Elemental and Isotopic Fingerprint of Argentinean Wheat. Matching Soil, Water, and Crop Composition to Differentiate Provenance

Natalia S. Podio,[†] María V. Baroni,[†] Raúl G. Badini,[‡] Marcela Inga,[‡] Héctor A. Ostera,[§] Mariana Cagnoni,[§] Eduardo A. Gautier,[⊥] Pilar Peral García,[‖] Jurian Hoogewerff,[#] and Daniel A. Wunderlin*,[†]

[†]Facultad de Cs. Químicas-ICYTAC, Universidad Nacional de Córdoba-CONICET, Ciudad Universitaria, 5000 Córdoba, Argentina

[‡]Ministerio de Ciencia y Tecnología Provincia de Córdoba-CEPROCOR, Santa María de Punilla, Córdoba, Argentina

[§]Facultad de Ciencias Exactas y Naturales-INGEIS, Universidad de Buenos Aires-CONICET, Ciudad Universitaria, Buenos Aires, Argentina

[⊥]Comisión Nacional de Energía Atómica, Gerencia Química, Centro Atómico Constituyentes, San Martín, Buenos Aires, Argentina

[‖]Facultad de Ciencias Veterinarias-IGEVET, Universidad Nacional de La Plata-CONICET, Ciudad Universitaria, La Plata, Argentina

[#]Department of Chemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

ABSTRACT: The aim of this study was to investigate if elemental and isotopic signatures of Argentinean wheat can be used to develop a reliable fingerprint to assess its geographical provenance. For this pilot study we used wheat cultivated at three different regions (Buenos Aires, Córdoba, and Entre Ríos), together with matching soil and water. Elemental composition was determined by ICP-MS. $\delta^{13}C$ and $\delta^{15}N$ were measured by isotopic ratio mass spectrometry, while $^{87}Sr/^{86}Sr$ ratio was determined using thermal ionization mass spectrometry. Wheat samples from three sampling sites were differentiated by the combination of 11 key variables (K/Rb, Ca/Sr, Ba, $^{87}Sr/^{86}Sr$, Co, Mo, Zn, Mn, Eu, $\delta^{13}C$, and Na), demonstrating differences among the three studied regions. The application of generalized Procrustes analysis showed 99.2% consensus between cultivation soil, irrigation water, and wheat samples, in addition to clear differences between studied areas. Furthermore, canonical correlation analysis showed significant correlation between the elemental and isotopic profiles of wheat and those corresponding to both soil and water ($r^2 = 0.97$, $p < 0.001$ and $r^2 = 0.96$, $p < 0.001$, respectively). To our knowledge, this is the first report demonstrating the correspondence between soil, water, and wheat samples using different statistical methods, showing that wheat elemental and isotopic compositions are mainly related to soil and irrigation water characteristics of the site of growth.

KEYWORDS: *wheat, multielement composition, isotopes, geographical origin, food authenticity*

■ INTRODUCTION

Wheat, maize, and rice are the most produced cereals in the world, with wheat being the most widely consumed grain in Western civilization from antiquity.[1] According to the FAO,[2] the world production of wheat was 694.8 million tonnes during the 2010−2011 harvest, 13.5 of which occurred in Argentina, with a cultivated area of 4.2 million hectares.

Different wheat varieties are commercially used for the production of a variety of foods, such as bread, noodles, cakes, biscuits, and cookies. In the past few years, with the development of a globalized food market and the increased interest shown by consumers to know the characteristics, quality, and geographical origin of foods, many countries have taken steps to guarantee the traceability of food. Denomination of Protected Origin (DOP) and the Denomination Origin Controlled (DOC) systems have been promoted and are applied to control and ultimately ensure the origin and quality of food and to prevent fraud.[3] One of the requirements to achieve DOP or DOC certification is to obtain a chemical characterization of the food. There is a wide range of techniques to establish food authenticity.[4] Some of these techniques are utilized to determine organic constituents[5,6] and others to determine inorganic constituents.[7,8] However, it is often difficult to verify the authenticity of a given food by just analyzing organic constituents, mainly because their qualitative

and quantitative profile is influenced by the use of fertilizers, weather conditions along with different production years, water stress, culinary or industrial practices, etc.[9] On the other hand, the inorganic pattern of a food reflects the local geochemistry of both soil and water, which is influenced by geology, temperature, climate, distance from the sea, elevation, latitude, food processing, etc.,[10−12] which can be assumed to be more stable in time compared to the organic constituents.

Trace element and isotopic contents of cereals are assumed to be related to the chemistry of the local water and soil layers in which they grow, although anthropogenic practices could have some influence. So far, as the soil layer reflects the underground rock, the isotopic and elemental composition of a crop should be correlated with the geological characteristics of the producing areas.[13]

The analysis of element composition is considered to be an effective primary tool. In addition to the essential elements that plants need to grow (B, N, Mg, P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, and Mo),[14] they can take in other bioavailable elements from soil and water from the production site. Elements, such as

alkaline metals, especially rubidium (Rb) and cesium (Cs), can be easily mobilized in the soil and transported into plants, being good indicators of geographical identity.[3] Element availability depends on several factors such as soil pH, humidity, porosity, clay, and the presence of humic complexes. Consequently, the range of soils present in addition to different agricultural practices (organic, traditional, etc.) and the bioavailability of soil constituents may supply unique fingerprints in the final food product that characterize its geographical origin.[3]

Considerable research has been carried out to establish the origin of vegetable foods using elemental analysis. Some of the studied commodities include wine,[15] pistachios,[16] and amaranth seeds,[17] among others.

On the other hand, isotope ratios may add to the element fingerprint essential information on plant type and geographical origin.[18] The carbon isotope ratio ($^{13}C/^{12}C$) of plant compounds is affected by the botanical group (discrimination between C_3 and C_4 plants), the plant age, and level of maturation,[19] in addition to several environmental factors such as relative humidity, temperature, amount of precipitation, and water stress.[20] The nitrogen ratio ($^{15}N/^{14}N$) depends on the botanical type of plant but also on the bacterial activity associated with its growth, which can also be affected by the temperature and agricultural practices (e.g., use of fertilizers).[10] Differences in the relative abundance of ^{87}Sr compared to ^{86}Sr in rocks and minerals are related to the geological age and the host's Rb/Sr ratio. Consequently, strontium isotope ratios ($^{87}Sr/^{86}Sr$) in soils vary according to the local geology (bedrock).[3] On the other hand, water interacts and equilibrates with the rocks and reflects the isotope composition of the minerals able to exchange with it. The isotope ratio of the bioavailable portion of Sr derived from bedrock is therefore found in plants.[3] Studies on the application of isotope analysis to assess the origin and adulteration of foods have been carried out on a range of products, including ciders,[21] potatoes,[6] and rice,[22] among others.

However, food authentication studies have shown that one single method, or one single parameter, is generally insufficient to indicate the origin and to enable the traceability of food.[21] Thus, our current knowledge indicates that it is necessary to evaluate at least two different groups of parameters (e.g., elements and isotopes), combining them using multivariate statistics, to achieve a good differentiation of food from various geographic origins. There are several studies that combine the use of isotope and element composition for designation of origin.[11,12,18,23−25] However, very few studies have been carried out to determine the provenance of wheat combining both element and isotopic composition.[10,13] Moreover, to our knowledge, there are no reports matching elemental and isotope composition from the growing soil and the irrigation water with those of the cereal, enabling a more certain characterization of wheat provenance.

Brescia et al.[26] differentiated samples of durum wheat semolina from Italy and other countries (Canada, Turkey, and Australia), evaluating isotopic signatures ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$), confirming the relationship between the isotopic content and the latitude. On the other hand, Branch et al.[13] carried out a preliminary investigation, using stable isotopes and elemental analysis ($\delta^{13}C$, $\delta^{15}N$, Cd, Pb, Se, and Sr), to identify the country of origin of commercial wheat samples from the USA, Canada, France, and Germany, applying discriminant analysis. They found that all wheat samples could be geographically classified using a single isotope ratio ($\delta^{13}C$). The combination of isotopic

signature of strontium ($^{87}Sr/^{86}Sr$), in addition to light element isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$) and multielemental profiling, allowed Goitom Asfaha et al.[10] to discriminate different European cereal samples according to the latitude of the sampling site/production area, the proximity to a marine environment, and the bed rock geology. Also Zhao et al.[27] presented a multivariate statistical method for determining the geographic origin of wheat from four major wheat-producing regions of China, taking into account different varieties and growing years. The method allowed classifying wheat according to their geographical growing regions using multielement analysis (Be, Na, Mg, Al, K, Ca, V, Mn, Fe, Cu, Zn, Mo, Cd, Ba, and Th); they also found that elemental composition may be influenced by cereal varieties, cultivation years, and agronomical practices.

