STABLE ISOTOPE AND MULTI-ELEMENT TECHNIQUES FOR DETERMINATION OF GEOGRAPHICAL ORIGIN OF TURKISH RICE

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Abstract

Osmancık-97 is an important Turkish rice cultivar with high quality and it is often targeted for the adulteration with imported varieties. Multiple elements and stable isotope profile were investigated for their use to trace geographical origin of Turkish rice (*Oryza sativa* L.) samples. Fourteen key variables Copper (Cu), Zinc (Zn), Iron (Fe), Nickel (Ni), Chromium(Cr), Aluminium (Al), Strontium (Sr), Carbon (C), Nitrogen (N), Oxygen (O) and Hydrogen (H) contents and stable isotopes (carbon, nitrogen, and oxygen isotopic compositions) were identified by "stepwise" canonical discriminate analysis (CDA) of Turkish rice. For all samples $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values are contributed from -25.99 to -28.15‰, +3.27 to +7.71‰ and +22.32 to 27.08‰, respectively. Thus, the comparison of isotope profile and multiple element contents of the rice is a good tool for rice authentication.

Keywords: Authenticity, geographical origin, IRMS, Osmancık 97, rice

TÜRK PİRİNCİNİN COĞRAFİ MENŞEİ BELİRLENMESİNDE DURAYLI İZOTOP VE MULTİ- ELEMENT TEKNİKLERİNİN KULLANIMI

Özet

Osmancık-97, yüksek kalitede bir Türk pirinç çeşidi olup ve genellikle düşük kalite ve fiyatlı ithal çeşitleri karıştırılarak satılmak suretiyle tağşiş gerçekleştirilen bir pirinç çeşidimizdir. Ülkemiz pirinç örneklerinin coğrafi orijinin belirlenmesi amacıyla gerçekleştirilen çalışmada çoklu element ve kararlı izotop oran analizler verileri birlikte kullanılmıştır. Araştırmada pirinç örnekleri Bakır (Cu), çinko (Zn), demir (Fe), Nikel (Ni), krom (Cr), alüminyum (Al), stronsiyum (Sr), karbon (C), azot (N), oksijen (O) ve hidrojen (H), olmak üzere on dört element bileşimi ve karbon, azot ve oksijen kararlı izotop oranı değerleri ölçülmüştür. Elde edilen veriler kanonik diskriminant analizi (CDA) ile istatistiksel olarak anlamlandırılarak gruplamalar gerçekleştirilmiştir. Pirinç örneklerine ait $\delta^{13}C$ için $\delta^{15}N$ ve $\delta^{18}O$ değerleri sırasıyla; -25,99 den -28,15‰, +3.27 ile +7.71‰ ve +22,32 ile + 27.08‰, olarak saptanmıştır. Bu değerlendirmeler ışığında pirinç örneklerinin $\delta^{13}C$, $\delta^{15}N$ ve $\delta^{18}O$ kararlı izotop oranları ve elemental bileşim oranlarına göre orijin sınıflandırmasının başarılı sonuç verdiği saptanmıştır.

Anahtar kelimeler: IRMS, coğrafik işaretleme, pirinç

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INTRODUCTION

Rice is the seed of a monocot plant "*Oryza sativa* L.*", from the grass family (Poaceae). It is an important stable food for Turkey as well as more than half of the world's population. Climate and soil provisions of Turkey are appropriate for raising this plant. Turkey has tended to increase rice production in recent years and has been the position of resellers of around the country as a result of it. In particular, Osmancık-97, a cultivar of Turkish rice is the highest quality rice and has sometimes been targeted for the adulteration. High quality Osmancık- 97 rice varieties are sold mixed with poor quality imported rice in the rice market, which distorts the image. Osmancık-97 rice is originally from Osmancık province of the city Çorum, Turkey and it is mostly grown under the rain-fed conditions. It was first released in 1997 and its growing region has been increasing over the years in all the rice growing regions in Turkey. Today's Osmancık-97 species constitute more than 80% of the rice cultivated area in Turkey (1). The expansion of global trade has also promoted the international trade of rice. As the global economy expands, agricultural products including rice are inevitably labelled with their geographical origin in many countries for consumer desire.

