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# Stable isotope ratio and elemental composition parameters in combination with discriminant analysis classification model to assign country of origin to commercial vegetables – A preliminary study





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#### ABSTRACT

Recently, increased public attention has been paid to the geographical authentication of food, including vegetables, which are considered to be one of the major health-promoting components in a balanced diet. The purpose of the present study was to investigate the suitability of the use of isotopic compositions of light elements ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{18}$ O,  $\delta^{34}$ S) in combination with multi-elemental fingerprinting (P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr) to provide rapid, robust and inexpensive screening methods for distinguishing lettuce, sweet pepper, and tomato samples according to their given country of origin (i.e., Slovenia, Austria, Spain, Morocco, Italy, Greece), and thus ensuring their traceability in terms of their authenticity. The classification efficiency of the proposed multivariate statistical models using supervised pattern-recognition analysis, namely multivariate discriminant analysis, was sufficient for rapid and robust screening purpose. The predictions of the suggested discriminant analysis models *per* kind using cross-validation leave-one-out were 86.2%, 71.1% and 74.4% for lettuce, sweet pepper and tomato, respectively. The first use of the proposed methodology on vegetable samples on European and Mediterranean scales provides a valuable and necessary contribution to the development and implementation of a new national surveillance system that can be used to trace the geographical origins of vegetables.

1. Introduction

Over the last few years, the identification and certification of the geographical origins of food have gained an increasing amount of attention. These can both be used as important factors for evaluation of food products and maintenance of the credibility of the food industry (Opara & Mazaud, 2001). Globally, large amounts of food are exchanged and transported on a daily basis, and for many consumers, geographical authentication of food is looked upon as an assurance of its quality and safety, which are both particularly weighty parameters in their conceptions and choice of food. However, safety is directly linked to traceability (also in terms of geographical origin) more often than quality, as traceability can

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provide rapider and more successful interventions in, e. g., outbreaks of foodborne situations. The aftermath of crisis situations is also economic and environmental in nature (Aung & Chang, 2014), and thus safety/quality control through the use of an appropriate geographical traceability system is more than welcome. The terms of quality and safety can refer also to different production regimes that can be linked with nutrient bioavailability, sensory qualities, microbiological contamination, and pesticide residues (Bourne & Prescott, 2002).

Regulation (EC) No 178/2002 of the European Parliament (2002) laid down the general principles and requirements of food laws as applied at all stages of the food chain (i.e., from production, processing, transport and distribution, to supply), and provided the relevant information to the appropriate authorities and to consumers. At the same time, three EU schemes provide protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialty guaranteed (TSG); these promote

and protect the names of quality agricultural products and foodstuffs from misuse and imitation (European Commission, EU quality logos, 2016).

As vegetables are one of the major components of a balanced and healthy diet, it is also important to be able to trace their geographical origin. In Slovenia, the quality of lettuce, sweet pepper and tomato is regulated according to the Rules on the Ouality of Vegetables (Official Gazette of the Republic of Slovenia 86/2000, 2000). These include not only the visual appearance of vegetables and their packaging, but also their labelling and designation of geographical origin. However, even though these regulations are in place, there are still no specified parameters and suitable analytical methodologies for monitoring the authenticity in terms of the geographical origin of production of vegetables. Moreover, no database for tracing the origins of vegetables has been created yet, even though there are several PDO and PGI designations, such as PDO for sweet peppers ("Pimiento de Herbón") and PGI for tomatoes ("Pachino"). The present study was carried out within the framework of the EU project "ISO-FOOD- Era chair for isotope techniques in food quality, safety and traceability" and Project V4-1408 with the title "Evaluation of quality and safety parameters of vegetables produced on different systems in Slovenia and abroad with the aim to establish a national quality scheme for vegetables". There are designed to initiate the creation of databanks and reliable prediction models for the identification and classification of unknown samples, and thus to control the geographical quality and safety of vegetables.

In any case, the development and/or setting-up of new approaches and systems for food traceability that can provide rapid and reliable mechanisms is needed, as well as robust analytical tools. Isotope ratio mass spectrometry (IRMS) is a technique that ensures rapid and simple accurate measurements of stable isotope compositions of bioactive elements (i.e., H, C, N, O, S; Benson, Lennard, Maynard, & Roux, 2006). On the other hand, the elemental content of macro-elements and micro-elements can be determined using energy dispersive X-ray fluorescence (EDXRF), which is a rapid, cheap and non-destructive method (Nečemer, Kump, & Vogel-Mikuš, 2010).