These results suggest that the discrimination of wheat samples according to their geographical origin is possible using multivariate statistical analysis.

Multivariate statistics analysis usually starts with using an exploratory nonsupervised method such as principal components analysis (PCA) or cluster analysis (CA) to evidence differences between the studied data matrix. A supervised method such as linear discriminant analysis (LDA) or partial least-square discriminant analysis (PLS-DA) is further used to confirm the first classification obtained by PCA and CA as well as finding patterns indicating differences between groups.[28]

The aim of this study was to obtain a reliable fingerprint to assess the origin of Argentinean wheat, considering both elemental composition and isotopic pattern from wheat of three different regions (Buenos Aires, Córdoba, and Entre Ríos). Additionally, we were interested in demonstrating the link between wheat, soil, and water by applying different chemometric tools. What sets this study apart from other studies is that we not only investigate the food product but try to understand and model the relation of the food with the local water and soil so that we can then scientifically predict the composition of food from water and soil parameters in the future, negating the need for maintaining large databases of all food produced.

■ MATERIALS AND METHODS

Reagents and Materials. Ultrapure water (<5 μg/L TOC) was obtained from an Arium 61316-RO purification system combined with an Arium 611 UV (Sartorius, Germany). The certified ICP-MS multielement Merck VI CertiPUR standard solution was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%), sub-boiling grade, was prepared from analytical grade acid using a distiller (Figmay sub-boiling distiller, Córdoba, Argentina). The purity of nitric acid was verified by ICP-MS. Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). Trace element certified reference material (CRM) wheat flour 1567a was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). The best estimate values for constituent elements in CRM 1567a are detailed in the accompanying certificate of analysis provided by the manufacturer. Ion exchange chromatography Sr Spec resin was obtained from Eichrom Technologies (USA). Strontium isotopic standard (NBS SRM 987) was employed to determine mass discrimination during the isotopic analyses by thermal ionization mass spectrometry (TIMS). All other reagents were analytical grade.

Sampling. Soil, water, and wheat samples were collected from three provinces of Argentina: Buenos Aires, Córdoba, and Entre Ríos. These provinces are the largest wheat-producing areas in Argentina (Figure 1). The sampling area in Buenos Aires is located between 37°25′ and 37°38′ S and 59°10′ and 59°14′ W; the Córdoba sampling
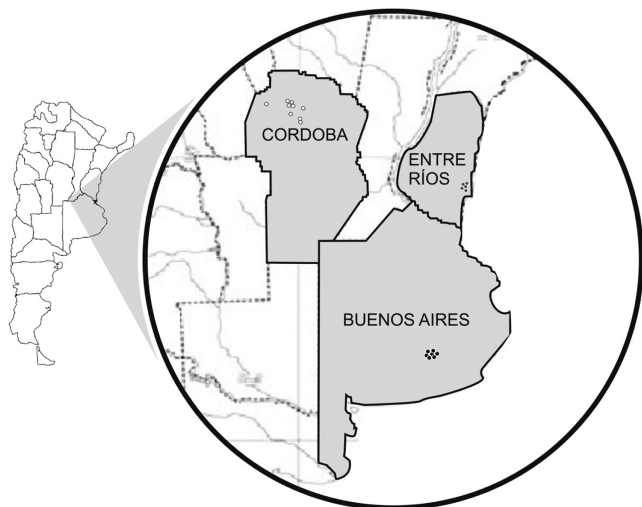
Figure 1. Map of three regions where wheat samples were collected: (white circles) farms located in Córdoba; (gray circles) farms located in Entre Ríos; (black circles) farms located in Buenos Aires.

area is located between 30°30′ and 31°08′ S and 63°34′ and 64°08′ W; while the Entre Ríos sampling area is located between 32°49′ and 32°53′ S and 58°32′ and 58°34′ W. The altitude varies from 460 m above sea level in Córdoba, to 240 m above sea level in Buenos Aires, to 30 m above sea level in Entre Ríos.

Samples (soil, water, and wheat) from three harvests (2007, 2008, and 2009) were collected from different farms: nine farms within Córdoba, seven within Buenos Aires, and five within Entre Ríos. At least two soil samples (1 kg each, 5−30 cm deep) were taken from each farm. Water samples (1 L each) were collected from small streams/rivers/channels within the area used for soil sampling. Water samples were filtrated (0.45 μm) in the field using a manual pump, acidified with 1 mL of nitric acid (sub-boiling grade), and stored in precleaned plastic bottles for transportation to the laboratory.

A total of 80 samples of wheat were collected, 27 samples were collected in Buenos Aires, 26 in Córdoba, and 27 in Entre Ríos. One kilogram of fresh grain was collected in plastic bags, separated from the chaff, transported to the laboratory within 48 h, dried at 40 °C during 4 days, and stored at 4−8 °C until analysis. Immediately before analysis, samples were cleaned, powdered, and homogenized, followed by further drying at 40 °C overnight.

Elemental Analyses. Wheat samples were mineralized using a microwave oven (Anton Paar Multiwave 3000, Vienna, Austria). Five hundred milligrams of dry wheat sample were introduced in quartz vessels, followed by the addition of 8 mL of concentrated nitric acid. Vessels were kept open until no fumes were observed. Afterward, vessels were cap-closed and heated using the following power sequence: a 15 min ramp to 400 W, held for 45 min (maximum temperature = 177 °C; maximum pressure = 75.7 bar), followed by a final step (15 min) disabling the power to reach pressure equilibration and room temperature. Mineralized samples were quantitatively transferred to 25 mL volumetric flasks, completing the volume with ultrapure water, followed by filtration using 0.45 μm filters. CRM 1567a was processed and measured in the same way used for wheat. Values obtained for the latter were between 85% and 105% of certified values.

Thirty-one elements were quantified in wheat samples: Al, B, Ba, Ca, Cd, Ce, Co, Cs, Cu, Eu, Ga, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Rb, Se, Sm, Sr, Tl, U, V, Yb, and Zn. The analysis was carried out by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) for all elements except for sodium. A Thermo-Elemental X7 series ICP-MS (Thermo Fisher Scientific, Bremen, Germany) equipped with an ASX-100 autosampler model (CETAC Technologies, Omaha, NE, USA) was used. The sample introduction system consisted of a microflow concentric nebulizer, Peltier-cooled spray chamber, and 1.5 mm i.d. fixed injector torch. The RF forward power

was 1350 W for all experiments, and the interface was fitted with Ni sampling and skimmer cones designed for low polyatomics formation. Two operation modes were used: with and without collision cell technology (CCT). CCT mode measurements were performed for Ba, Ca, Cd, Co, Cu, Ga, K, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sr, U, V, and Zn. The collision cell was flushed with 7% H_2 in a He high-purity mixture. The elements Al, B, Ce, Cs, Eu, La, Li, Lu, Nd, Sm, Tl, and Yb were measured without operating the collision cell with gas, and thus full sensitivity was obtained. The oxide ratio and double-charged species were maintained below 1% for both modes of operation. All the Q-ICP-MS measurements were performed using Sc, In, and Re as internal standards. Sodium measurements were carried out by flame atomic absorption spectrometry using a Perkin-Elmer 3030 (Boston, MA, USA) using an air-acetylene flame. All samples (mineralized) were diluted 10-fold using a 1% HNO_3−0.5% HCl mixture before Q-ICP-MS measurements. Standards and blanks were prepared using the same mixture (1% HNO_3−0.5% HCl). Instrumental and procedural blanks were determined together with samples, and the mean of five runs was obtained for each sample. Full quantitative analysis was performed against calibration standards for each element. Precision (% CV) was below 5% considering five measures on the same sample.

Stable Isotope Analysis. $\delta^{13}C$ and $\delta^{15}N$ were measured on defatted samples. The fat was extracted in a Soxhlet apparatus with hexane. The dry sample was placed in an extraction thimble, and the fat was extracted during 6 h. The fat free mass was dried in a fume hood overnight until constant weight and stored in screw-capped recipients until measurement. Defatted wheat samples (5−8 mg) were weighed into tin capsules for dual C and N analysis. The capsule was sealed and dropped into the reaction tube of a Carlo Elba elemental analyzer. The combustion took place at 1020 °C, and the products were separated on a packed gas chromatographic (GC) column, using helium as carrier gas. The GC effluent was transferred to the stable isotope ratio mass spectrometer (Thermo Fisher Scientific DELTA V Plus) using a ConFlo IV interface. The isotope ratios were normalized using laboratory-working standards, calibrated with international standards supplied by the International Atomic Energy Agency (NBS 22 and IAEA N-1). In each case, duplicate samples were measured. Isotope data were expressed using the international delta notation (δ, ‰) and are referred to Vienna Pee Dee Belimnite (V-PDB) for $\delta^{13}C$ and to air-N_2 for $\delta^{15}N$. The uncertainty of the carbon and nitrogen isotopic determinations was ±0.2‰.

