DETERMINATION OF THE GEOGRAPHICAL ORIGIN OF SLOVENIAN POTATO AND GARLIC, BASED ON STABLE ISOTOPE AND ELEMENTAL ANALYSES - PRELIMINARY RESULTS

Anja Mahne Opatić[1,2], *Marijan Nečemer*[3], *Sonja Lojen*[1]

[1]Department of Environmental Sciences O-2, "Jožef Stefan" Institute, Jamova cesta 39, Ljubljana, Slovenia, anja.mahne00@gmail.com
[2]International Postgraduate School Jožef Stefan, Jamova cesta 39, Ljubljana, Slovenia, anja.mahne00@gmail.com
[3]Department of Low and Medium Energy Physics F-2, "Jožef Stefan" Institute, Jamova cesta 39, Ljubljana, Slovenia, marijan.necemer@ijs.si
[1]Department of Environmental Sciences O-2, "Jožef Stefan" Institute, Jamova cesta 39, Ljubljana, Slovenia, sonja.lojen@ijs.si

Abstract – In this study, the applicability of stable isotope (δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O) and multi-element (K, P, S, Cl, Ca, Si, Zn, Br, Rb, Sr, Ti) data for determining the geographical origin of Slovenian organic garlic (*Allium sativum* L.) and potato (*Solanum tuberosum* L.) was examined. The discriminant analysis (DA) of obtained data suggested a distinction of four macroregions (the Alpine, the Dinaric, the Mediterranean and the Pannonian). The most significant parameters for separation were δ^{13}C, δ^{34}S, Zn, P and Cl in garlic and δ^{13}C, δ^{18}O, δ^{34}S, K, Cl and Ca in potato.

Key words: Stable isotopes; Elemental composition; Geographical origin; Slovenia

1. INTRODUCTION

In recent years, proof of provenance has become a significant issue in food safety and quality surveillance programmes and also affects consumers rights in accordance with national legislation and international standards and guidelines. Geographical origin determination is another fundamental factor used for evaluating the quality of a product [1]. Lately, the main goal of research in this field is to provide fast and robust analytical tool to check the authenticity and traceability of food products. One of the techniques, where the scope of geographical assignment of food products can be remarkably extended, is a combination of isotopic and elemental fingerprinting. An important prerequisite is the availability of databases, based on large number of authentic samples, collected over a number of years. Additionally, basic knowledge on stable isotope fractionation effects in nature also need to be increased [2].

The carbon isotopes in plants are mostly affected by metabolism (i.e. carbon fixation process such as the C-3, C-4 or crassulacean acid metabolism). Various factors can be reflected applying stable carbon isotope ratios, including isotopic compositions of source materials, assimilation processes, geological conditions (e.g. climate, altitude) [3].

Since the isotopic composition of O and H in plant water directly reflects that of its source, i.e. groundwater or precipitation, it can be used for estimation of their provenance. Namely, the isotopes in precipitation across a continent reveal consistent predictable patterns, depending on the temperature, altitude, latitude, distance from the ocean etc. [4].

The sulfur stable isotope ratio analysis of the bulk matter or defined fractions of plant could be an ideal and reliable tool for food origin and authenticity proof due to sulfur assimilation in most plants is an one-way process, which means that the total ingested element remains completely in the plant and does not depend on biochemical conversions with isotope fractionation and binding forms. Therefore, the bulk sulfur isotope ratio preserves and reflects the isotopic property of the primary material which provides sulfur as an ideal biomarker for determination of geographical origin of the sample [5].

Nitrogen isotope ratios of plant tissues are in correlation with the source nitrogen, i.e. soil nitrogen and fertilizer used, and therefore usually used to discriminate between conventional and organic production [6,7].

Similar to isotopes, the elemental composition of plants reflects the soil composition, which, on the other hand, reflects the spatial variability of the elemental composition of bedrock and soil type [8].

Slovenia is a small country, characterized by rich geological, climatological and biological diversity. Its rather small territory is situated between the Alps, the Dinaric Mountains, the Pannonian plain and the Mediterranean and for this reason very interesting for investigating geographical influences. The aim of our study was to investigate the suitability of stable isotopes ratio and multi-element analyses for classifying Slovenian organic garlic (*Allium sativum* L.) and potato (*Solanum tuberosum* L.) into geographical macroregions.