The characterization of the geographical origin of foodstuff is continuously gaining interest from the consumers and the producers since it may be used as a criterion for the denomination of the material as a "traditional product". One of the most reliable techniques for determination of authenticity is to measure isotopes. Most elements occur in natural bio-systems as mixtures of stable isotopes. Thus, various types of analytical techniques have been developed to arrange of the source or geographical origin of agricultural products. Most commonly applied analytical methods include isotope ratio mass spectrometry (IRMS) and the isotopic composition of light elements such as H, C, N, O and S. Stable isotope ratio analysis has a wide range of applications and unlike quantitative methods, it does not focus directly on the amount of certain compounds present in the food, but studies the isotopic composition of selected elements to infer information on the product's origin (2). This techniques provide interesting information about the origin of food products, since they are governed by the geo-climatic condition under which the plant (3). The isotopic composition of oxygen in plants is the most complicated among light isotopes since there are multiple sources of this element, which is absorbed via leaf system as atmospheric O_2 and CO_2 and via the root system as liquid H_2O. The dependence of the oxygen isotopic ratio on geo-climatic conditions is at the base of the geographical origin profiling of food products, while the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios and hydrogen and oxygen contents of agricultural products generally show climatic characteristics of cultivation areas (4). Recently identification of isotopic composition of fruit juices, wine, olive oil, fish, meat and dairy products has been used to prevent adulteration (5,6).

The level of trace elements depends on the topography and soil characteristics; hence for the application of this to geographic origin studies, a unique soil map is needed for every country (3). Trace element composition in rice is primarily affected by the water and soil composition as well as the composition of any fertilizer used (7). The significant influence of the soil mineral nutrients on plants has led to many studies of the geographical origins of foods using multi-elements ICP-OES analysis. Studies have demonstrated that the inclusion of elemental composition in rice grains is capable of providing good geographical discrimination of rice (3, 7, 8). Most of the studies concerning the geographical origins of rice were limited to rice samples from a single country, therefore with the current extent of globalization in trade; there is a need to establish rice geographical origin data across the world (2, 9).To improve the protection level of consumer and honest producers, an analytical technique capable of objective determination of the indicated geographical origin of rice is fundamental for the implementation of the law.

In this study, the selected analytical approach involved the use of multi-element isotope and trace elements analysis by IRMS and ICP/OES, respectively. Also an additional comparison study was performed between the carbon isotope measurements by conventional mass spectrometry versus spectroscopic techniques. The aim of this work is to evaluate the discrimination of the geographical origin of Turkish (Osmancık-97)

and imported rice sample in the Turkish market using combined data from multi-element isotope and trace element analysis and with the processing by statistical approaches.

MATERIALS AND METHODS

Materials

The study was conducted on 50 rice samples with varying origins 19 of which were from local producers in Osmancık, Çorum, Turkey (Local Osmancık), 20 of which were local variety from Turkish market place (Turkish variety) and 11 of which were imported varieties obtained from Turkish market (Imported). These rice samples are classified in three groups as indicated. The production areas of the Turkish samples were distributed throughout 37 prefectures out of 39 was included. Each of the samples was taken from a different lot. The large number of Turkish samples, compared to those from other countries is more or less proportional to the ratio production according to Turkish Statistical Institute (TUIK) in the last ten years. According to the TUIK data also emerged that about 20% of Turkish Osmancık-97 rice was produced in northern Turkey.

