

## Multielement Analysis of Argentinean Lemon Juices by Instrumental Neutronic Activation Analysis and Their Classification According to Geographical Origin

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Multielement analysis of lemon juices from different Argentinean regions was carried out by instrumental neutronic activation analysis (INAA) with the aim at developing a reliable method in the traceability of the origin of lemon juices. This work presents a characterization of 44 lemon juice samples selected from three different geographical origins in the Northwest region of Argentina. Multivariate chemometric techniques such principal component analysis and lineal discriminant analysis (LDA) were used with the aim of classifying the juices and identifying the most significant parameters. Eleven elements were determined (Br, As, Na, Rb, La, Cr, Sc, Fe, Co, Zn, and Sb). The analytical method was validated by analyzing the standard reference material IAEA V-10 (hay powder); the results are within  $\pm 10\%$  of the reported values for the majority of the elements. Biplots of LDA scores for the INAA data illustrate clear separation between each sample.

**KEYWORDS:** INAA; lemon juices; principal components analysis; linear discriminant analysis; mineral profile

### INTRODUCTION

Inorganic element levels in lemon juice have been used to monitor quality, authenticity, and country of origin. Mineral profiles in juices could vary according to the place of origin in terms of element contents, based on the differences of chemical compositions between lemons grown in different production areas (1). In addition, it has been reported that different species of citrus have individual profiles (2, 3), making these elements potentially useful for controlling citrus products for truth-in-labeling (5). Several works have been carried out to study the characterization of citrus fruits (including lemons), based on organic constituents (5, 6), mineral contents or compositions (7–10), isotope ratios, or their combination (11–14).

Multivariate statistical methods appear to be the most competent tools for characterizing and classifying multielemental

data, related to classifying the different types of links as geographical origins or botanical varieties. Moreover, some of these methods can let one know which variables govern the welding process and which are responsible for the different behaviors of the different types during this process. Linear discriminant analysis (LDA) is used to find theoretical values resulting in the best possible discrimination between a priori established groups. This is a parametric pattern recognition method, which establishes simple linear functions as a model for discrimination (15).

In this work, mineral element concentrations were determined by instrumental neutron activation analysis (INAA); this technique could be considered a powerful tool not only because of its well-known high sensitivity and accuracy but also because it allows multielement analysis for each single sampling of a complex matrix such as lemon juice. The quality control and method validation were done by analysis of standard reference materials (IAEA V-10, hay powder).

Finally, the goal of the present work is the study of the mineral contents of 11 elements (As, Br, Co, Cr, Fe, La, Na, Rb, Sb, Sc, and Zn) on single-strength lemon juices from four different botanical varieties and three major producing regions of Argentina (Tucumán, Salta, and Jujuy provinces). This region is the major lemon-producing area in this country (94% of total

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production), and no published parameters of characterization allowing one to ensure the genuineness of the production of juices exist at present (16).

## MATERIALS AND METHODS

**Sampling.** Lemon fruits [*Citrus limon* (L.) Burm] were harvested from the Northwest region of Argentina, located between parallels  $-22^\circ$  and  $-29^\circ$  latitude and  $-68^\circ$  and  $-65^\circ$  west longitude. This is at present the major lemon-producing area in Argentina with 1277000 tons of annual lemon production (94% of total production of this country).

This region was divided into three principal zones, Northern, Middle, and Southern. The Northern zone included the producing region of the Jujuy province. The Middle zone was formed by the southern part of Salta province and the Tafi del Valle department corresponding to the northern limit of the Tucuman province. Finally, the Southern zone was formed by the central portion of the Tucuman province. Four different botanical varieties of lemons [*C. limon* (L.) Burm] were chosen as follows: cv. Génova Nuclear EEAT (GN-EEAT), cv. Lisboa Limoneira 8A (LL-8A), cv. Lisboa Frost Nuclear (LFN), and cv. Eureka Frost Nuclear (EFN). Rootstocks were Citrus Volkameriano. All fruits were harvested during the 2004–2005 seasons between June and October. The juices were placed in sealed amber bottles (250 mL) and kept frozen at  $-14^\circ\text{C}$  until analyzed; the storage time did not exceed 1 month.