2. MATERIALS AND METHODS

2.1. Stable isotope analysis

Samples for carbon, nitrogen and sulfur isotope ratio analysis were dried in an oven at 60°C until constant weight. The analysis was carried out using an Elementar vario Pyrocube linked to IsoPrime™ continuous flow IRMS. About 10 mg of homogenized powdered garlic and potato samples for simultaneously $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ analysis were weighed, folded and compressed in a tin capsule and then introduced into autosampler. Each sample was analysed in duplicate and the average was adopted. If the difference between the replicates for any of the elements exceeded 0.2‰, the analysis was repeated.

Fresh samples of plant water for oxygen stable isotope analysis were squeezed through a gauze in order to obtain the liquid. Oxygen stable isotope ratio was measured using an IsoPrime™ IRMS and MultiFlow preparation system (IsoPrime, Cheadle, UK). The $\delta^{18}O$ values were measured in garlic and potatoe liquid after equilibration (40°C, 6h) with 5% CO_2+ He mixture.

The δ notation is used to report the difference between the heavy-to-light isotope ratio of the sample and that of the international standard, expressed in per mil (‰) as follows "(1)":

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

R is the heavy-to-light isotope ratio (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$ etc.) of the sample (R_{sample}) and the reference material ($R_{standard}$), respectively. Results are reported vs. IAEA VSMOW for oxygen, VPDB for carbon, VCDT for sulfur and atmospheric nitrogen (Air) for nitrogen. By definition, the δ value of each standard is 0‰. A positive δ-value means that the ratio of the heavy to the light isotope is higher in the sample than the standard and vice versa for the negative δ value.

The accuracy of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotopic analysis was checked with the certified reference material Protein (Casein) Standard OAS (Sercon) ($\delta^{13}C$ -26.98 ± 0.13‰, $\delta^{15}N$ +5.94 ± 0.08‰, $\delta^{34}S$ +6.32 ± 0.8‰), Sorghum Flour OAS (Sercon) ($\delta^{13}C$ -13.68 ± 0.19‰, $\delta^{15}N$ +1.58 ± 0.15‰, $\delta^{34}S$ +10.11 ± 1‰) and Wheat Flour Standard OAS (Sercon) ($\delta^{13}C$ -27.21 ± 0.13‰, $\delta^{15}N$ 2.85 ± 0.17‰, $\delta^{34}S$ -1.42 ± 0.80‰), respectively. The accuracy of the $\delta^{18}O$ isotopic analysis was checked with in-house reference materials W-3869- Marine water ($\delta^{18}O$ +0.34 ± 0.07‰), W-3870- MiliQ ($\delta^{18}O$ -9.12 ± 0.07‰) and W-3871- Snow ($\delta^{18}O$ -19.73 ± 0.09‰), respectively.

2.2. Elemental analysis

Multielement determination of macro (P, S, Cl, K, Ca, Si) and micro (Zn, Rb, Br, Sr, Ti) elemental content was performed by non-destructive Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF). The pellets were prepared from 0.5 to 1.0 g of powdered sample material by a pellet die and hydraulic press. For excitation, the disc radioisotope excitation source of Fe-55 (25 mCi) from Eckert and Ziegler was used. The emitted fluorescence radiation was measured by an energy dispersive X-ray spectrometer constituted of a Si(Li) detector (Canaberra), a spectroscopy amplifier (Canberra M2024), ADC (Canberra M8075) and PC based MCA (S-100 Canberra). The spectrometer was equipped with a vacuum chamber (Fe-55) for measurement of light elements P-Cl, and the energy resolution of the spectrometer was 175 eV at 5.9 keV. The analysis of complex X-ray spectra was performed by AXIL spectral analysis software. To evaluate the uncertainty of this procedure, it is required to include the statistical uncertainty of measured intensities and the uncertainty of the mathematical fitting procedure. The QAES (Quantitative Analysis of Environmental Samples) software, developed in our laboratory [9,10] was used. The estimated uncertainty of the analysis was 5% to 10%.

2.3. Statistical analysis

Statistical calculations were carried out using XL-STAT software package (Addinsoft, New York, USA). Discriminant analysis (DA) was used to differentiate between four geographical Slovenian regions by the multivariate model.

3. RESULTS

The garlic and potato data sets (δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O, Si, P, S, Cl, K, Ca, Ti) were subjected to the DA.

