and Na, were sufficient to discriminate between Lanzerote and La Palma wines, and this result is confirmed by cluster analysis using the Ward method and the Euclidean distance. It was possible to differentiate Canary Islands and Malaga sweet wines using only their content of Mg (*18*). The significance of Mg is demonstrated in a multivariate data analysis in the classification of must and wine from chemical measurements (*19*).

We have chosen to use ICP in this study because it can yield trace element concentrations. The trace element composition of the spirits analyzed may depend on the unique geologic, edaphic, and climatic conditions of the spirit production areas of Cyprus. Therefore, their specific composition may characterize the local spirits and especially the Cypriot alcoholic beverage zivania. This technique can serve as a complementary technique for other spectroscopic methods currently used in our laboratories as IR, Raman, and NMR.

MATERIALS AND METHODS

Samples of Distillings. The study concerns 68 alcoholic beverages from Cyprus (zivania) and other countries, such as tsipouro, tsikoudia, ouzo, grappa, tsuica, rakea, vodka, gin, and eau de vie. The various samples are presented in **Table 1**. The choice of these samples was dictated by the fact that they are seen as competitors to zivania, whereas the European Union classifies them in the relevant legislation in the category of spirits best suited to zivania. Furthermore, eau de vie has been chosen because it is often used as a cheap substitute for zivania. In the context of the present work, eau de vie samples used originated from northern European countries. In addition to European samples, several others were also included of extra-European origin, for the purposes of comparison. The origin of various samples is shown in **Table 1**. All samples are fruit-based (for example, the Romanian beverage tsuica is made mainly from peaches), with the exception of that of Hong Kong origin, which was rice-based.

Preparation of Samples. Fifty milliliters of each sample was subjected to freeze-drying using a Christ, Alpha 1-2 freeze-drier. The condenser temperature was 233 K and the final pressure in the drying chamber 3 mPa. The freeze-drying required 24 h, and the residue was dissolved in 5 mL of HNO_3 (BDH-Spectrosol 10% v/v), filtered through a folded filter paper, and stored in a polypropylene bottle for ICP analysis.

Analytical Determinations. Samples were analyzed using a Shimadzu ICP-7500 spectrometer to determine the following metals: iron (Fe), copper (Cu), aluminum (Al), manganese (Mn), magnesium (Mg), zinc (Zn), antimony (Sb), arsenic (As), selenium (Se), chromium (Cr), cadmium (Cd), lead (Pb), calcium (Ca), sodium (Na), potassium (K), and barium (Ba).

ICP spectroscopy has proved to be a suitable and convenient technique for the determination of metal content in the samples. The sample solution was introduced into the core of an inductively coupled argon plasma at a temperature of ∼8270 K. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. The light was collected by the spectrometer and resolved into a spectrum of its constituent wavelengths, amplified to yield an intensity measurement, which converted to an elemental concentration by comparison with three calibration standards for each element. The advantage of using ICP spectroscopy was the determination and simultaneous detection of 16 metals in a single sample analysis. All determinations were made twice, and the mean was retained.

Preparation of Standard Solutions. Standard solutions were prepared from monoelemental stock solutions (BDH-Spectrosol). Each solution contained HNO_3 (BDH-Spectrosol 10% v/v), and the concentration ranges were 0−1 mg/L, the range within which sample concentrations were expected to be and where they were indeed found to be. An exception to this was calcium, for which a second set of standards in the range of 1−30 mg/L was additionally used. The concentrations of the 16 metals in the samples analyzed are shown in a table submitted as Supporting Information.

The typical background equivalent concentrations and detection limits were calculated with the measured solutions and the blank solution. The amount of the metals was calculated from the standard calibration curve. To ensure consistency of data, repeatability of measurements was checked, as usual, by reanalyzing some of the earlier samples, stored at 3−4 °C, before analysis of the new samples was begun.