Before analysis, about 150 g of juice was placed in a previously weighed porcelain crucible and heated (first in a stove and then in a furnace) from 120 to  $550^\circ\text{C}$ . The resulting white ash was put in polyethylene boxes for further analyses.

**Analytical Determinations.** INAA was used for mineral elements determination. This was because it is one of the most sensitive and accurate tools for chemical characterization available (17, 18). The ashes together with comparative standards and suitable control materials were put in polyethylene boxes, wrapped in aluminum foil, and packed in aluminum capsules for irradiation. They were irradiated for 4 h in the RA-3 reactor (8.5 MW,  $6 \times 10^{13}$  thermal neutron fluxes) at Ezeiza Atomic Centre, National Commission of Atomic Energy, of Argentina. The samples, standards, and control materials were measured in a hipper pure germanium detector (HPGe, 30% efficiency, 1.8 keV resolutions for the 1332.5 60Co peak); Gamma Vison software was used for data acquisition. The element concentrations were calculated using software developed at the laboratory. A reference material was used for analytical quality control, IAEA V-10 (hay powder).

**Statistical and Multivariate Analysis.** Prior to chemometric processing, data were autoscaled by subtracting their mean values and dividing into the standard deviations (SDs). At first, a series of statistical test were realized to explode the distribution of the matrix data.

First of all, a descriptive analysis of the variables was carried out. Normality of the data was verified by means of the Kolmogorov test. Second, an analysis of variance was made (one-way ANOVA) to detect if the combined factor precedence variety was significantly diverse. As a third step, we proceeded to carry out a study of the bivariate correlations between all of the variables. The Bartlett test of sphericity and the KMO test (Kaiser–Meyer–Olkin measure of sampling adequacy) were also performed to study the correlations matrix.

A nonparametric pattern recognition method was used (principal components analysis, PCA) that is a procedure for finding hypothetical variables (components) that account for as much of the variance in the multidimensional data as possible. These new variables were linear combinations of the original variables. This method identified redundancy or correlation among a set of measurements or variables. Quite generally, reducing the number of variables used to describe data led to some loss of information. PCA operated in a way that made this loss minimal, in a sense that will be given a precise meaning. Therefore, PCA may be regarded as a dimensionality reduction technique.

Lastly, a stepwise LDA was made with the aim of determining which of them discriminated best between the lemon juice varieties analyzed, as well as establishing a mathematical model for this purpose. The LDA method was a tool to obtain vectors showing the maximal resolution among categories, maximal separation, and compactness of the categories. The statistical significance of each discriminant func-

**Table 1.** Concentrations (in  $\mu\text{g/g}$ ) Obtained for the IAEA Reference Material IAEA-V-10, Hay (Powder), as Compared to Certified Values

element	measured value	certified value	certified range
As	ND $\leq$ 0.16	NR	
Br	$8.46 \pm 0.12$	8	7–11
Co	$0.16 \pm 0.019$	0.13	0.11–0.14
Cr	$6.5 \pm 0.8$	6.5	5.6–7.1
Fe	$202 \pm 14$	186	177–190
La	$0.075 \pm 0.0076$	0.07	0.06–0.09
Na	$503 \pm 18$	500	440–570
Rb	$8.5 \pm 1.5$	7.6	7.3–7.8
Sb	$0.0184 \pm 0.0047$	0.019	0.018–0.020
Sc	$0.0134 \pm 0.0007$	0.014	0.01–0.015
Zn	$23.7 \pm 1.2$	24	23–25

tion was evaluated on the basis of the Wilks'  $\lambda$  factor after the function was removed. The separation among groups in the discriminant space was checked by plotting the first and the second functions. Finally, to verify the power and the stability of the model, a "leave-one-out" cross-validation discriminant analysis was performed (19). The Statistical package for Social Science (SPSS) was used for these objectives.

## RESULTS AND DISCUSSION

**Chemical Analysis.** The detection power of the INAA technique was able to give good data for the determination of elements of interest in lemon juice samples. The accuracy and precision of the technique were assured by analyzing the IAEA reference material IAEA V-10 (hay powder). The reference material was prepared under the same conditions as the samples. Three replicates of the standard were analyzed. A comparison of our data with the certified/literature values (in parentheses) shows good agreement, within  $\pm 5$ –10% in most cases. SDs, in most cases, were small and well within  $\pm 10\%$  (Table 1). Therefore, it is presumed that all of the elemental concentrations in the samples should be accurate and precise. The concentrations of the elements in the samples under investigation analyzed by INAA are listed in Table 2.