Strontium Isotope Analysis. Wheat samples were processed by the dry-ashing technique. An aliquot of 5 g of dry sample was placed in porcelain crucibles in a high-temperature muffle furnace, heating at 550−600 °C for 18 h until ash formed. After cooling, residues were treated with concentrated nitric acid on a hot plate. After that, samples were transferred to the muffle furnace, operated as previously described. White ashes obtained were dissolved in 1 M nitric acid and loaded onto an ion exchange chromatography column.

Sr fractions were dried down and stored for analysis. Sample manipulation was carried out in a clean environment (Class 100 laboratory). To prevent contamination, all laboratory-ware was soaked in 50% (v/v) nitric acid for at least 24 h, rinsed several times with ultrapure water, and dried in a Class 100 laminar flow exhaust hood.

Strontium isotopic ratios were measured using a Finnigan MAT-262 TIMS (Thermo Fischer Scientific, Bremen, Germany). It is equipped with a sample magazine for 13 samples, using 7 Faraday cups and a secondary electron multiplier as collectors. Measurements were carried out using a double-filament rhenium ion source. NBS SRM 987 was employed as standard to determine the instrumental bias for each set of 12 samples to evaluate the reproducibility and accuracy of the measurements. The Eimer & Amend standard (Massachusetts Institute of Technology)[29] was also regularly analyzed to check for proper operation of the mass spectrometer. Measured ratios were corrected for mass fractionation using $^{88}Sr/^{86}Sr$ = 8.375 209.[30] ^{85}Rb was monitored at each block of data to quantify any interference from ^{87}Rb. During the course of measurements for this study, SRM 987 gave a value of 0.710 08 ± 0.000 26 (n = 50; ±1σ).

The methodology and the results of soil and water samples in the regions under study were published by Baroni et al. (2011).[12] Briefly,

Table 1. Means and Standard Deviations of Measured Elements (μg/kg) and Isotopic Ratios (δ units, ‰ per mil) Corresponding to Wheat According to Region of Origin[a]

variable	Buenos Aires	Córdoba	Entre Ríos
n	27	26	27
Al	4000 ± 2100 b	8000 ± 7000 a	3100 ± 1500 b
B	670 ± 140 b	1000 ± 500 a	430 ± 200 c
Ba	9000 ± 5000 a	5800 ± 2000 b	8000 ± 3000 a
Ca	$(42 \pm 14) \times 10^4$ b	$(39 \pm 7) \times 10^4$ b	$(45 \pm 6) \times 10^4$ a
Cd	9 ± 5 c	12 ± 6 b	18 ± 8 a
Ce	4 ± 3 b	7 ± 7 a	3 ± 7 b
Co	9 ± 10 b	22 ± 12 a	10 ± 6 b
Cs	<LOQ c	3 ± 3 b	15 ± 10 a
Cu	4500 ± 700 b	3900 ± 800 c	5300 ± 500 a
Eu	2.7 ± 0.7 a	1.7 ± 0.8 b	1.1 ± 0.4 c
Ga	11.4 ± 0.8 a	<LOD	<LOD
K	$(41 \pm 8) \times 10^5$ a	$(40 \pm 6) \times 10^5$ b	$(40 \pm 6) \times 10^5$ a
La	4 ± 4 b	5 ± 6 a	3 ± 5 b
Li	8.8 ± 2.3 c	70 ± 40 a	24 ± 5 b
Lu	<LOD	<LOD	<LOD
Mg	$(12.5 \pm 1.3) \times 10^5$ a	$(12.1 \pm 1.8) \times 10^5$ b	$(12.3 \pm 1.8) \times 10^5$ a
Mn	$(40 \pm 12) \times 10^3$ b	$(42 \pm 12) \times 10^3$ a	$(41 \pm 10) \times 10^3$ a
Mo	420 ± 210 b	900 ± 300 a	800 ± 250 a
Na	$(19 \pm 8) \times 10^3$ a	$(12 \pm 6) \times 10^3$ b	$(9 \pm 3) \times 10^3$ b
Nd	1.9 ± 1.5 b	4 ± 3 a	2 ± 3 b
Ni	500 ± 700 a	290 ± 70 b	390 ± 130 a
Pb	13 ± 40 b	30 ± 80 a	14 ± 18 b
Rb	$(20 \pm 5) \times 10^2$ b	$(13 \pm 4) \times 10^2$ c	$(12 \pm 5) \times 10^3$ a
Se	<LOQ	190 ± 140 a	<LOQ
Sm	<LOQ	<LOQ	<LOQ
Sr	2800 ± 900 c	4700 ± 1500 a	3500 ± 800 b
Tl	<LOD	<LOD	<LOD
U	<LOD	<LOD	<LOD
V	<LOD	<LOD	<LOD
Yb	1.37 ± 0.10 a	0.9 ± 0.7 b	<LOD
Zn	$(30 \pm 4) \times 10^3$ a	$(24 \pm 6) \times 10^3$ b	$(26 \pm 5) \times 10^3$ b
Ca/Sr	170 ± 90 a	90 ± 30 c	130 ± 19 b
K/Rb	2100 ± 600 b	3200 ± 600 a	390 ± 170 c
δ^{13}C	−24.4 ± 1.1 b	−23.9 ± 0.8 a	−24.7 ± 0.9 b
δ^{15}N	3.3 ± 1.0 a	2.9 ± 0.9 b	3.8 ± 0.9 a
^{87}Sr/^{86}Sr	0.7073 ± 0.0005 c	0.7089 ± 0.0005 a	0.7080 ± 0.0004 b

[a]<LOD, below limit of detection. <LOQ, below limit of quantification. IDL (μg/kg): Ga (1.5), Lu (0.05), Tl (0.5), U (2.5), V (50), and Yb (0.15). IQL (μg/kg): Se (170) and Sm (0.8). Different letters (a > b > c) in the same row indicate significant differences ($p < 0.05$).

bioavailable soil fractions were prepared from soil dried at 40 °C, homogenized, and sieved through a 2 mm acrylic sieve, followed by further drying at 40 °C overnight. Twenty grams of dried sieved soil was weighed into an Erlenmeyer flask, adding 50 mL of 1 M NH_4NO_3, shaken 2 h at room temperature, allowed to settle for 1 h, filtered through a 0.45 μm filter (HAWG04756, Millipore), and acidified with 0.5 mL of nitric acid (sub-boiling grade). Some samples were spiked to verify recovery percentages of different elements. Variable amounts of individual standard solutions (1000 mg/L in 1% nitric acid) were added to 40 g of dried sieved soil sample to double the starting concentration for each element. Recoveries were between 80% and 120%. Water samples were acidified with ultrapure nitric acid and filtered using 0.45 μm (HAWG04756, Millipore) filters before measuring. All samples were prepared in duplicate.

Statistical Analysis. Data were treated using the statistical package Statistica 8.0 from StatSoft Inc. (2007) and the Infostat software package.[31]

Analysis of Variance. ANOVA was performed using mixed models;[32] in the case of significance ($p < 0.05$), a DGC[33] comparison test was performed to reveal paired differences between means.

To explore and classify different samples, CA and PCA were performed. The amalgamation criterion to CA was Ward's minimum variance, and the distance method was Gower (*sqrt(1-S)*). PCA was based on the correlation matrix, with no rotation factor (this is the suggested option for PCA) and average values (each missing value is replaced by the average value of that variable).

Discriminant Analysis (DA). DA was performed to evaluate if wheat samples could be mathematically distinguished according to their geographical origin. The selection of the most significant variables was performed by forward stepwise analysis considering the F value. The robustness of the classification model was evaluated by a cross-validation test, using the "leave-one-out" procedure.

Generalized Procrustes Analysis (GPA). GPA was applied to assess the relationship between wheat, soil, and water data. Specifically, GPA constructs the consensus configuration of a group of data sets by applying transforms in an attempt to superimpose them. In this work we used the Grower algorithm that minimizes within-samples variance by applying translation, scaling, and rotation to generate a *p*-dimensional average configuration *Yc*. Following this, a *q*-dimensional group average space ($q \leq p$) is constructed from *Yc* by PCA.

Therefore, GPA theory and algorithms can be applied to match wheat elemental and isotopic data to the corresponding soil and water data.

Canonical Correlation Analysis (CCA). CCA was used for assessing the relationship between data sets by a more robust mathematical-statistical method. Specifically, this method allowed us to verify the relationship between soil, water, and wheat studied during this work by GPA.