Biostatistical Methods. Two kinds of groupings were used to analyze the behavior of metal elements within each distilling. The first kind comprises six groups according to the type of sample and the biological origin. In this grouping all zivanias belong to three groups referred to as Kykkotiki (i.e., produced by the Kykko Winery), red (also produced by the Kykko Winery but also containing cinnamon), or other. Eau de vie comprises its own group, whereas all of the Greek distillings belong to one group. The other distillings from various parts of the world such as vodkas and tequilas belong to a separate group (see **Table 1**). The other grouping is more detailed in some respects but agglomerates the zivanias into one group except the red ones, which comprise another group. The Greek distillings fall into three groups according to the geographic area, specifically tsipouro (mainland Greece), ouzo (aniseed flavored), and tsikoudia (Crete). Eau de vie has its own group, and distillings from other parts of the world form a separate group. In this way, 25 groups were formed.

The biostatistical analytical methods employed were of two different types, each providing a specific insight into the data. The first type is the canonical discriminant analysis (CDA) (*20*), where the a priori groups are delimited by linear functions in a space of dimensionality, which is determined by Wilk's λ criterion (*21*); the dimensions of the discriminant space are uncorrelated, and the amount of variation depicted in the CDA configuration was determined by the estimated eigenvalues, which are accepted in addition to Wilk's λ criterion if they account for >90% of the original variation. This is a prerequisite posed by us to ensure that the resulting separation algorithm possesses

Table 1. CDA and CBT Groupings of the Samples of Distillings[a]

serial no.	sample label	CDA grouping 1	serial no. of grouping 1	CDA grouping 2	serial no. of grouping 2	CBT estimate	origin
1	S1	zivania kykkotiki	1	zivania	1	5	Kykkotiki
2	S2	zivania kykkotiki	1	zivania	1	*1*	Kykkotiki
3	S10	zivania kykkotiki	1	zivania	1	5	Kykkotiki
4	S61	zivania kykkotiki	1	zivania	1	5	Kykkotiki
5	S62	zivania kykkotiki	1	zivania	1	5	Kykkotiki
6	S92	zivania kykkotiki	1	zivania	1	5	Kykkotiki
7	S8	zivania other	2	zivania	1	6	Pachna
8	S19	zivania other	2	zivania	1	6	Kaminaria
9	S20	zivania other	2	zivania	1	6	Kellaki
10	S21	zivania other	2	zivania	1	6	Chrysoroyiatissa
11	S22	zivania other	2	zivania	1	6	Omodos
12	S31	zivania other	2	zivania	1	6	Ag. Varvara
13	S33	zivania other	2	zivania	1	6	Lofou
14	S35	zivania other	2	zivania	1	6	Archimandrita
15	S38	zivania other	2	zivania	1	6	Panagia
16	S42	zivania other	2	zivania	1	6	V. Kilaniou
17	S43	zivania other	2	zivania	1	6	Pelendri
18	S44	zivania other	2	zivania	1	5	Platanis
19	S45	zivania other	2	zivania	1	6	Loel
20	S46	zivania other	2	zivania	1	*4*	Sodap
21	S50	zivania other	2	zivania	1	5	Crusaders
22	S51	zivania other	2	zivania	1	6	Hadjipavlou
23	S59	zivania other	2	zivania	1	*4*	YeniRaki
24	S66	zivania other	2	zivania	1	6	Keo
25	S86	zivania other	2	zivania	1	6	Lania
26	S84	zivania other	2	zivania	1	6	Kel. Soultani
27	S3	zivania red	3	zivania red	2	2	red
28	S4	zivania red	3	zivania red	2	2	red
29	S93	zivania red	3	zivania red	2	2	red
30	S94	zivania red	3	zivania red	2	2	red
31	S95	zivania red	3	zivania red	2	2	red
32	S96	zivania red	3	zivania red	2	2	red
33	S5	various distillings	4	vodka abs	3	1	Russia
34	S6	various distillings	4	grappa	4	*4*	Italy
35	S13	various distillings	4	YUK	5	1	Hong Kong
36	S12	various distillings	4	tsouika	6	1	Romania
37	S15	various distillings	4	arak	7	1	Jordan
38	S23	various distillings	4	vodka	8	1	Russia
39	S24	various distillings	4	vodka	8	1	Russia
40	S25	various distillings	4	gin	9	1	Cyprus
41	S26	various distillings	4	rakea	10	1	Bulgaria
42	S30	various distillings	4	germana	11	1	Brazil
43	S32	various distillings	4	tequila	12	*6*	Mexico
44	S53	various distillings	4	chancella	13	1	Portugal
45	S54	various distillings	4	lagle	14	*4*	Austria
46	S55	various distillings	4	barack	15	1	Hungary
47	S56	various distillings	4	barberino	16	1	Italy
48	S57	various distillings	4	segrel	17	1	Spain
49	S58	various distillings	4	becherovka	18	*4*	Czech
50	S67	various distillings	4	vodka	8	1	Sweden
51	S72	various distillings	4	sambuca	19	1	Italy
52	S90	various distillings	4	abouraad	20	1	Libanon
53	S7a	Greek distillings	5	tsipouro	21	4	Greece
54	S7b	Greek distillings	5	tsipouro	21	4	Greece
55	S9a	Greek distillings	5	tsikoudia	22	4	Greece (Crete)
56	S9b	Greek distillings	5	tsikoudia	22	4	Greece (Crete)
57	S16	Greek distillings	5	tsipouro	21	4	Greece
58	S17a	Greek distillings	5	ouzo	23	4	Greece
59	S17b	Greek distillings	5	ouzo	23	4	Greece
60	S25	Greek distillings	5	tsipouro	21	4	Greece
61	S52	Greek distillings	5	tsipouro	21	4	Greece
62	S97	Greek distillings	5	tsipouro	21	4	Greece
63	S11a	eau de vie	6	6 years	24	3	vat 4
64	S18a	eau de vie	6	1 year	25	3	vat 17
65	S11b	eau de vie	6	6 years	24	3	vat 4a
66	S18b	eau de vie	6	1 year	25	3	vat 17a
67	S11c	eau de vie	6	1 year	25	3	vat 4b
68	S18c	eau de vie	6	6 years	24	3	vat 17b