A limited survey was carried out to compare other citrus juices from other countries. The results of mineral elements in Argentinean fresh lemon juices by INAA analysis are in good agreement with the medium composition of squeezed lemon juice from Brazil, Australia, and the United States (4, 8).

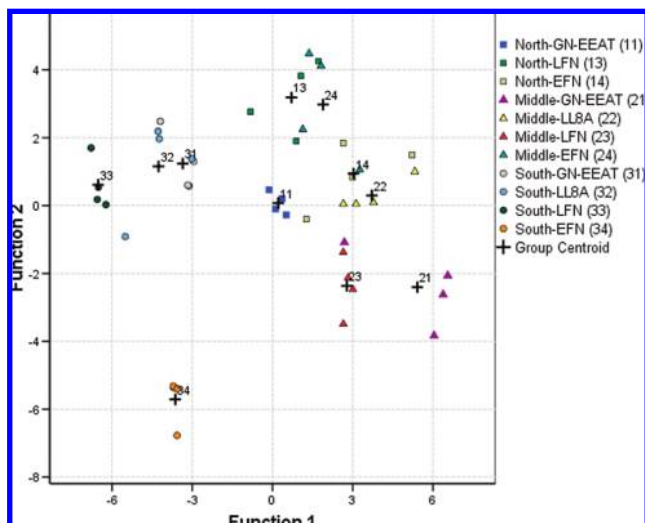
**Exploratory Data Analysis.** A total of 44 lemon juice samples representing three geographic regions and four varieties from each were analyzed. From the results of the Kolmogorov test on the normality of sample distributions, it can be concluded that all of them were significantly normal ( $p$  value  $>$  0.05). The one-way ANOVA, considering the factor precedence-variety at 11 levels, showed that, in general, this factor is significant except for the cases of variables Cr, Sb, and Zn. The KMO and Bartlett tests show that the KMO index (0.582) and the Bartlett sphericity test are significant ( $p <$  0.001). This fact indicates that the data matrix is suitable for proceeding with a principal component analysis (PCA).

On the basis of eigenvalues  $>$  1, PCA evolved three principal components explaining about 65.5% of the total variance. According to the eigenvalue criterion, only the PCs with eigenvalues greater than one are considered important. The scatter plot for the first principal components assumes a very widespread distribution scores. Visual inspection of the obtained plot indicates a poor separation between groups. Only one cluster formed by samples corresponding to the varieties cultivated in the southern region with regard to the other varieties could be distinguished. The separation by these categories is not necessarily expected since PCs are measures of total sample variation