Determination of Isotope Ratios and Multi-element Concentrations

Dried rice representative samples (100–150 g) were ground to a fine powder before analysis. For carbon and nitrogen, the powdered rice was weighed 7 mg into tin capsule. Then, each sample was analyzed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) using a Vario ISOTOPE cube. For oxygen, the powdered rice was weighed 1 mg into silver capsule. Then, oxygen isotope analysis was carried out by using Vario PYRO cube+IRMS. The δ notation is used to describe the isotopic difference between the sample and the international standard, and defined as the following equation (1),

$$\delta\ (‰) = (R_{sample} - R_{standart}) / R_{standart} \times 1000 \qquad (1)$$

Where R_{sample} is the isotope ratio (*i.e.*, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$) of the sample, and $R_{standart}$ is the isotope ratio of the international standards: for carbon: Peedee Belemnite (PDB); for nitrogen: Air; and for oxygen: Standard Mean Ocean Water (SMOW). Each isotope value is given as per mile, ‰. C, N and O isotopic compositions can normally

be measured with analytical uncertainty of ±0.2 to 0.3‰. Additionally sample values were corrected using running standards, i.e. Benzoic Acid (IAEA-601 and IAEA-602) with certified IAEA were used for carbon, nitrogen and Oxygen, respectively. All of the samples were analyzed three times with reliable reproducibility. C, O, N and H (by difference) contents are given % of dry w/w.

Determination of trace element concentrations

Rice samples were ground to a fine powder before analysis, with particle size lower than 250 μm, before sample digestion. The microwave-assisted treatment was adapted from that of Gonzálvez (7) according to the microwave laboratory system manufacturer recommendations Cu, Zn, Fe, Ni, Cr, Al, and Sr analysis of previously digested rice samples were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES Perkin Elmer Model Optima 7000 spectrometer). The operating conditions of the ICP-OES equipment were as follow: 15 L/min plasma gas flow rate, 0.2 L/min auxiliary gas flow rate, 0.8 L/min nebuliser gas flow rate, 1300 W RF power, 1.5 mL/min sample flow rate with 15 s time flush.

Statistical analysis

The canonical discriminate analysis (CDA) method were applied to combined isotopes and element data obtained from Osmancık-97 collected from Osmancık-Çorum, Osmancık-97 collected from the rest of Turkey and imported samples (from USA, Europe and Thailand *etc.*) collected from markets. This approach was used to find combination of functions based on isotope and multi element values that maximized the separation between countries of origin. The CDA was employed for fourteen variable quantities, Cu, Zn, Fe, Ni, Cr, Al, Sr, C, N, O and H contents and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values with using the PASW® Statistics Ver.18.0. For the analysis, data were standardized as mean values of one and variations of one. The Holdout Cross-validation was used to check the reliability of the chemometric models in terms of classification ability (10).

RESULTS AND DISCUSSION

Table 1 shows the mean value of isotope ratios and mineral concentrations in collected rice samples from Turkish origin and imported groups. Compared to Turkish samples (Local Osmancık and Turkish variety) the mean concentrations of mineral nutrients such as Fe, Al and Sr are rather high in the Local Osmancık and imported samples (Imported). Therefore, strontium contents of the Turkish rice samples obtained in this study can be regarded as a reasonable representation of the discrimination from imported sample (Fig 1). We found that the comparison of Ni is also difficult because there is lack of information for group Turkish variety and Imported. It has been reported that Ni is a highly mobile element (11). In general, elemental concentration range of Turkish rice is similar to the imported rice. The nitrogen and carbon contents in all groups of the samples were found to be centred at about 1.16%, and 37.80% respectively. The oxygen content showed a slight variation at around 47% among the group of samples. Similarly, the hydrogen content in group Turkish variety and imported were almost the same at 13.90 and 14.10% but that of Local Osmancık samples were slightly lower 12.56 % (P<0.05).

The δ^{15}N variation of the rice samples ranged from 1.53‰ to 8.08‰, using a box plot (Fig. 2) it was observed the variation in the δ^{15}N values is larger than that of the δ^{15}N values in general and one striking observation is that the rice sample

from group Turkish variety had the highest δ^{15}N values. The observation that rice samples from group Imported had δ^{15}N values lower than the mean whereas those from group Local Osmancık and Turkish variety had δ^{15}N values higher than the mean (Fig 4) may suggest different agricultural practice. Organic fertilizers generally raise the ^{15}N content in soil and plants whereas the uses of synthetic fertilizers decrease it (6). The δ^{18}O variation of the rice samples ranged from 22.32‰ to 27.08‰ In general there was a significant variation in the δ^{18}O values in the groups. As the Figure 3 indicates, an interesting observation is that the rice samples from Turkey (Local Osmancık and Turkish variety) posses noticeably higher δ^{18}O value than from import samples (Imported).