[a] *Italic* font demarcates a misclassification in CBT. All samples have grapes or other fruit as raw material except 35 (rice).

all of the important data estimated in the laboratory. The application of the CDA was the forward stepwise one—interactive mode with metals entered if they were found to minimize Wilk's λ or the Mahalanobis squared distance D^2—whatever comes first—while having a partial F value of at least 4.00. Where metals had a partial F value lower than 3.84, they were removed from the CDA descriptive variable set.

The second type belongs to the family of methods referred to as classification binary trees (CBT) (*22*). The presentation tree produced is called a mobile according to Wilkinson (*23*) and shows a density diagram of the distribution of distilling samples in a set of groups (here six groups). Unlike CDA, CBTs produce a set of simple rules for predicting the group affiliation of a specific sample and classify all of the samples in meaningful hierarchical groups according to their metal content. A loss function is used to monitor the reduction of the error in the classification. Here the "towing" loss function coefficient of Breiman et al. (*24*) is used, because of the better reduction in error it produced. CBTs contain also a geometric interpretation, very close to that of CDA, with the desired property of the separator surfaces being perpendicular to the axes of the dimensional space produced, unlike CDA, which produces compartments with hyperplane separators generally at an angle with the axes. Profoundly, this is the reason for the production of simpler classification solutions by CBTs and similarly simpler predicting algorithms of new samples.

Because no linear curves are used to delineate groups, the underlying model of a CBT can accommodate some nonlinearity and discontinuity in the data. CBTs, unlike CDA, are able to predict the metals responsible for the separation of two specific groups, whereas CDA indicates the respective metals for the separation of all groups simultaneously. The prediction algorithm can be readily transferred to a computer program for implementation. We have implemented it into a visual basic for applications (VBA) algorithm (not shown here) to find easily the groups of our samples, but the same can be done in every set of samples whatever their size or complexity. A new run of the CBT on the augmented data set can train "better" the set of rules and results in another prediction algorithm and program. In this way the CBT possesses an intrinsic nature of artificial intelligence decision support system.