■ RESULTS AND DISCUSSION

Wheat Composition. Table 1 shows mean values and standard deviations of elemental and isotopic constituents of wheat from three studied regions. In addition to elemental and isotopic composition, we included K/Rb and Ca/Sr ratios. The K/Rb ratio can greatly differ among various rocks and soils.[34] On the other hand, the Ca/Sr ratio has been used as a chemical tracer in geochemistry, hydrogeochemistry, and bioavailability studies.[35]

Multielement Contents. Six out of 31 elements analyzed showed values below the detection limit, while three showed values below the quantification limit in wheat, so these elements were rejected for further statistical analysis. Potassium (K) was the most abundant element in studied wheat, followed by Mg, Ca, Mn, Zn, and Na. These results were consistent with previous reports for wheat from China[27] and Denmark.[14] Many elements showed different patterns in accordance with wheat provenance. Seven elements (Sr, Li, B, Cu, Rb, Cd, and Eu) were significantly different (ANOVA, $p < 0.05$) among three studied regions. Concentrations of Sr, Li, and B were higher in wheat from Córdoba. On the other hand, Cu, Rb, and Cd showed higher values in wheat from Entre Ríos, while Eu presented higher values in Buenos Aires. Additionally, Al, Co, La, Ce, Nd, and Pb presented higher values in Córdoba, whereas Entre Ríos and Buenos Aires had similar low values. Conversely, Mg, K, Ni, and Ba showed major concentrations in Buenos Aires and Entre Ríos compared with Córdoba. On the other hand, Buenos Aires presented higher concentrations of Na and Zn compared with Córdoba and Entre Ríos, whereas Entre Ríos showed the highest value in Ca. Variations were also found in the Ca/Sr and K/Rb ratios. Ca/Sr was higher in Buenos Aires, intermediate in Entre Ríos, and lower in Córdoba, whereas K/Rb was lower in Entre Ríos, intermediate in Buenos Aires, and higher in Córdoba. It is known that Sr and Ca have geochemical affinity, while Rb has affinity to the essential element K; thus they can compete for identical binding sites or reactive centers in roots/plants. However, while Ca and K are essential elements for plant life, Sr and Rb have no known biological role.[36] In general, it seems that neither Sr or Rb can replace Ca and K in biochemical functions.[37] Most biological systems prefer the uptake of essential elements over others without biological roles.[38] However, this uptake depends greatly on soil properties, such as Ca and K availability (the higher the content of Ca and K in the soil, the lower the uptake of Sr and Rb), pH, and adsorption capacity.[34]

Isotope Composition. The isotopic compositions of plant materials reflect various factors such as isotopic compositions of source materials, their assimilation processes, and growth environments.[24]

Carbon Isotopic Composition. The carbon atoms present in plant carbohydrates are derived from the atmospheric carbon dioxide (CO_2). However, $\delta^{13}C$ values in plants are different from atmospheric CO_2.[39] Discrimination against ^{13}C occurs during the photosynthetic cycle, and isotope ratios are also affected by environmental factors such as light intensity, temperature, and relative humidity.[20]

C_3 plants have $\delta^{13}C$ values of about −25‰ (range: −22 to −33‰), whereas C_4 plants have $\delta^{13}C$ values about −12‰ (range: −10‰ to −20‰).[40] It is known that wheat is a C_3 plant, which is in agreement with our results (−24.7‰ to −23.91‰). Nevertheless, $\delta^{13}C$ values in samples from Córdoba were significantly higher than values observed in samples from Entre Ríos and Buenos Aires (Table 1). As previously stated, this variation could be related to the geographical environment as well as to climatic conditions. $\delta^{13}C$ values in leaves increase with increasing number of sunshine days, increasing mean temperature, and decreasing average humidity.[20,41] This correlation trend indicates that the leaf $\delta^{13}C$ is under the control of water-use efficiency.[41]

The highest $\delta^{13}C$ values from Córdoba samples could be related to an evaporation effect due to its low average humidity with respect to Buenos Aires and Entre Ríos. This is because the cultivation area of Córdoba is located close to a semiarid region, which could produce a shift of $\delta^{13}C$ toward values characteristics of C_4 plants, which predominate in semiarid or salty environments, where water availability limits photosynthesis.[42] Changes in $\delta^{13}C$ of wheat due to differences in $\delta^{13}C$ of soils should be discarded, since the main source of carbon for plants, particularly for wheat, arises from the atmospheric CO_2, with less incidence of soil carbon.[43]

Nitrogen Isotopic Composition. $\delta^{15}N$ of whole plant and leaves reflect the isotope ratio of the nitrogen source (soil). The sources of nitrogen in soils are the atmosphere ($\delta^{15}N = 0‰$), the fertilizers produced from atmospheric nitrogen ($\delta^{15}N = 0−3‰$), and animal manure ($\delta^{15}N = +10‰$ to $+25‰$). Rock/mineral sources of nitrogen are usually considered negligible.[44] When the nutrient concentration is lower than vegetation needs, absorption is the limiting step, and there is no isotopic fractionation during nitrogen uptake. In this case, many studies have shown that ^{15}N can act as a tracer, providing information about regional agricultural practices.[45] The nitrogen isotopic composition of a soil is influenced by many factors, such as fertilizer use and climatic and geographical conditions.[12] It has also been reported that tillage practices can affect $\delta^{15}N$, showing lower grain $\delta^{15}N$ under no-till.[46]

Our current results show that wheat samples from Buenos Aires (3.3‰ ± 1.0‰) and Entre Ríos (3.8‰ ± 0.9‰) had $\delta^{15}N$ values higher than samples from Córdoba (2.9‰ ± 0.9‰) (Table 1). Lower $\delta^{15}N$ values for Córdoba could be attributed to the use of chemical fertilizers, which are depleted in ^{15}N relative to organic fertilizers.[11]

Similar results were previously found for meat samples from the same regions.[12] Baroni et al.[12] found that $\delta^{15}N$ values in cattle were influenced by local feeding practices. Animals fed in regions where organic fertilizers were used (Buenos Aires and Entre Ríos) had $\delta^{15}N$ values greater than animals fed in regions where synthetic nitrogen-based fertilizers were more widely used (Córdoba).

So far, the $\delta^{15}N$ values of wheat from Córdoba seem to be more strongly linked to agricultural practices than to geo-climatic conditions.

Strontium Isotopic Composition. The determination of the Sr isotope ratio is one of the methods by which the provenance of food can be identified, reflecting local geochronological and lithological settings.[7] Differences in the relative abundance of ^{87}Sr vary with geological age, and the Rb/Sr ratio and consequently strontium isotope ratios in bedrock and soil
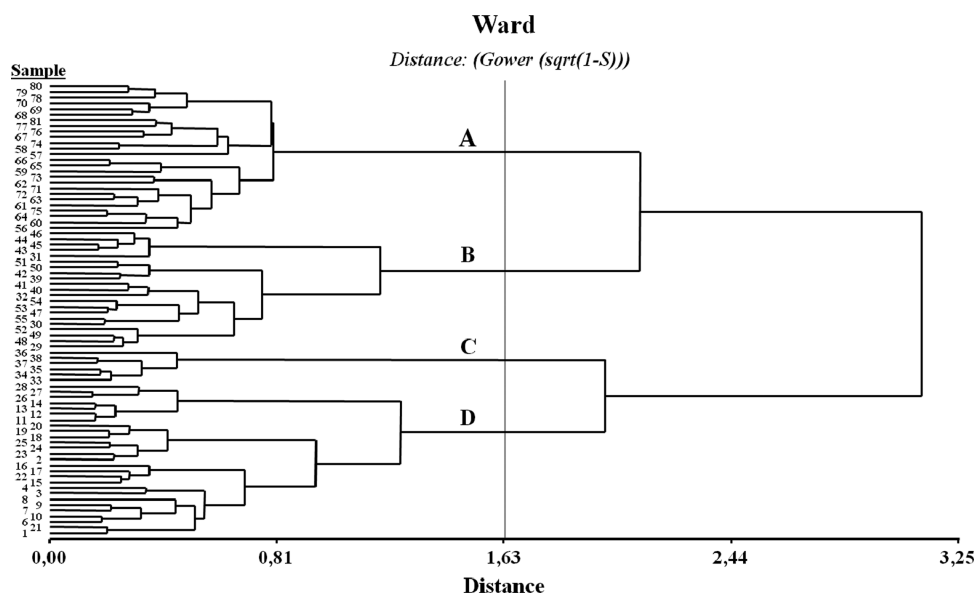
Figure 2. Dendrogram plot of wheat samples. The variables used were Al, B, Ba, Ca, Cd, Ce, Co, Cu, Eu, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, Pb, Rb, Sr, Zn, Ca/Sr, K/Rb, δ^{13}C, δ^{15}N, and ^{87}Sr/^{86}Sr.