**Table 2.** Concentrations of 11 Elements on Single-Strength Lemon Juice<sup>a</sup>

province/ subregion	variety	n	As (ppb)	Br (ppm)	Co (ppb)	Cr (ppb)	Fe (ppm)	La (ppb)	Na (ppm)	Rb (ppm)	Sb (ppb)	Sc (ppb)	Zn (ppm)
Northern	GN-EEAT	4	1.8 (1.5)	0.10 (0.01)	1.9 (1.2)	17.1 (8.5)	0.71 (0.17)	0.6 (0.4)	7.3 (4.7)	0.98 (0.08)	0.004 (0.001)	0.049 (0.023)	0.33 (0.18)
Northern	LFN	4	1.2 (0.2)	0.03 (<0.01)	2.2 (0.1)	27.6 (6.9)	0.82 (0.21)	0.8 (0.3)	11.5 (4.9)	0.91 (0.04)	0.022 (0.012)	0.109 (0.073)	0.33 (0.05)
Northern	EFN	4	1.2 (0.3)	0.04 (0.02)	1.6 (0.9)	22.9 (9.9)	0.88 (0.09)	1.1 (0.2)	13.3 (0.2)	0.84 (0.03)	0.018 (0.015)	0.097 (0.074)	0.23 (0.02)
Middle	GN-EEAT	4	2.7 (1.6)	0.09 (0.03)	1.6 (0.2)	21.8 (8.4)	0.66 (0.02)	1.2 (<0.1)	8.7 (1.7)	0.68 (0.31)	0.013 (0.004)	0.090 (0.001)	0.24 (<0.01)
Middle	LL8A	4	3.3 (1.2)	0.04 (0.01)	1.8 (0.1)	46.0 (21.1)	0.71 (0.02)	1.1 (0.1)	15.4 (4.3)	0.67 (0.29)	0.023 (0.004)	0.083 (<0.001)	0.31 (0.21)
Middle	LFN	4	2.8 (0.3)	0.04 (0.03)	1.6 (0.2)	23.2 (5.5)	0.59 (0.02)	1.0 (0.2)	10.1 (4.5)	0.87 (0.24)	0.016 (0.003)	0.049 (0.002)	0.31 (0.10)
Middle	EFN	4	2.1 (1.3)	0.05 (0.02)	1.8 (0.2)	28.3 (7.1)	0.77 (0.03)	0.8 (0.2)	6.5 (1.3)	0.92 (0.30)	0.029 (0.004)	0.109 (0.003)	0.24 (0.02)
Southern	GN-EEAT	4	3.0 (0.8)	0.03 (0.01)	1.4 (<0.1)	41.8 (10.2)	0.73 (<0.01)	0.5 (<0.1)	5.3 (0.1)	1.44 (1.39)	0.013 (0.001)	0.045 (0.014)	0.31 (0.07)
Southern	LL8A	4	2.7 (0.2)	0.03 (<0.01)	1.2 (0.7)	35.3 (26.2)	0.82 (0.04)	0.5 (<0.1)	7.1 (2.4)	0.75 (0.11)	0.008 (0.003)	0.049 (0.016)	0.30 (0.01)
Southern	LFN	4	3.8 (0.8)	0.03 (0.01)	0.8 (0.0)	21.1 (2.7)	0.64 (0.15)	0.3 (<0.1)	5.5 (0.2)	2.94 (1.47)	0.013 (0.004)	0.033 (0.012)	0.31 (<0.01)
Southern	EFN	4	2.2 (0.1)	0.03 (0.02)	0.1 (<0.1)	20.8 (5.0)	0.54 (0.06)	0.7 (<0.1)	7.4 (1.9)	0.53 (0.10)	0.010 (0.001)	0.035 (0.023)	0.32 (0.06)

<sup>a</sup> GN-EEAT, Genova Nuclear EEAT; LL8A, Lisboa Frost Nuclear; LFN, Lisboa Limoneira 8A; and EFN, Eureka Frost Nuclear.



**Figure 1.** LDA plot for 44 lemon juice samples based on concentrations of 11 elements and using 11 groups of **Table 2** as input a priori.

and do not explicitly take into account variation between groups (locations–varieties) of interest. Finally, the first PC accounting for 32% of the total variance was principally correlated (loading > 0.70) with Na, Fe, and La. The second PC accounting for 17.3% of total variance was correlated with Br, As, and Co.

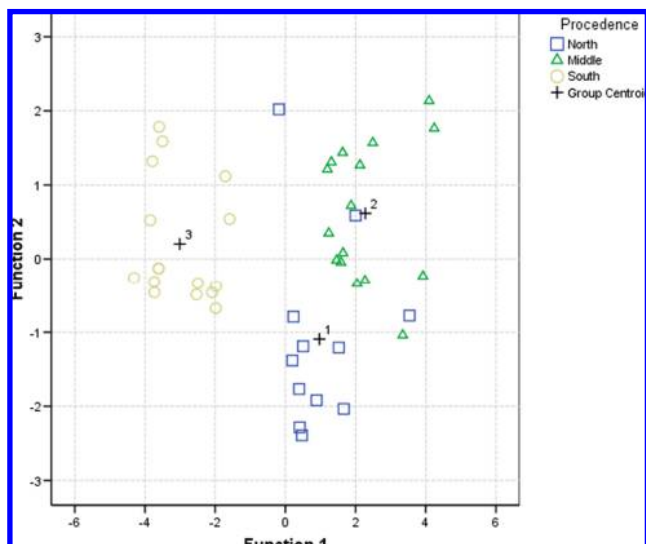
#### Discriminating Variables and Classification of Samples.