Although several methods pertain to the matter of detection of responsible metals, we selected CDA and CBTs because of several features they possess. They work on samples belonging to a priori groups, and they are actually classification techniques (a) involving tests for the determination of the dimensionality (CDA), (b) employing selection of variables according to their significance in describing the separation of not only the groups but also the arrangement of samples within each group (CDA and CBT), and (c) constructing an algorithm (CBT) or a set of linear functions (CDA) of the original variables capable in predicting the affiliation of samples. Unlike principal component analysis (PCA) and principal coordinate analysis (PCO), which ordinate samples according to axes of maximum variation alone, these two techniques take into account also the grouping of samples, and they do not merely reveal others inherent to the data set grouping but perform tests—in a second run—about their validity (CDA) or depict the group content in terms of simple algebraic expressions of the concentration of various metals. One thing that is not evident to the researchers is that CDA (*20*) and CBT (*22*) have the ability to cope with the existence of many metals and select the most informative ones, whereas PCA and PCO usually perform badly when too many descriptive variables exist (*21*).

RESULTS AND DISCUSSION

Table 1 shows the CDA and CBT groupings for the samples of distillings used in the study. The user a priori assigned the groupings of samples used in CDA. However, in the case of CBT, the algorithm, using the methods described in the text below, estimated the grouping assignments. The CDA treatment using the first grouping of six groups as described above was very successful. The first three eigenvalues accounted for 100% of the variation, and all axes were significant (Wilk's $\lambda = 0.813$, $\chi^2 = 12.969$, df = 3, $P < 10^{-4}$). The results of the treatment are shown in **Figure 1**, where only the two axes are shown, whereas the analysis was based on three axes. The 68 samples analyzed were assigned to one of six groups and are depicted in the figure in a CDA bidimensional space (97.84%). The first discriminant axis accounts for 94.33% and the second axis for 3.52% of the original variation. The six groups are well separated in the cloud of points except the fourth group, consisting of various distillings, which is scattered along the second axis remaining unaffected by both discriminant axes, which successfully separate the other groups. Examination of **Figure 1** shows that the groups (i) of other zivanias and (ii) of the Greek distillings are separated on the first axis (CDF I). CDF I has a high coefficient of Mg (−1.160) and a high positive one of Zn (0.801). CDF II is negatively correlated with Zn (coeff = −5.480), whereas CDF III has a high coefficient of Zn (0.890).

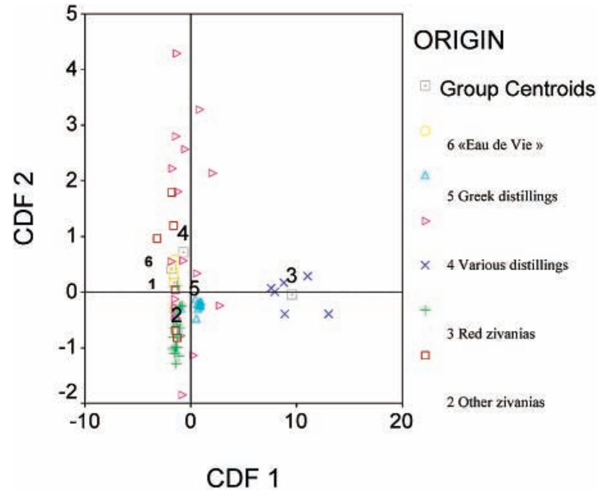

Figure 1. The 68 samples allocated in six groups, depicted in a CDA bidimensional space (97.84%).

Table 2. Metals Entered in the CDA That Achieved the High Percentages Presented by the Classification Table

	F value of the entered metal	Wilk's λ	approx *F* value	*P* <
Mg	107.195	0.104	107.195	0.001
Zn	5.113	0.073	32.935	0.001
Cu	4.491	0.053	20.970	0.001

The metals needed to produce a satisfactory CDA configuration are therefore only three, namely, Mg, Zn, and Cu. **Table 2** shows the parameters obtained for these metals, as they were entered at various steps of the CDA analysis. Notably, there is a marked decrease in the value of Wilk's λ criterion as more metals are added. Furthermore, the high statistical significance of this should be noted. All three of these metals have a significant biological role, but Mg is the most important of the three in view of its presence in the chlorophyll molecule.