vary according to the local geology. Isotopic abundances of all the Sr isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr) can also vary due to mass-dependent isotopic fractionation through various physicochemical reactions in nature (e.g., weathering). However, in the conventional isotopic analysis, ^{87}Sr/^{86}Sr ratio is normalized against a fixed ^{88}Sr/^{86}Sr ratio[47] and with the employment of the isotopic reference standard. They correct any experimental or natural mass-dependent fractionation, making ^{87}Sr/^{86}Sr a good tracer of source. Table 1 shows ^{87}Sr/^{86}Sr values for the three studied regions. We can see that wheat samples from Buenos Aires present the lowest value (0.7073 ± 0.0005), whereas samples from Córdoba showed the highest value (0.7089 ± 0.0005). Samples from Entre Ríos have a ^{87}Sr/^{86}Sr value between these two groups (0.7080 ± 0.0004). These results are in agreement with ^{87}Sr/^{86}Sr values previously reported for soil samples of similar sampling areas (Buenos Aires = 0.7066 ± 0.0004; Córdoba = 0.7100 ± 0.0030; and Entre Ríos = 0.7076 ± 0.0002).[12] Moreover, ^{87}Sr/^{86}Sr values for water of the same regions showed a similar trend (Buenos Aires = 0.7072 ± 0.0002; Córdoba = 0.7133 ± 0.0020; and Entre Ríos = 0.7078 ± 0.0004).[12] These isotope ratios can be explained by the differences in the parental soil material of each region. Volcanic ash falls constitute an important contributor to South American loess, which is the parent material of the modern cultivated soils of Argentina. The Sr isotope composition of the Pampean loess is very close to that of the south central Andes volcanic rocks (0.704−0.707), suggesting that they were derived from such a source.[12] The Tandilia and Ventania Ranges (Province of Buenos Aires) provided minor amounts of material to the soils and loess sediments of the surrounding areas,[12] which is in agreement with Buenos Aires data. However, several studies based on grain size and mineralogy have highlighted the possibility of other sources for the Argentinean loess, such as Pampean Ranges and Parana−Uruguay Rivers basin,[12] which could be more relevant for other sites such as Entre Ríos. The higher radiogenic Sr isotope ratios of Córdoba suggest the contribution of loess particles derived from sediments originated in the Pampean Ranges, the main source of which

are Paleozoic granitic rocks with Sr isotope ranges of 0.707−0.809.

On the other hand, wheat samples from Córdoba have ^{87}Sr/^{86}Sr ratios lower than soils and waters. Agriculture in Córdoba is characterized by the use of fertilizers, and phosphate fertilizers are produced by processing phosphorite with ^{87}Sr/^{86}Sr ratios between 0.7073 and 0.7078. The isotope results obtained using a simplifying bulk mixing model of two components between soil (water) and phosphorite are consistent with the strontium isotope ratios obtained for wheat samples. It is a reasonable possibility that fertilizer-derived Sr has been supplied to soil and water and put into solution.

So far, elemental and isotopic compositions of wheat show different patterns for diverse agricultural production areas in agreement with geology, hydrogeology, and environmental issues in addition to different agricultural practices.

Geographical Discrimination of Wheat Samples by Multivariate Data Treatment. Elemental and isotopic profiles of wheat (Al, B, Ba, Ca, Cd, Ce, Co, Cu, Eu, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, Pb, Rb, Sr, Zn, Ca/Sr, K/Rb, δ^{13}C, δ^{15}N, and ^{87}Sr/^{86}Sr) were subsequently analyzed using chemometrics to investigate if it was possible to discriminate samples from different regions.

Cluster Analysis. A first exploratory evaluation of the data structure was carried out by CA. Results obtained are presented as a dendrogram, which is shown in Figure 2. It can be observed that the sample arrangement is not random. Samples from different regions were separated into four clusters (A−D) formed at a Gower distance of 1.63 (50% of total linkage distance). Samples included in cluster A were all from Córdoba, whereas cluster D included samples from Entre Ríos. Samples from Buenos Aires were divided in two clusters; most of them were in cluster B, while a few were in cluster C. The latter ones show more similarity to samples from Entre Ríos than samples from Buenos Aires. So far, it looks like wheat from the three studied areas can be differentiated at least at an exploratory level.
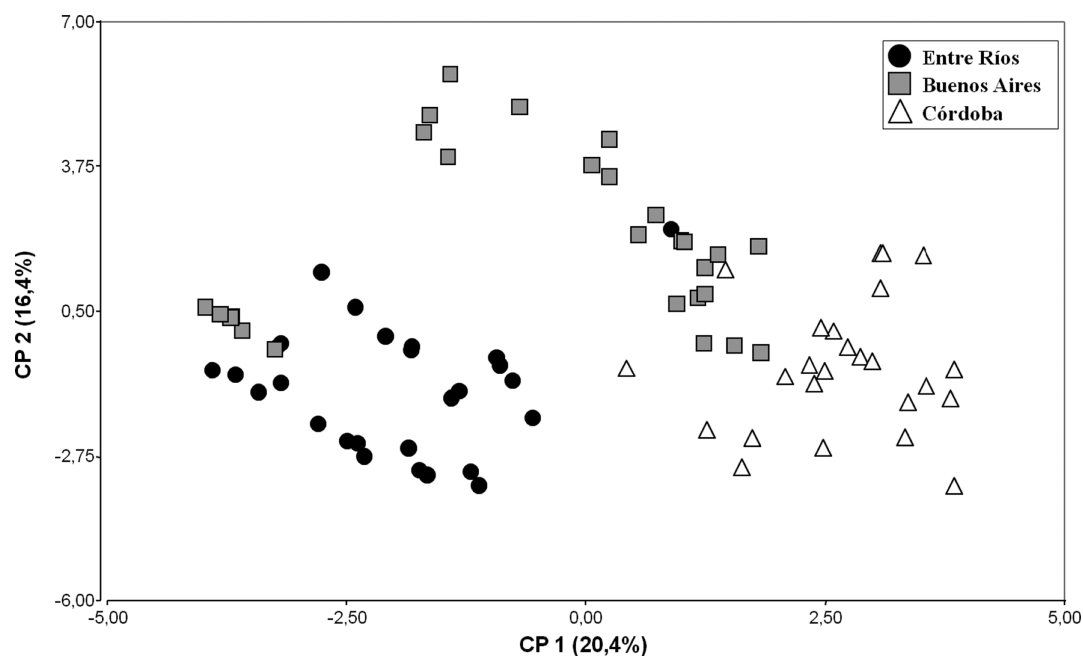
Figure 3. Principal components plot of wheat samples. The variables used were Al, B, Ba, Ca, Cd, Ce, Co, Cu, Eu, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, Pb, Rb, Sr, Zn, Ca/Sr, K/Rb, δ^{13}C, δ^{15}N, and ^{87}Sr/^{86}Sr.
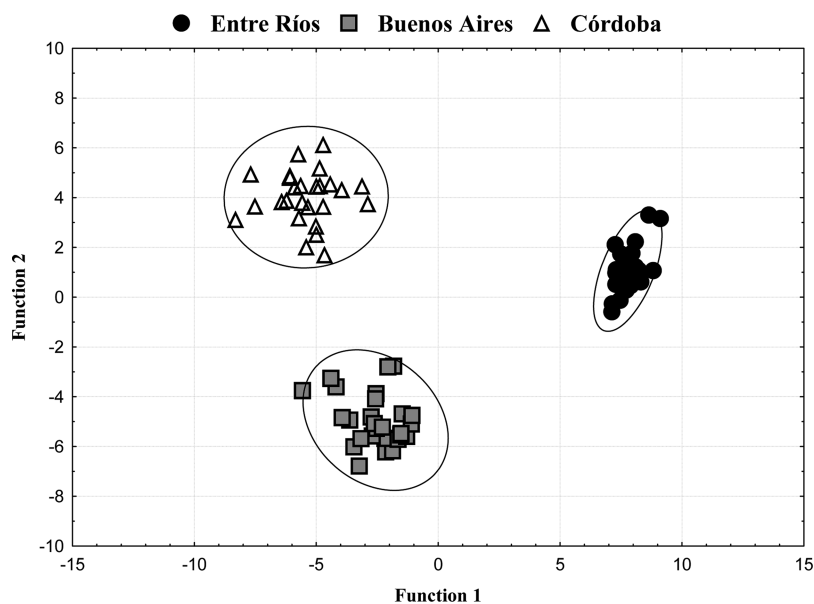
Figure 4. Scatter plot of two discriminant functions showing separation between wheat samples from different regions of origin.

Principal Components Analysis. The PCA model was obtained using four principal components (PCs), which explained 61% of the variance. PC1 accounts for 20% of the variance and was mainly characterized by K/Rb, Ca, Rb, and Cu. PC2 contributes to 17% of the variance and was mainly characterized by Eu, Na, and Mo. The scatter plot obtained from PCA scores is illustrated in Figure 3, showing that samples are separated into three ill-defined clusters.