Techniques used for discrimination of plant or animal foods with respect to their geographical origins have mostly included stable isotopes or elemental composition analyses, either independently (Almeida & Vasconcelos, 2003; Brescia et al., 2002; Chung et al., 2016; Crittenden et al., 2007; Di Giacomo, Del Signore, & Giaccio, 2007; Furia, Naccarato, Sindona, Stabile, & Tagarelli, 2011; Luo et al., 2015; Osorio, Moloney, Schmidt, & Monahan, 2011; Schmidt, Quilter, Bahar, Moloney, & Monahan, 2005b; Schellenberg et al., 2010; Smith, 2005; Palacios-Morillo, Jurado, Alcazar, & De Pablos, 2014; Rummel, Hoelzl, Horn, Rossmann, & Schlicht, 2010; Wu et al., 2015; Zhang, Pan, & Zhu, 2012), or in combination (Bontempo et al., 2011; Kropf et al., 2010; Nečemer, Potočnik, & Ogrinc, 2016; Pilgrim, Watling, & Grice, 2010; Pillonel et al., 2003; Suzuki, Chikaraishi, Ogawa, Ohkouchi, & Korenaga, 2008). Longobardi, Casiello, Sacco, Tedone, and Sacco (2011) used the combination of stable isotopes and volatile compounds to trace the geographical origin of Italian potatoes. The application of these techniques is justified because they are influenced by various region-specific factors, such as climatic and geographical conditions in the case of stable isotopes, and lithology and soil characteristics in the case of elements. Nevertheless, stable isotopes and elemental content analysis approaches in combination have rarely been tried, especially in terms of the determination of the geographical origins of vegetables. To the best of our knowledge, the literature data related to the geographical origins of vegetables using the particular combination of proposed approaches (Bontempo et al., 2011) are very limited.

Recent investigations into the provenance of olive oils, cereals and honey through the use of stable isotopes alone or in combination with elemental concentrations (Camin et al., 2010; Goitom Asfaha et al., 2011; Schellenberg et al., 2010) have shown that Europe (at the scale of the continent) is characterised by a wide range of geographical, geological and climatological characteristics with large spatial variability. This makes Europe an interesting and demanding area of research.

The aim of this study was to investigate the suitability of the use of stable isotope ratios of light elements (i.e., C, N, O, S) in bulk vegetable samples in combination with multi-elemental analyses to provide rapid, robust and inexpensive screening methods for the control of geographical origin. This was thus based on distinguishing lettuce, sweet pepper and tomato obtained from a Slovenian market according to their given countries of origin (i.e., Slovenia, Austria, Spain, Morocco, Italy, Greece). Based on the measured parameters and the application of discriminant analysis (DA), we provide the first example in Europe and the Mediterranean of the characterisation and classification of vegetable samples into groups in terms of their geographical origins. To simulate the real conditions in the market, the regime for their production (e.g., field *vs.* greenhouse), transportation and storage conditions were not taken into account, considering the limited availability and/or access to this information.

#### 2. Materials and methods

#### 2.1. Sampling and sample preparation

Samples were bought in grocery shops in Slovenia from January to July, 2016. In total, 58 samples of lettuce, 38 samples of sweet pepper, and 43 samples of tomato from different countries (i.e., Slovenia, Austria, Spain, Morocco, Italy, Greece) were analysed (Table 1). The edible parts of the samples were oven-dried at 60 °C until constant weight, and ground to a fine powder.

#### 2.2. Stable isotope analysis

The stable isotope analysis was performed using an Elementar Vario Pyrocube elemental analyser (OH/CNS Pyrolyser/Elemental Analyser) linked to IsoPrime100 continuous flow IRMS (IsoPrime, Cheadle, Hulme, UK). About 10 mg homogenised powder were weighed and folded into a tin capsule, which was then introduced into the autosampler for simultaneous  $\delta^{13}$ C,  $\delta^{15}$ N and  $\delta^{34}$ S analyses. Each sample was analysed in three repetitions and the mean was adopted. The differences between the replicates for any of the elements did not exceed 0.2‰.

For stable isotope analysis of the oxygen in fresh samples of plant water, these were squeezed through a gauze to obtain the

Table 1
Details of the lettuce, sweet pepper and tomato samples.

Sample	Country of origin	Number of samples
Lettuce (Lactuca sativa L.)	Slovenia	14
	Austria	8
	Italy	21
	Spain	15
	Total	58
Sweet pepper	Slovenia	10
(Capsicum annuum L.)	Italy	4
	Spain	13
	Morocco	6
	Greece	5
	Total	38
Tomato	Slovenia	10
(Solanum lycopersicum L.)	Italy	8
	Spain	11
	Morocco	14
	Total	43

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