3.1. Garlic samples

The most efficient parameters for classifying garlic samples with respect to their geographical origin were stable isotopes of carbon (with average δ^{13}C -27.1‰ in Alpine, -26.4‰ in Dinaric, -27.5‰ in Pannonian and –26.4‰ in Mediterranean garlic), sulfur (with average δ^{34}S 3.5‰ in Alpine, 4.1‰ in Dinaric, 2.3‰ in Pannonian and 1.5 ‰ in Mediterranean), Zn (2.6 mg/ 100 g in the Dinaric, 2.5 mg/ 100 g in the Mediterranean, 2.4 mg/ 100 g in the Pannonian and 2.3 mg/ 100 g in the Alpine) and P (513 mg/ 100 g in the Mediterranean, 511 mg/ 100 g in the Pannonian, 409 mg/ 100 g in the Alpine and 356 in the Dinaric) in F1 and δ^{13}C, δ^{34}S, P and Cl (51.4 mg/ 100 g in the Mediterranean, 28.2mg/ 100 g in the Dinaric, 26.2 mg/100 g in the Alpine and 22.2 mg/ 100 g in the Pannonian) in F2.

Results obtained showed a slight overlap among garlic samples originated from the Alpine and Dinaric geographical regions, while the groups of samples from the Mediterranean and Dinaric regions were well separated (Fig. 1). Function 1 explained 60.83% of total variance, function 2 27.34%. Total prediction ability for garlic was 97.37%.

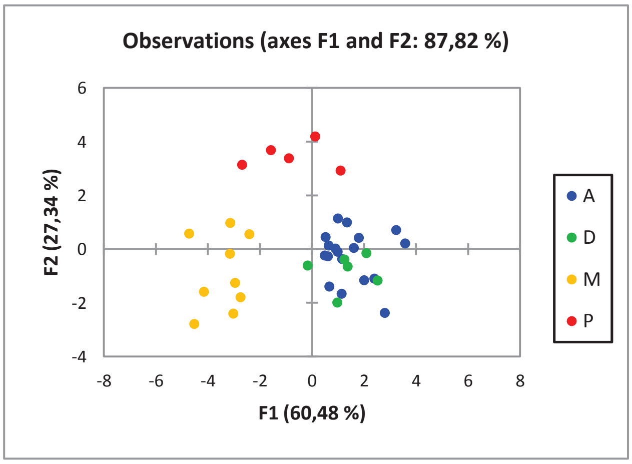

Fig. 1. Discriminant score plots of 38 Slovenian garlic samples (A = Alpine, D = Dinaric, M = Mediterranean, P = Pannonian).

3.2. Potato samples

Potato samples originated from different macroregions differed mainly in stable isotopes of carbon (with average δ^{13}C -25.0‰ in Alpine, -24.1‰ in Dinaric, -24.3‰ in Pannonian and -23.3‰ in Mediterranean potato), oxygen (with average δ^{18}O -4.5‰ in Alpine, -3.4‰ in Dinaric, -4.1‰ in Pannonian and -2.3‰ in Mediterranean garlic) and sulfur (with average δ^{34}S 4.6‰ in Alpine, 3.5‰ in Dinaric, 4.3‰ in Pannonian and 3.1‰ in Mediterranean garlic) and K (2041 mg/ 100 g in the Alpine, 1644 mg/ 100 g in the Dinaric, 1458 mg/ 100 g in the Pannonian and 1878 mg/ 100 g in the Mediterranean potato) in F1 and δ^{18}O, Cl (123 mg/ 100 g in the Alpine, 123 mg/ 100 g in the Dinaric, 190 mg/ 100 g in the Mediterranean and 70.2 mg/ 100 g in the Pannonian region) and Ca (21.0 mg/ 100 g in the Alpine, 22.5 mg/ 100 g in the Dinaric, 35.7 mg/ 100 g in the Mediterranean and18.4 mg/ 100 g in the Pannonian region) in F2.

In Fig. 2, plots of the first two functions obtained by DA for Slovenian ecological potato samples are presented. It can be observed that potato samples are successfully separated into four groups due to their geographical origin. 78.42% of total variance was explained by function 1 and 19.52% by function 2. Total prediction ability was 97.22%.

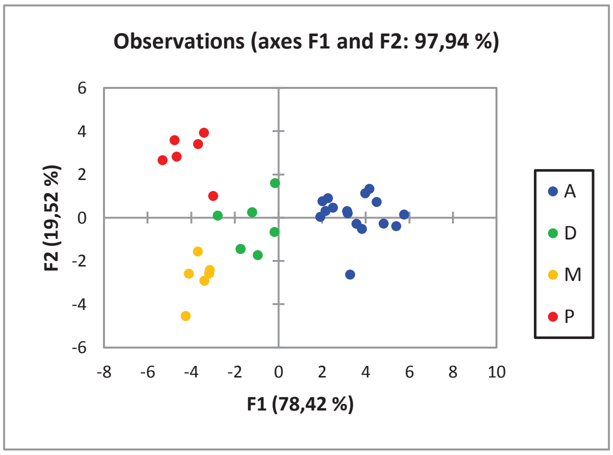

Fig. 2. Discriminant score plots of 36 Slovenian potato samples (A = Alpine, D = Dinaric, M = Mediterranean, P = Pannonian).