Overall, CA and PCA results were generally in agreement with the geographical origin of samples, confirming that multielement and isotopic information could be suitable to classify wheat samples from three studied regions.

Discriminant Analysis. The application of forward stepwise discriminant analysis (F to enter = 6; T = 0.01; N step = 27)

allowed predicting the origin of all wheat samples with 100% accuracy using two independent discriminant functions. Eleven out of 27 variables give the major contribution to sample discrimination, with K/Rb as the most significant variable, followed by Ca/Sr, Ba, ^{87}Sr/^{86}Sr, Co, Mo, Zn, Mn, Eu, δ^{13}C, and Na. The scatter plot corresponding to discriminant functions (Figure 4) confirms the usefulness of this method to classify samples according to their growing region. The first function (explaining 69.9% of the total variance, mainly determined by K/Rb) separated Entre Ríos samples from Buenos Aires and Córdoba, whereas function 2 (explaining 30.1% of total variance, mainly determined by Eu) improved the discrimination between samples from Córdoba and Buenos Aires.

So far, K/Rb, Ca/Sr, $^{87}Sr/^{86}Sr$, and Eu showed a different profile in the three studied regions. Barium, Co, and $\delta^{13}C$ allowed the differentiation of samples from Córdoba from those of Buenos Aires and Entre Ríos. On the other hand, Mo, Mn, Zn, and Na allowed the differentiation of samples from Buenos Aires from those arising from Córdoba and Entre Ríos (Table 1).

As far as we know, there are no previous studies using K/Rb and Ca/Sr ratios as tracers of the geographical origin of wheat samples. Di Paola-Naranjo et al.[11] found that the Ca/Sr ratio contributed to the differentiation of wine samples from three different regions of Argentina. On the other hand, Baroni et al.[12] also found that this ratio contributed to the differentiation of meat samples from different regions of Argentina.

The $^{87}Sr/^{86}Sr$ ratio has been widely employed as a geochemical tracer. However, there are few reports on the use of Sr isotope ratios for food or beverage authentication.[7,11,12,21,22] Furthermore, to our knowledge, there is only one report using this parameter to discriminate the origin of wheat samples.[10]

On the other hand, Brescia et al.[26] found that $\delta^{13}C$, $\delta^{18}O$, and $\delta^{15}N$ contributed to the discrimination of wheat samples according to their geographical origin. However, Branch et al.[13] did not find $\delta^{15}N$ as a good indicator of geographical origin. In fact, the simplest DA model tested by Branch et al.[13] involved only $\delta^{13}C$, in good agreement with our current results.

We cannot disregard that wheat samples from Argentina have $\delta^{13}C$ mean values similar to samples from Australia and the Mediterranean region (Spain and Italy) due to the influence of the climate on $\delta^{13}C$ values.

Ba and Mn are directly linked to soil geochemistry[48] and are important elements when the authentication of geographical origin is based only on multielemental data.[17] Furthermore, Laursen et al.[14] found significantly different Ba concentrations in wheat samples arising from different Danish locations. Also Zhao et al.[27] found that Ba and Mn were good markers to discriminate wheat samples from four regions of China.

Finally, the concentration of Na is known to be associated with the occurrence of sea-spray and the deposition of sea-salt.[10] In our case, samples from Buenos Aires presented higher values of Na in comparison to Córdoba and Entre Ríos (Table 1). This could be attributed to the proximity of the sampling area in Buenos Aires to the ocean coast, in addition to the influence of winds from the sea (Figure 1).

Correlation between Soil, Water, and Wheat Composition. It is suggested that element and isotope composition in wheat reflects the local geochemistry of the soil and irrigation water but can also be influenced by cereal variety, climate, and agriculture practices.[10,49] Therefore, we were interested in evaluating the association between chemical-inorganic profiles of soil and water from the production area with the chemical-inorganic profile of wheat samples.

Some elements and isotopes exhibited a good correspondence between soil, water, and wheat in the three studied regions. For instance, wheat, soil, and water samples from Córdoba show the highest values in $^{87}Sr/^{86}Sr$, samples from Buenos Aires present the lowest, while Entre Ríos samples show intermediate values. Figure 5A clearly shows this correspondence (standardized values). In addition, Mg presented the same profile in samples from soil and wheat but not in water (Figure 5B), while Cu (Figure 5C), Li, and Mn presented good correspondence between water and wheat samples but not soil.
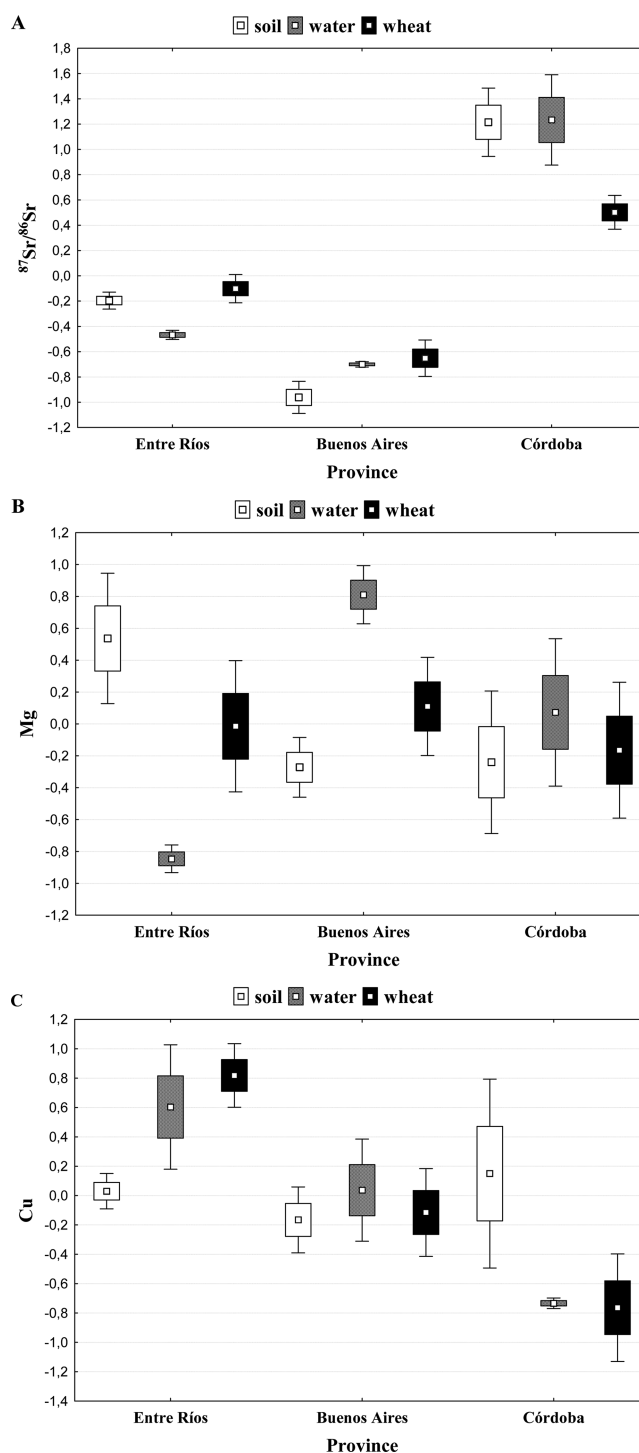

Figure 5. Correspondence between levels of several elements and isotopes in soil, water, and wheat samples from different geographical areas. Standardized values. Mean ± SE.

Looking for additional evidence on the correspondence between the three studied matrices, we decided to apply GPA. In Figure 6, the consensus configuration projected onto the plane defined by its first and second principal axes is shown, explaining 100% of variability between samples.