In order to illustrate the contribution of O-C elements to the canonical discrimination, the δ^{18}O and δ^{13}C values of rice samples have been plotted as an X-Y scatter graphs in Fig 4. The
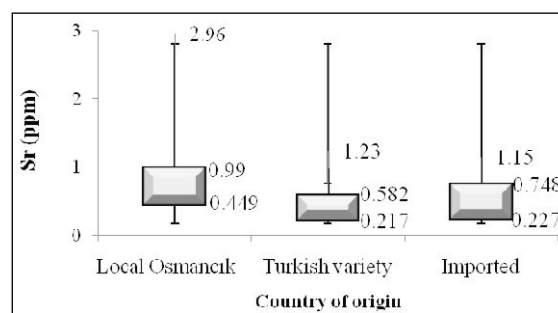

Fig 1. Box plot showing Sr (ppm) variations of for all rice according to the groups

Table 1. The mean analytical results of rice sample from each group and results of multiple comparisons between groups*

Isotopes and elements	Local Osmancık (n=19, Mean±SD)	Turkish variety (n= 20, Mean±SD)	Imported (n=11, Mean±SD)
_15N	+5.04±0.9d	+6.28±1.0e	+4.27±1.1d
_13C	-26.69±0.3a	-26.45±0.3a	-27.11±1.0b
_18O	+26.00±0.6f	+25.26±0.9g	+24.28± 0.03h
N %	1.12±0.09c	1.16±0.08c	1.16±0.06c
C %	38.63±2.2i	37.37±3.7i	37.4 5±3.9i
O %	47.64±0.5j	47.55±1,1j	47.27±0.7j
**H %	12.56±2.4e	13.90±4.1e	14.10±0.4e
***Cu	6.232±0.9v	5.865±0.8v	5.286±0.9v
***Ni	5.497±0.2k	nd±l	nd±m
***Zn	28.48±0.8r	33.85±0.7r	32.31±0.4r
***Cr	0.690±0.1n	0.826±0.5n	0.407±0.7n
***Al	4.326±0.2s	1.850±0.8t	2.699±0.5s
***Sr	0.990±0.8p	0.582±0.5p	0.748±0.5p
***Fe	4.803±0.6u	4.014±0.5u	3.629±0.9u

*Different letters mean significantly different results (P<0.05): SD means standard deviation.
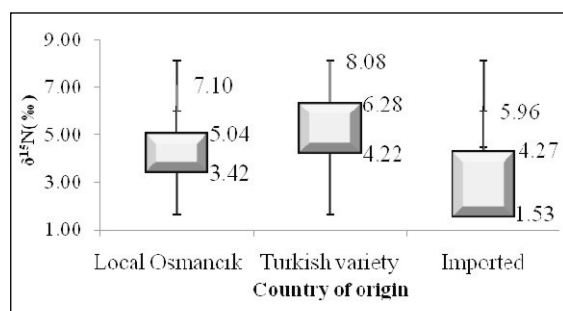** Obtained by difference
***mg/kg

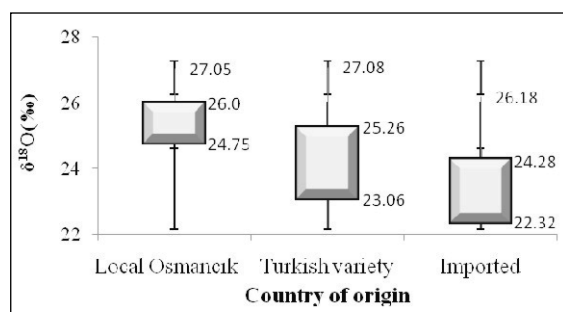Fig 2. Box plot showing δ¹⁵N (‰) variations of for all rice according to the groups