The classification results obtained by the CDA method are shown in **Table 3**. The most important figure in this table is the amount of distillings correctly classified as zivania (93.75%). The predictability of the table is satisfactorily good, because 82.35% or 56 of 68 samples were correctly classified. The group of "various distillings" was scattered to a large degree, with a classification success of only 65%. This was not unexpected, given the wide provenance of these samples. It should be noted that one sample has been misidentified as zivania, whereas it was classified as "other". This sample was no. 25 in **Table 1** and was of Hong Kong origin and is rice-based. The misidentification as zivania is not particularly significant, given that other analytical parameters, such as FTIR or NMR profiles or higher alcohol content, correctly distinguish the sample from zivania.

Turning to the second groupings regime consisting of 25 groups of distillings, the overall picture changes to some extent.

Table 3. Classification Results for the CDA[a]

	actual origin	predicted sample origin 1	2	3	4	5	6	total
zivania kykkotiki	1	*4*	**2**	0	0	0	0	6
zivania various	2	**1**	*17*	0	0	**2**	0	20
zivania red	3	0	0	*6*	0	0	0	6
various distillings	4	**1**	0	0	*13*	**6**	0	20
Greek distillings	5	0	0	0	0	*10*	0	10
eau de vie	6	0	0	0	0	0	*6*	6
total		7	21	9	17	23	12	68
		Percentages						
zivania kykkotiki	1	*66.67*	**33.33**	0	0	0	0	
zivania various	2	**5**	*85*	0	0	10	0	
zivania red	3	0	0	*100*	0	0	0	
various distillings	4	**5**	0	0	*65*	**30**	0	
Greek distillings	5	0	0	0	0	*100*	0	
eau de vie	6	0	0	0	0	0	*100*	
% of original grouped samples correctly classified								82.35
no. of sampes correctly classified								56
% of sampes correctly classified as zivania								93.75

[a] Italic values are numbers of correct distilling classifications, and bold off-diagonal values are numbers of misclassified distillings.

Table 4. Importance of Metals as Discriminators in the 25 Groups Regime

metal	*F* value	Wilk's λ	approx *F* value	$P < 10^{-4}$
Ba	298.733	0.006	298.7333	*
Fe	49.759	0.0002	121.2127	*
Al	50.408	0	90.5875	*
Ca	36.144	0	72.6595	*
Mg	6.527	0	46.7838	*
Cd	4.808	0	33.3227	*

The variation of the original data accommodated here by the first three discriminant axes is 91.30%, whereas Wilk's λ for the overall analysis is highly significant (Wilk's $\lambda = 0.0003$, $F_{approx} = 33.465$, df1 = 144, df2 = 229, $P < 10^{-4}$). Six metals, in general different from the ones of the previous CDA, are important here as can be seen in **Table 4**. Only Mg is common to both CDA analyses and here with very low *F* values. Nevertheless, it is important to note that all six metals reduce Wilk's λ criterion, as shown in the last column of probabilities (**Table 4**). The configuration thus obtained is not meaningful, as can be seen in **Figure 2**, because there are six outliers causing the rest of the points to swarm together. The six outliers were Tsuica (no. 6 in **Table 1**), YUK (no. 7), Chancella (no. 42), Barack (no. 44), Becherovka (no. 47), and the Swedish vodka (no. 54). All outliers were classified as "various distillings" and originate from various parts of the world.

However, the classification ability of the analysis is very high (~93%) as shown in **Table 5**. In **Table 5**, the columns are the groups predicted by the model and the rows correspond to actual groups. A perfect classification would demand all values to be on the main diagonal. The overall classification is very good (almost 93%). It must be noted that all zivanias here are grouped together. For zivanias the classification is very accurate except for the two "zivania other", which were classified as "vodka absolute". Both samples were homemade in small quantities with use of a nonstandard manufacturing method, namely, double distillation. The red zivanias were perfectly classified. The jack-knifing of the samples observed in **Table 5** has resulted in very low predictability for samples other than zivania, reflecting the diversity of the distillings. Furthermore, many groups in **Table 5** consisted of only one sample, meaning that