As shown in the Figure 6, three geographical origins are well separated on the basis of multielements and isotopes from soil, water, and wheat samples. GPA results show that data obtained from wheat have a significant consensus (99.2%) with those
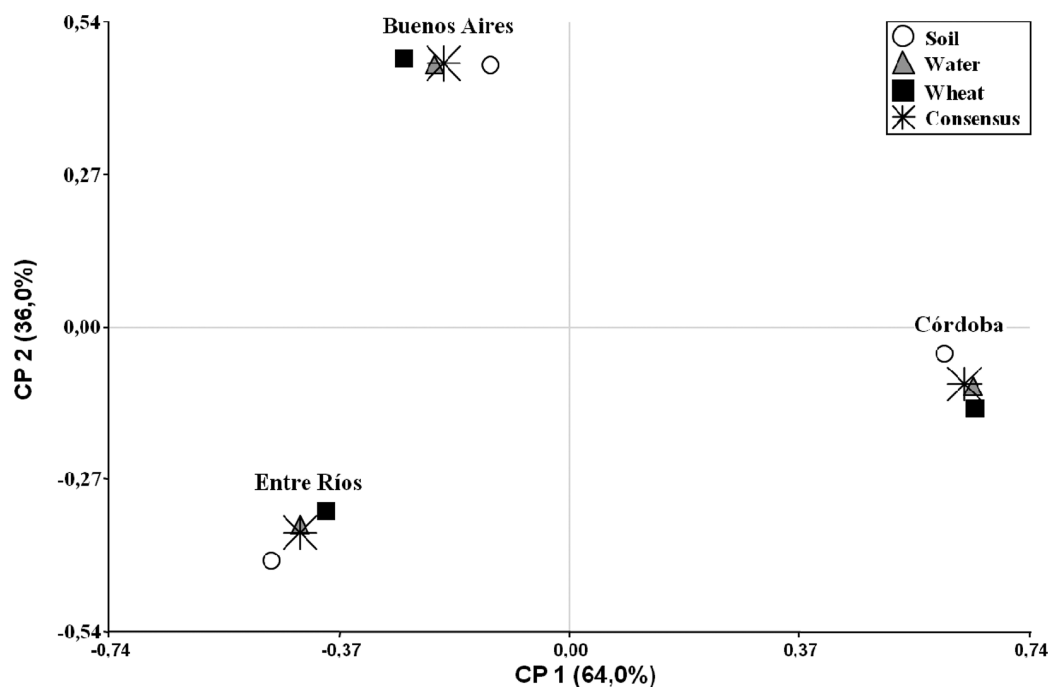
Figure 6. Consensus space from generalized Procrustes analysis: plot in the plane defined by the first two dimensions.

obtained from soil and water, as three data sets project the regions in the same way onto the plane defined by its first and second principal axes. This last result gives further indication for the connection between the three studied matrices.

Finally, we applied CCA to assess the correspondence between soil, water, and wheat composition using a more formal mathematical−statistical approach. For this purpose, four sets of variables were defined taking into account common variables between soil and wheat and between water and wheat. The first CCA was calculated between soil and wheat data sets using 23 variables (Al, B, Ba, Ca, Ce, Co, Cu, Eu, K, La, Li, Mg, Mn, Na, Nd, Rb, Sr, Zn, Ca/Sr, K/Rb, δ^{13}C, δ^{15}N, and ^{87}Sr/^{86}Sr). This CCA showed a significant correlation (r^2 = 0.97, p < 0.001) between soil and wheat. Contents of Eu, Ba, and K/Rb in soil as well as Mn, Cu, and Rb in wheat showed substantial loadings on the first canonical factor; that is, they correlated highly with this factor, meaning that these variables were those that mainly contribute to the correlation between soil and wheat. The second CCA was calculated using water and wheat data sets with 15 variables (B, Ba, Ca, Cu, Eu, K, Li, Mg, Mn, Na, Sr, Zn, Ca/Sr, K/Rb, and ^{87}Sr/^{86}Sr). This second CCA showed a significant correlation (r^2 = 0.96, p < 0.001) between water and wheat. According to the loadings on the first canonical factor, Ca, B, and Mg contents in water as well as K/Rb and Ca content in wheat were the variables that mainly contribute to the correlation between water and wheat. Therefore, both GPA and CCA coincide, showing the influence of growth environment (soil and water) on wheat composition.

All in all, isotope ratios and element concentrations in soil, water, and wheat from different geographical origin were determined. Some of the parameters show significant differences depending on the region, allowing geographical discrimination of wheat samples by suitable multivariate statistical analysis. Discriminant analysis enabled the correct classification (100%) of wheat samples coming from different regions when considering Ba, ^{87}Sr/^{86}Sr, Co, Mo, Zn, Mn, Eu, δ^{13}C, and Na as original variables. K/Rb and Ca/Sr were added,

as they proved to be the most significantly derived variables, demonstrating the importance of including these ratios in determining the origin of wheat samples.

To our knowledge, this is the first report demonstrating the correspondence between soil, water, and wheat samples by appropriate statistical methods. This shows that wheat composition is closely related to the soil and water composition. So far, generalized Procrustes analysis and canonical correlations were useful tools to evaluate such correspondence, which could be used for the analysis of wheat or any other food from diverse sources.

■ AUTHOR INFORMATION

Corresponding Author
*Tel/Fax: +54 351 433 3193/94. E-mail: dwunder@fcq.unc.edu.ar.

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Kent, N. L. Cereal crops: economics, statistics and uses. In *Technology of Cereals: Introduction for Students of Food Science and Agriculture*, fourth ed.; Kent, N. L., Evers, A. D., Eds.; Woodhead Publishing Publishers, Elsevier Science Press: Langford Lane, UK, 1994; pp 1−28.
(2) Food and Agriculture Organization. *Crop Prospects and Food Situation*. FAO's Global Information and Early Warning System: Rome, Italy, 2011; p 4.
(3) Kelly, S.; Heaton, K.; Hoogewerff, J. Tracing the geographical origin of food: the application of multi-element and analysis. *Trends Food Sci. Technol.* 2005, 16, 555−567.

(4) Peres, B.; Barlet, N.; Loiseau, G.; Montet, D. Review of the current methods of analytical traceability allowing determination of the origin of foodstuffs. *Food Control* **2007**, *18*, 228−235.

(5) Castro-Vázquez, L.; Díaz-Maroto, M. C.; De Torres, C.; Pérez-Coello, M. S. Effect of geographical origin on the chemical and sensory characteristics of chestnut honeys. *Food Res. Int.* **2010**, *43*, 2335−2340.

(6) Longobardi, F.; Casiello, G.; Sacco, D.; Tedone, L.; Sacco, A. Characterization of the geographical origin of Italian potatoes, based on stable isotope and volatile compound analyses. *Food Chem.* **2011**, *124*, 1708−1713.

(7) Voerkelius, S.; Lorenz, G. D.; Rummel, S.; Quétel, C. R.; Heiss, G.; Baxter, M.; Brach-Papa, C.; Deters-Itzelsberger, P.; Hoelzl, S.; Hoogewerff, J.; Ponzevera, E.; Van Bocxstaele, M.; Ueckermann, H. Strontium isotopic signatures of natural mineral waters, the reference to a simple geological map and its potential for authentication of food. *Food Chem.* **2010**, *118*, 933−940.

(8) Sun, S.; Guo, B.; Wei, Y.; Fan, M. Multi-element analysis for determining the geographical origin of mutton from different regions of China. *Food Chem.* **2011**, *124*, 1151−1156.

(9) Gonzalvez, A.; Armenta, S.; de la Guardia, M. Trace-element composition and stable-isotope ratio for discrimination of foods with protected designation of origin. *Trends Anal. Chem.* **2009**, *28*, 1295−1311.

(10) Goitom Asfaha, D.; Quétel, C. R.; Thomas, F.; Horacek, M.; Wimmer, B.; Heiss, G.; Dekant, C.; Deters-Itzelsberger, P.; Hoelzl, S.; Rummel, S.; Brach-Papa, C.; Van Bocxstaele, M.; Jamin, E.; Baxter, M.; Heinrich, K.; Kelly, S.; Bertoldi, D.; Bontempo, L.; Camin, F.; Larcher, R.; Perini, M.; Rossmann, A.; Schellenberg, A.; Schlicht, C.; Froeschl, H.; Hoogewerff, J.; Ueckermann, H. Combining isotopic signatures of n(87Sr)/n(86Sr) and light stable elements (C, N, O, S) with multi-elemental profiling for the authentication of provenance of European cereal samples. *J. Cereal Sci.* **2011**, *53*, 170−177.

(11) Di Paola-Naranjo, R. D.; Baroni, M. V.; Podio, N. S.; Rubinstein, H. R.; Fabani, M. P.; Badini, R. G.; Inga, M.; Ostera, H. A.; Cagnoni, M.; Gallegos, E.; Gautier, E.; Peral-García, P.; Hoogewerff, J.; Wunderlin, D. A. Fingerprints for main varieties of argentinean wines: terroir differentiation by inorganic, organic, and stable isotopic analyses coupled to chemometrics. *J. Agric. Food Chem.* **2011**, *59*, 7854−7865.

(12) Baroni, M. V.; Podio, N. S.; Badini, R. G.; Inga, M.; Ostera, H. A.; Cagnoni, M.; Gallegos, E.; Gautier, E.; Peral-García, P.; Hoogewerff, J.; Wunderlin, D. A. How much do soil and water contribute to the composition of meat? a case study: meat from three areas of Argentina. *J. Agric. Food Chem.* **2011**, *59*, 11117−11128.

(13) Branch, S.; Burke, S.; Evans, P.; Fairman, B.; Wolff Briche, C. S. J. A preliminary study in determining the geographical origin of wheat using isotope ratio inductively coupled plasma mass spectrometry with 13C, 15N mass spectrometry. *J. Anal. At. Spectrom.* **2003**, *18*, 17−22.