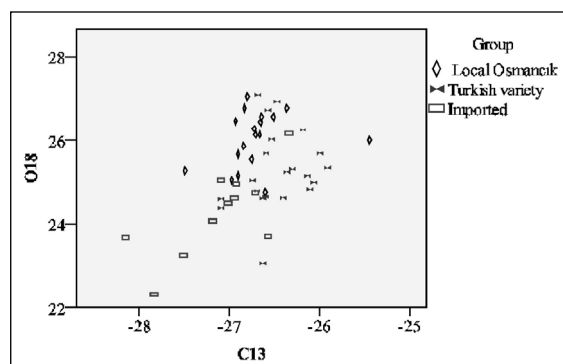Fig 3. Box plot showing δ¹⁸O (‰) variations of for all rice according to the groups

Fig 4. Distributions of ¹⁸O/¹⁶O, and ¹³C/¹²C isotopic composition of whole rice samples.

mean value of $\delta^{18}O$ is found to be +24.28‰ for imported samples followed by the Turkish variety of samples which have the next highest set of $\delta^{18}O$ values with a mean value of +25.26‰. The rice samples classified in group Local Osmancık collected from Çorum-Osmancık have the highest mean $\delta^{18}O$ values of +26.00‰. The imported rice samples have relatively lower $\delta^{13}C$ values with a mean value of -27.11‰. The group Turkish variety samples relatively enriched in $\delta^{13}C$ with a mean of -26.45‰ compared to the imported samples. The Local Osmancık rice samples are the most enriched one in terms of 13C with a mean $\delta^{13}C$ value of -26.69‰.

The main principle of CDA method is to find a combination of functions based on isotope and elemental values that maximize the separation of Osmancık-97 (Local Osmancık and Turkish variety) and other group of samples. The concentrations of isotopes were significantly different (P<0.05) between groups as determined by multiple comparisons. For all samples C, N, O and H contents and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values are contributed from 29.16 to 40.26%, 0.96 to 1.37%, 46.03 to 51.60%, 9.99 to 24.23%, -25.99 to -28.15‰, +3.27 to +7.71‰ and +22.32 to +27.08‰, respectively. These C, N, O and H contents and isotopic compositions are consistent with those of general plant material (12). Turkish rice samples (Local Osmancık and Turkish variety) showed a higher $\delta^{15}N$ value than import rice samples and clearly discriminated from group Imported samples. Nitrogen isotopic composition of rice is thought to depend mainly on soil nutrition. Although, organic fertilizers generally increase the $\delta^{15}N$ content in soil and plants, the utilization of artificial fertilizers decreases it. In fact, in Australia, many farmers rotate the rice crops with pasture crops over several years. This agricultural cycle in Australia may be consistent with the high $\delta^{15}N$ value of the Australian rice as determined several researchers (2, 13). The first group of rice samples (*n*=19) collected from ten different cultivation regions have average values of +5.04±0.9 ‰ in $\delta^{15}N$, -26.69±0.3‰ in $\delta^{13}C$, and +26.0±0.6‰ in $\delta^{18}O$. When the average value of each region is compared, the heavier $\delta^{15}N$ is found in the Turkish variety samples with the value of +6.28‰ whereas the lighter value is found in imported samples (Imported) with +4.27‰. When the $\delta^{13}C$ values are compared, it has been found that the heaviest value of -26.45‰ is observed in Turkish variety group, whereas the lightest value of -27.11‰ is seen in group Imported samples. For $\delta^{18}O$ values the heaviest value of +26.00‰ is found in the Local Osmancık group samples, whereas the lightest value of +24.28‰ is found in imported samples. Notably, Local Osmancık sample was enriched in $\delta^{18}O$, whereas imported sample was enriched in $\delta^{12}C$, $\delta^{14}N$, and $\delta^{16}O$. Because the Local Osmancık group sample has higher value of $\delta^{18}O$ compared to other group of samples, they can clearly be distinguished from the second and third group of samples.