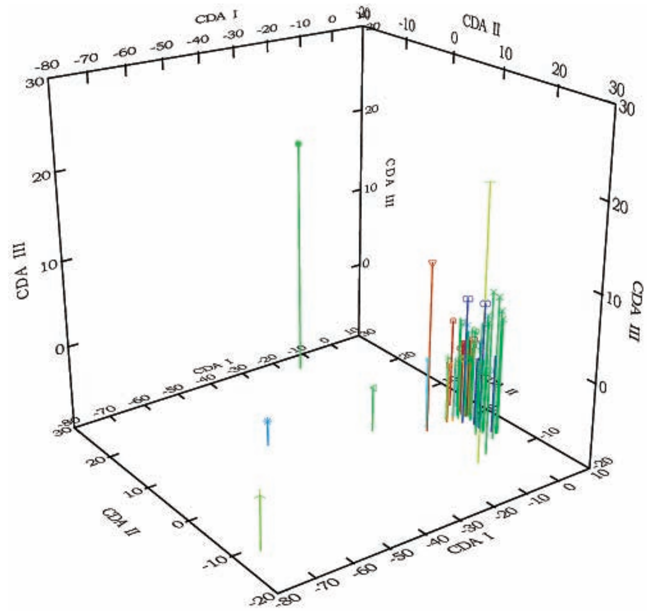

Figure 2. Discriminant configuration of the 25 distilling samples. The cases Romanian tsouika (serial no. 36 in **Table 1**), Hong Kong YUK (no. 35), Portugese chancella (no. 44), Hungarian barack (no. 46), Czech becherovka (no. 49), and the Swedish vodka (no. 50) are outliers and not predicted by the CDA. All outliers come from "various distillings" from various parts of the world.

the groups were not very sharply defined, a fact that was evident in the set of included metals and the classification table of CDA. However, the significant factor for the present work was that all zivanias were grouped together.

The CBT analysis results are shown in **Figure 3** and were very illuminating. The figure shows the binary classification tree as a "mobile" of the 68 samples incorporating from the beginning all metals. The mobile shown in **Figure 3** achieves a reduction in error of 76.19%, which is considered to be very high (*23*), although no statistical test is available for its significance. The classification results at 89.71% can also be considered as very accurate (see also **Table 1**). Among all possible trees this has the highest improvement in classification and predicts the grouping with an accuracy of 76.2%. Mg and Cu, which offer the best discrimination in the CDA, are included here together with K, Al, Pb, and Ca. Zn is excluded. The mobile has eight terminal nodes indicating the number of simple rules involved; this number appears to be sufficient to accommodate the six recognizable groups. The number of diagnostic metals involved is also moderate. Six metals (**Figure 3**) are judged to be important separators, among them Mg, Cu, and the highly variable K and Ca. There are no single figures that estimate the importance of each metal except the prediction shown in **Figure 3**. The combinations of metals that form prediction rules are the differences Mg − Cu and K − Ca and the sums K + Cu and Mg + Al. The highly variable metals K and Ca are thus included in the separation of samples. The diagram in **Figure 3** for each resulting and a priori unknown group can produce a set of simple rules. Importantly, groups can be predicted in more than one way, such as group 1, involving different metals in each prediction indicating either the heterogeneity of the group or the multiple predicting role of widely distributed metals such as Mg and K in plants and Ca, Al, and Cu in soils and plants (*24*). An important feature of this mobile is its ability to form core terminal groups of samples, which correspond to one origin, although not inclusively, and contain the mainstream of distillings and hazy groups composed of distillings of various origins.

Table 5. Classification Table of the CDA Involving 25 Sample Groups

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	no. of samples	% correct
1	*24*		**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	92
2	0	*6*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	100
3	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
4	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
5	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
6	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
7	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
8	0	0	**1**	0	0	0	0	*2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	67
9	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
10	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
11	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
12	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
13	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	0	1	100
14	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	0	1	100
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	0	1	100
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	0	1	100
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	0	1	100
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	0	1	100
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	0	1	100
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*	0	0	0	0	0	1	100
21	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*4*	0	0	**1**	0	6	67
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*2*	0	0	0	2	100
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*2*	0	0	2	100
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*3*	0	3	100
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*3*	3	100
	24	6	5	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	4	2	2	4	3	68	93