(14) Laursen, K. H.; Schjoerring, J. K.; Olesen, J. E.; Askegaard, M.; Halekoh, U.; Husted, S. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *J. Agric. Food Chem.* **2011**, *59*, 4385−4396.

(15) Fabani, M. P.; Arrúa, R. C.; Vázquez, F.; Diaz, M. P.; Baroni, M. V.; Wunderlin, D. A. Evaluation of elemental profile coupled to chemometrics to assess the geographical origin of Argentinean wines. *Food Chem.* **2010**, *119*, 372−379.

(16) Anderson, K. A.; Smith, B. W. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. *J. Agric. Food Chem.* **2005**, *53*, 410−8.

(17) Aguilar, E. G.; Cantarelli, M. A.; Marchevsky, E. J.; Escudero, N. L.; Cami, M. Multielemental analysis and classification of amaranth seeds according to their botanical origin. *J. Agric. Food Chem.* **2011**, *59*, 9059−9064.

(18) Camin, F.; Larcher, R.; Nicolini, G.; Bontempo, L.; Bertoldi, D.; Perini, M.; Schlicht, C.; Schellenberg, A.; Thomas, F.; Heinrich, K.; Voerkelius, S.; Horacek, M.; Ueckermann, H.; Froeschl, H.; Wimmer, B.; Heiss, G.; Baxter, M.; Rossmann, A.; Hoogewerff, J. Isotopic and elemental data for tracing the origin of European olive oils. *J. Agric. Food Chem.* **2010**, *58*, 570−577.

(19) Farquhar, G. D.; Ehleringer, J. R.; Hubick, K. T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 503−537.

(20) O'Leary, M. H. Environmental effects on carbon isotope fractionation in terrestrial plant. In *Stable Isotopes in the Biosphere*; Wada, E., Yoneyama, T., Mingawa, M., Ando, T., Fry, B. D., Eds.; Kyoto University Press: Kyoto, JA, 1995; pp 78−91.

(21) García-Ruiz, S.; Moldovan, M.; Fortunato, G.; Wunderli, S.; García Alonso, J. I. Evaluation of strontium isotope abundance ratios in combination with multi-elemental analysis as a possible tool to study the geographical origin of ciders. *Anal. Chim. Acta* **2007**, *590*, 55−66.

(22) Kawasaki, A.; Oda, H.; Hirata, T. Determination of strontium isotope ratio of brown rice for estimating its provenance. *Soil Sci. Plant Nutr.* **2002**, *48*, 635−640.

(23) Camin, F.; Larcher, R.; Perini, M.; Bontempo, L.; Bertoldi, D.; Gagliano, G.; Nicolini, G.; Versini, G. Characterisation of authentic Italian extra-virgin olive oils by stable isotope ratios of C, O and H and mineral composition. *Food Chem.* **2010**, *118*, 901−909.

(24) Suzuki, Y.; Chikaraishi, Y.; Ogawa, N. O.; Ohkouchi, N.; Korenaga, T. Geographical origin of polished rice based on multiple element and stable isotope analyses. *Food Chem.* **2008**, *109*, 470−475.

(25) Pilgrim, T. S.; Watling, R. J.; Grice, K. Application of trace element and stable isotope signatures to determine the provenance of tea (*Camellia sinensis*) samples. *Food Chem.* **2010**, *118*, 921−926.

(26) Brescia, M. A.; Di Martino, G.; Fares, C.; Di Fonzo, N.; Platani, C.; Ghelli, S.; Reniero, F.; Sacco, A. Characterization of Italian Durum Wheat Semolina by Means of Chemical Analytical and Spectroscopic Determinations. *Cereal Chem.* **2002**, *79*, 238−242.

(27) Zhao, H.; Guo, B.; Wei, Y.; Zhang, B.; Sun, S.; Zhang, L.; Yan, J. Determining the geographic origin of wheat using multielement analysis and multivariate statistics. *J. Agric. Food Chem.* **2011**, *59*, 4397−4402.

(28) Wunderlin, D. A.; Díaz, M. P.; Amé, M. V.; Pesce, S. F.; Hued, A. C.; Bistone, M.Á. Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquía River Basin (Córdoba − Argentina). *Wat. Res.* **2001**, *35*, 2881−2894.

(29) Faure, G. *Origin of Igneous Rocks: The Isotopic Evidence*, 1st ed.; Springer-Verlag: Berlin, Germany, 2001.

(30) Steiger, R. H.; Jäeger, E. Subcommission on Geochronology: convention on the use of decay constants in geo- and cosmochronology. *Earth Planet. Sci. Lett.* **1977**, *36*, 359−362.

(31) Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W. *Infostat Versión*; Grupo InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina, 2010.

(32) Di Rienzo, J. A.; Macchiavelli, R.; Casanoves, F. Modelos Mixtos en InfoStat. *Infostat Versión*; Grupo InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina, 2010.

(33) Di Rienzo, J. A.; Guzmán, A. W.; Casanoves, F. A multiple comparisons method based on the distribution of the root node distance of a binary tree. *J. Agric. Biol. Environ. Stat.* **2002**, *7*, 1−14.

(34) Peltola, P.; Brun, C.; Mats, A.; Tomilina, O. High K/Rb ratios in stream waters—exploring plant litter decay, ground water and lithology as potential controlling mechanisms. *Chem. Geol.* **2008**, *257*, 92−100.

(35) Pett-Ridge, J. C.; Derry, L. A.; Barrows, J. K. Ca/Sr and 87Sr/86Sr ratios as tracers of Ca and Sr cycling in the Rio Icacos watershed, Luquillo Mountains, Puerto Rico. *Chem. Geol.* **2009**, *267*, 32−45.

(36) Dunn, C. E. Biogeochemistry in Mineral Exploration. *Handb. Explor. Environ. Geochem.* **2007**, *9*, 1−460.

(37) Kabata-Pendias, A. In *Trace Elements in Soils and Plants*, 3rd ed.; CRC Press: Boca Raton, FL, 2001.

(38) Burton, J. H.; Price, T. D.; Middleton, W. D. Correlation of Bone Ba/Ca and Sr/Ca due to Biological Purification of Calcium. *J. Archaeol. Sci.* **1999**, *26*, 609−616.

(39) Deines, P. The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry*; Fritz, A. P., Fontes, J. C., Eds.; Elsevier: Amsterdam, 1980; Vol. *1*, pp 329−406.

(40) Bullen, T. D.; Kendall, C. Tracing of Weathering Reactions and Water Flowpaths: A Multi-isotope Approach. In *Isotope Tracers in Catchment Hydrology*; Kendall, C., McDonell, J. J., Eds.; Elsevier Science Press: Amsterdam, 1998; pp 611−646.

(41) Jonasson, S.; Medrano, H.; Flexas, J. Variation in leaf longevity of *Pistacia lentiscus* and its relationship to sex and drought stress inferred from leaf δ^{13}C. *Funct. Ecol.* **1997**, *11*, 282−289.

(42) Werth, M.; Kuzyakov, Y. ^{13}C fractionation at the root-microorganisms-soil interface: a review and outlook for partitioning studies. *Soil Biol. Biochem.* **2010**, *42*, 1372−1384.

(43) Larsen, T.; Gorissen, A.; Henning Krogh, P.; Ventura, M.; Magid, J. Assimilation dynamics of soil carbon and nitrogen by wheat roots and Collembola. *Plant Soil* **2007**, *295*, 253−264.

(44) Evans, R. D. Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci.* **2001**, *6*, 121−126.

(45) Fraser, R. A.; Bogaard, A.; Heaton, T.; Charles, M.; Jones, G.; Christensen, B. T.; Halstead, P.; Merbach, I.; Poulton, P. R.; Sparkes, D.; Styring, A. K. Manuring and stable nitrogen isotope ratios in cereals and pulses: towards a new archaeobotanical approach to the inference of land use and dietary practices. *J. Archaeol. Sci.* **2011**, *38*, 2790−2804.

(46) Dalal, R. C.; Strong, W. M.; Cooper, J. E.; King, A. J. Relationship between water use and nitrogen use efficiency discerned by ^{13}C discrimination and ^{15}N isotope ratio in bread wheat grown under no-till. *Soil Tillage Res.* **2013**, *128*, 110−118.

(47) Faure, G. *Principles of Isotope Geology*, 2nd ed.; Wiley: NewYork, 1986.

(48) Kment, P.; Mihaljevic, M.; Ettler, V.; Sebek, O.; Strnad, L.; Rohlová, L. Differentiation of Czech wines using multielement composition−a comparison with vineyard soil. *Food Chem.* **2005**, *91*, 157−165.

(49) Zhao, X. L. Effects of nitrogen and phosphorus fertilization and sowing date on dynamic changes of grain sedimentation value during grain filling stage of spring wheat. *Chin. J. Appl. Ecol.* **2006**, *17*, 640−646.