In this study, fourteen variables including $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$, Cu, Zn, Fe, Ni, Cr, Al, Sr, C, N, O and H contents were selected by the software for discrimination. Function 1 (70.4% of variance) which provides the main separation between the group Local Osmancık and Turkish variety rice samples was primarily correlated with $\delta^{13}C$, $\delta^{15}N$, carbon and nitrogen contents. Function 2 (29.6% of variance) which provides the discrimination between the group Local Osmancık and Imported rice samples was primarily correlated with $\delta^{18}O$, $\delta^{15}N$, Sr, and Ni contents. According to Box's of equality of covariance matrices test results of CDA, for the groups to determine the geographical origin of the rice sample is homogeneous in its inside, and it can be used for this purpose.

In 2008, Suzuki et al. (2), Investigated the geographical origins of a single rice cultivar, the Koshihikari rice, from Australia, Japan and USA. In the study, rice samples from different origins were clearly discriminated from Japanese rice by high $\delta^{15}N$ or high $\delta^{18}O$ values. The other geographical traceability study of rice was carried out by Gonzálvez et al. (7), on the "Arròs de Valencia" rice grain using mineral element composition. From the study, lanthanides, cadmium and cobalt have been identified as the most influential indicators of the geographical origin of rice samples (4).

The results of the CDA analysis, 89.5% of the samples in group Local Osmancık, 80.0% of samples in group Turkish variety and 81.8% of samples in group Imported can correctly be classified. According to the CDA analysis results 84.4% of original grouped cases correctly classified. The inclusion of the stable isotope ratio results, together with the multi-element data, in the geographical origin discrimination of the rice samples by CDA was found to be more powerful in providing distinction by group of origin slight improvement to the rice geographical differentiation. This was supported statistically by the increase of the original grouped cases correctly classified from 83.3% to 84.4%. All rice samples can be successfully classified into three groups representing corresponding origins during model training process. Holdout Cross-validation was selected during the model cross-validation procedure to evaluate the robustness of the developed model. As shown in Table 2, 92.3% of the samples in group cases selected Local Osmancık, 64.3% of samples in group cases selected Turkish variety and 100.0% of samples in group cases selected Imported can correctly be classified. According to the cross-validation results 82.4% of original grouped cases correctly classified.

CONCLUSIONS

Our study demonstrated that both trace-element composition and stable isotope profile are important parameters for establishing scientifically the geographic origin of foods by selecting appropriate chemometric data treatments. The 11 elements and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ isotopic ratios were selected for stable isotopes and the multi-element compositions for discriminate analysis. The elemental variations in the rice

Table 2. Classification of rice sample from each group and percentages of observations correctly classified[a,b]

		Group	Predicted Group Membership			
			Local Osmancık	Turkish variety	Imported	Total
Cases Selected	Original Count	Local Osmancık	12	1	0	13
		Turkish variety	1	9	4	14
		Imported	0	0	7	7
	%	Local Osmancık	92.3	7.7	0	100.0
		Turkish variety	7.1	64.3	28.6	100.0
		Imported	.0	.0	100.0	100.0
Cases Not Selected	Original Count	Local Osmancık	5	1	0	6
		Turkish variety	0	6	0	6
		Imported	0	1	3	4
	%	Local Osmancık	83.3	16.7	.0	100.0
		Turkish variety	.0	100.0	.0	100.0
		Imported	.0	25.0	75.0	100.0

[a] 82.4 % of selected original grouped cases correctly classified.
[b] 87.5 % of unselected original grouped cases correctly classified.

samples of the different origins could possibly improve the discriminate study. The rice from Osmancık (Local Osmancık) and other part of Turkey (Turkish variety) are clearly distinguished from imported samples (Imported) by high $\delta^{15}N$ or high $\delta^{18}O$ values. Thus comparison of Cu, Zn, Fe, Ni, Cr, Al, Sr, C, N, O, and H contents and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values would potentially be applied for rapid and routine analyses of geographical origin of rice. However, further investigation is still needed to test whether this discrimination method based on the observed difference in elemental and isotopic compositions can be generalized to all Turkish rice as well as other cultivars. At least, the proposed methods clearly indicate that it can be used as a tool to discriminate geographical origin of Turkish, "Osmancık-97" rice.

Acknowledgements

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