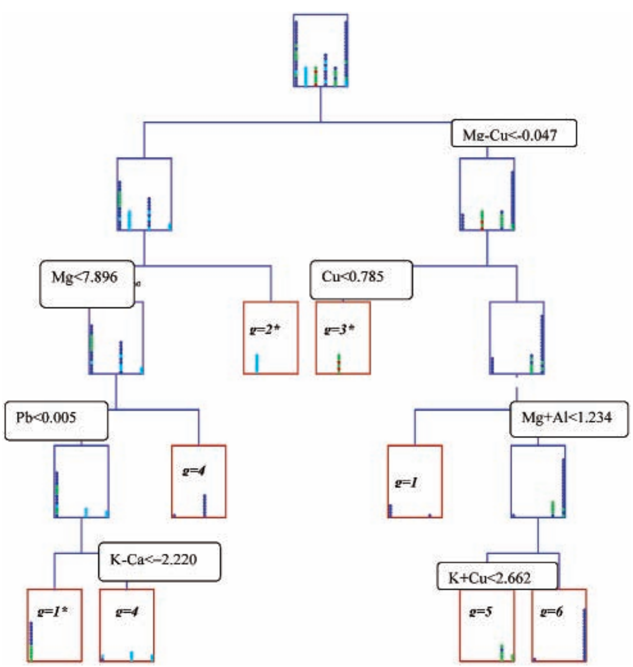

Figure 3. CBT binary classification tree shown as a "mobile" of the 68 samples incorporating from the beginning all metals. Underscoring denotes main classification origins, and the asterisks (∗) denote perfect terminal origin groups with no distillings of other origin, although not having all samples of its own origin.

All of these groups can be reached according to terms of metals written on the mobile trees (**Figure 3**).

CONCLUSION

The content of metal elements of alcoholic beverages was used to differentiate between zivania and samples from other spirits. CDA and CBT analyses revealed the occurrence of grouping between the analyzed samples, according to their metal content, which corresponded well to their geographic origin. The classification was very accurate, and only six metals were sufficient to predict the origin of each distilling. The predictive ability of the database will be augmented when more data are added in an artificial intelligence decision support system. Other methodologies are currently applied that will serve as complementary to ICP. The presence of copper and its significance in the discrimination of zivania from other samples can be explained by the use of copper distillation vessels. Furthermore, the combined Mg, Zn, and Cu content of soils in Cyprus is deemed to be characteristic (*25*−*27*).

ACKNOWLEDGMENT

We are grateful to the Reverend Bishop of Kykkos Nikiforos for his support and to Dr. C. Fournaris and M. Ktistis for useful discussions. We thank Dr. A. Charalambides and M. Lyssandrou (Department of Geological Survey) for the helpful laboratory assistance.

Supporting Information Available: Concentrations of the 16 metals in the samples analyzed. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Abbot Nikiforos of Holy Monastery of Kykkos. Alcoholic drink made from extract of grapes with flavour and taste of cinnamon. Eur. Patent Appl. 0-708-822, 1995.

(2) Abbot Nikiforos of Holy Monastery of Kykkos. Alcoholic drink made from extract of grapes with flavour and taste of cinnamon. WO 95 29227, 1995.

(3) Kokkinofta, R. Ph.D. Thesis, University of Cyprus, 2003.

(4) Ashurst, P. R.; Dennis, M. J. *Food Authentication*; Blackie Academic and Professional: Glasgow, Scotland, 1996.

(5) Defernez, M. Methods Based on Principal Component Analysis of Mid-Infrared Spectra: A New Approach for the Classification and Authentication of Fruit Products. Ph.D. thesis, University of East Anglia, Norwich, U.K., 1996.

(6) Kemsley, E. K. *Discriminant Analysis and Glass Modelling of Spectroscopic Data*; Wiley: New York, 1998.

(7) Latorre, M. J.; Jares, C. G.; Medina, B.; Herrero, C. Pattern Recognition Analysis Applied to Classification of Wines from Galicia with Certified Brand of Origin. *J. Agric. Food Chem.* **1994**, *42*, 1451−1455.

(8) Versini, G.; Orriols, I.; Dalla Serra, A. Aroma components of Galician Albarino, Loureira and Godello wines. *Vitis* **1994**, *33*, 165−170.