After PCA, stepwise LDA was applied. In the first place, a model was constructed considering the 11 group categories obtained by combining the different botanical varieties of cultivars from the three different producing regions.

The bidimensional plot (**Figure 1**) of the first two roots shows total overlapping between the proposed categories but a small visual trend of autoscores corresponding to samples from the southern region. These were characterized by low values of function 1. All of them were clearly separate from the rest. From the available data, it seems that the four cultivars cannot be distinguished accurately by this approach. Other classification techniques were applied to improve the separation of cultivars, such as cluster analysis or quadratic discrimination analysis, with similar results to those obtained by LDA.

As a consequence, we decided to focus on the geographic origin of samples; furthermore, the majority of the industrialized lemon juice produced in Argentina is obtained from the juice concentration of different lemon varieties.

On the ground of these considerations, the forward stepwise LDA was again performed on the concentrations of the selected 11 elements using as input a priori three groups only, that is, those corresponding to the lemon-producing areas



**Figure 2.** LDA plot for 44 lemon juice samples based on concentrations of 11 elements and using as input a priori three groups, that is, those corresponding to the lemon-producing areas.

**Table 3.** LDA Functions

function	eigenvalue	% of variance	cumulative %	vanonical correlation	Wilks $\lambda$	<i>p</i> value
1	18203.5	87.66	87.66	0.9953	0.000162	<0.0001
2	2563.47	12.37	100.0	0.9911	0.017598	<0.0001

(**Figure 2**). The stepwise analysis (probability of *F* to remove = 0.10 and to enter = 0.05) has eliminated six variables (Sc, Fe, Zn, Sb, Na, and Cr) and generated a plot in which it is possible to distinguish clearly three clusters for the three production zones of Northwest region of Argentina, respectively.

The LDA shows significant differences between groups (**Table 3**); in fact, the Wilks' value is near zero (0.097); this indicates that group means differ. In addition, the probability of a correct classification is very high, because the *p* level is lower than 0.10. From the results of the LDA and cross-validation that we obtained, original grouped cases (93.2%) were correctly classified, and 84.1% of cross-validated grouped cases were correctly classified. Furthermore, it should be noted that 100.0% of cross-validated grouped Southern region cases were correctly classified.

Then, to verify the reliability of the model, the method was tested using known samples as unknown variables. In particular, a set of three samples, composed of one sample for each production area, was randomly removed for five times, and the

**Table 4.** LDA Structure Matrix of Discriminant Functions

	function	
	1	2
Br	-0.7645	-0.5624
Rb	-4.9675	4.2216
Na	-0.8599	-0.3162
Zn	-12.8922	25.1686
Sb	-1.6080	2.3729
Th	-0.0714	1.9841
Cr	-15.8318	17.6946
Cs	7.6590	0.5091
Sc	4.1103	-4.9860
Fe	1.2144	-28.9730
Co	5.8717	0.5856
Sm	-0.9374	4.4137
La	-10.4579	10.3937
Ce	3.7926	-4.1933
Eu	9.0055	-1.2454

model was recalculated. At all events, the samples were correctly classified; therefore, the system can be considered stable and can be used for real samples.

Finally, the "structure matrix" (Table 4) of the discriminant functions showed the six variables chosen for the discrimination were, ranked in order of highest correlation first: function 1, Ithantunum, bromine, and rubidium; and function 2, arsenic and cobalt. Regarding the selection of elemental concentration variables, the bioavailability of metals depends on several factors such as pH, humidity, porosity, clay and humic complex, etc. The LDA identified rubidium, an element that is easily mobilized in the soil and readily transported into plants, which has previously been identified as a good indicator of geographical identity in rice (20).

**Conclusion.** The results presented above show that the discrimination between lemon juices coming from different regions can be afforded by a unique multielemental technique, such as INAA, which allows a safe determination of 11 elements present in the foodstuff. The method can be extended to build up a set of data to trace the geographical origin of Argentinean lemon juices based on reliable clues. Future extensions require the construction of databases for each producing zone. Work is in progress to further substantiate the effects of seasonal and lemon variety influences.

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