4. CONCLUSIONS

Stable isotopic compositions of four elements (δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O) and elemental composition (K, P, S, Si, Ca, Cl, Zn, Rb, Br, Ti, Sr) were determined in order to differentiate between geographical origin of Slovenian ecological garlic and potato samples. Results obtained exhibited definite tendency towards their respective Slovenian macroregion. A slight overlap among some samples could be attributed to similar geological and climate conditions, as well as to the natural variability of samples. The main contributions to differentiate garlic samples according to their geographical origin were $\delta^{13}C$, $\delta^{34}S$, Zn, P and Cl, while $\delta^{13}C$, $\delta^{18}O$, $\delta^{34}S$, K, Cl and Ca in potato samples were the parameters that contributed the most. Considering the results obtained, we can conclude that EA-IRMS method in combination with elemental composition determinations could be a rapid, simple and powerful method to investigate large number of samples. However, some limitations also exist. It should be noticed that further research based on large number of samples is extremely important in order to establish reliable databases and annual differences in plant materials should also be taken into account, therefore, samples need to be collected over a number of years. Nevertheless, expanded databases require more complex interpretations due to increased number of samples and their natural variation in stable isotope ratios and elemental composition.

ACKNOWLEDGMENTS

This study was financially supported by European Commission - Community Research and Development Information Service in the scope of project 621329 ISO-FOOD ERA Chair for isotope techniques in food quality, safety and traceability, and research programme P1-0143 financed by the Slovenian Research Agency.

REFERENCES

[1] Y. Zhao, B. Zhang, G. Chen, A. Chen, S. Yang, Z. Ye. *"Recent developments in application of stable isotope analysis on agro-product authenticity and traceability",* Food Chemistry, vol. 145, pp. 300-305, August 2013.

[2] L.M.A.M. Dion, S. M. van Ruth, *"An overview of analytical methods for determining the geographical origin of food products"*, Food chemistry, vol. 107, pp. 897-911, 2008.

[3] B. N. Smith, S. Epstein, *»Influence of the diet on the distribution of carbon isotope fractionation«,* Plant Physiology, 47, pp. 380-384, 1971.

[4] C. Kendall, M. G. Sklash, Bullen T.D. "*Isotope Tracers of Water and Solute Sources in Catchments"*, In: *Solute Modelling in Catchment Systems*, J. Wiley & Sons, New York, pp. 261- 303, 1995.

[5] N. Krivachy (Tanz), A. Rossman, H.-L. Schmidt. *"Potentials and caveats with oxygen and sulfur stable isotope analyses in authenticity and origin checks of food commodities"*, Food control, vol. 48, pp. 143-150, June 2015.

[6] F. Camin, M. Perini, L. Bontempo, S. Fabroni, W. Faedi, S. Magnani. *"Potential isotopic and chemical markers for characterising organic fruits"*, Food Chemistry, vol. 125, pp. 1072-1082, 2011.

[7] M. Šturm, S. Lojen. *"Nitrogen isotopic signature of vegetables from Slovenian market and its suitability as an indicator of organic production"*, Isotopes in Environmental and Health Studies, vol. 47, pp. 214-220, 2011.

[8] K. W. Kim, I. Thorthon. *»Influence of Ordovician uranieferous black shales on the trace element composition of soils and food crops«*, Applied Geochemistry, vol. 2, pp. 249-255, 1993.

[9] M. Nečemer, P. Kump, J. Ščančar, R. Jacimovic, J. Simčič, P. Pelicon, M. Budnar, Z. Jeran, P. Pongrac, , M. Regvar, , K. Vogel- Mikuš. *"Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies"*, Spectrochimica Acta Part B, vol. 63, pp.- 1240-1247, August 2008.

[10] M. Nečemer, P. Kump, K. Vogel-Mikuš, "*Use of X-ray fluorescence-based analytical techniques in phytoremediation"*, Handbook of Phytoremediation, I. A. Golubev (ed.), pp. 1-28, 2010.