(9) Vogels, J. T. W. E.; Tas, A. C.; van den Berg, F.; van der Greef, J. A new method for classification of wines based on proton and carbon-13 NMR spectroscopy in combination with pattern recognition techniques. *Lab. Info. Manage.* **1993**, *21*, 249−258.

(10) Moret, I.; Scarponi, G.; Cescon, P. Chemometric Characterization of Five Venetian White Wines. *J. Agric. Food Chem.* **1994**, *42*, 1143−1153.

(11) Kosir, I.; Kocjancic, M.; Ogrinc, N.; Kidric, J. Use of SNIF-NMR and IRMS in combination with chemometric methods for the determination of capitalization and geographical origin of wines. *Anal. Chim. Acta* **2001**, *429*, 195−206.

(12) Martin, G. J. Natural abundance deuterium NMR as a tool for studying mechanistic and environmental effects. *Semin. Food Anal.* **1998**, *3*, 251−265.

(13) Miralles, G. J. E.; Salazar, D. M.; Solana, I. Regional origin assignment of red wines from Valencia by hydrogen-2 NMR and carbon-13 IRMS stable isotopic analysis of fermentative ethanol. *J. Agric. Food Chem.* **1999**, *47*, 2645−2652.

(14) Rodushkin, I.; Odman, F.; Appelblad, P. K. Multielement determination and lead isotope ratio measurement in alcoholic beverages by high-resolution inductively coupled plasma mass spectrometry. *J. Food Compos. Anal.* **1999**, *12* (*4*), 243−257.

(15) Kallithraka, S.; Arvanitoyiannis, I. S.; Kefalas, P.; Zajouli, E. I.; Soufleros, E. Instrumental and sensory analysis of Greek wines. Implementation of principal component analysis (PCA) for classification according to geographic origin. *Food Chem.* **2001**, *79* (*4*), 501−514.

(16) Panossian, A.; Mamikonyan, G.; Torrosyan, M.; Gabrielyan, E.; Mkhitaryan, S. Analysis of aromatic aldehydes in brandy and wine by high performance capillary electrophoresis. *Anal. Chem.* **2001**, *73*, 4379−4383.

(17) Boyce, M. C.; Spickett, E. E. Solid-phase microextraction in food analysis with particular reference to wine. *Food Aust.* **2002**, *54* (*8*), 350−356.

(18) Frias, S.; Trujillo, J. P. P.; Pena, E. M.; Conde, J. E. Classification and differentiation of bottled sweet wines of Canary Islands (Spain) by their metallic content. *Eur. Food Res. Technol.* **2001**, *213*, 145−149.

(19) Gonzalez, G.; Pena-Mendez, E. M. Multivariate data analysis in classification of must and wine from chemical measurements. *Eur. Food Res. Technol.* **2000**, *212*, 100−107.

(20) Johnson, R. A.; Wichern, D. W. *Applied Multivariate Statistical Analysis* Prentice Hall: Englewood Cliffs, NJ, 1998.

(21) Morrison, D. F. *Multivariate Statistical Methods*; McGraw-Hill: Singapore, 1984.

(22) Breiman, L.; Friedman, J.; Olshen, R.; Stone, C. *Classification Regression Trees*; Kluwer Academic Publishers: New York, 1984.

(23) Wilkinson, L. *Mobiles*; Northwestern University, Department of Statistics: Evanston, IL, 1985.

(24) Bidwell, R. G. S. *Plant Physiology*; Collier-Macmillan: London, U.K., 1979.

(25) European Soil Bureau. *Geo-referenced Soil Database for Europe*, version 1.0; Ispra, Italy, 1998.

(26) Hadjiparaskevas C. *Options Méditerranéennes, Ser. B* **2003**, *No. 34*, 101−114

(27) Hall, J. M.; Walls, C. C.; Yang, J.-S. Constructional features of the Troodos ophiolite and implications for the distribution of ore bodies and the generation of ocean crust: Reply. *Can. J. Earth Sci.* **1990**, *27*, 1139−1141.

Received for review May 8, 2003. Revised manuscript received July 31, 2003. Accepted August 12, 2003. This research is financially supported by the Cyprus Foundation for the Promotion of Research and by the Kykko Monastery Winery.

JF034476O