Food Chemistry 275 (2019) 333–338

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Geographical discrimination of red garlic (*Allium sativum* L.) produced in Italy by means of multivariate statistical analysis of ICP-OES data

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ARTICLE INFO

Keywords:
Garlic
ICP-OES
Mineral composition
Geographical classification
Class-modelling

ABSTRACT

Sixty-five samples of red garlic (*Allium sativum* L.) coming from four different production territories of Italy were analysed by means of inductively coupled plasma optical emission spectrometry. The garlic samples were discriminated according to the geographical origin using the content of seven elements (Ba, Ca, Fe, Mg, Mn, Na and Sr). Both classification and class modelling methods by using linear discriminant analysis (LDA) and soft independent model class analogy (SIMCA), respectively, were applied. Classification ability and modelling efficiency were evaluated on an external prediction set (21 garlic samples) designed by application of duplex Kennard-Stone algorithm. All the calibration and prediction samples were correctly classified by means of LDA. The class models developed using SIMCA exhibited high sensitivity (almost all the calibration and external samples were accepted by the respective classes) and good specificity (the majority of extraneous samples were refused by each class model).

1. Introduction

Garlic (*Allium sativum* L.) is cultivated since ancient times and has been used for centuries as food condiment and both preventive and curative agent in traditional medicine. Garlic pungent aroma and a wide variety of medical properties, such as anticancer, anti-inflammatory, antimicrobial, cardioprotective and antidiabetic activities, are attributed to unique volatile organo-sulfur compounds originated by complex biochemical reactions (Block, 1992; Corzo-Martínez, Corzo, & Villamiel, 2007; Sendl, 1995). Alliin (S-allyl-L-cysteine sulfoxide) and other *S*-alk(en)yl-L-cysteine-*S*-oxides located in the cytoplasm are the precursors of the active sulfur components of garlic (Lanzotti, 2006). These compounds are exposed to the action of the enzyme alliinase when garlic is cut or crushed, promoting the initial formation of sulfinates that transform rapidly into thiosulfinates. Thiosulfinates undergo further rearrangements leading to the pool of organo-sulfur volatiles, including di-, tri-sulfur compounds and thiosulfonates, responsible for the characteristic flavour and taste of fresh garlic.

The composition of garlic and concentration of bio- and odor-active metabolites, in particular, are dependent on the kind of cultivar, the geographic origin and growing conditions (Baghalian, Naghavi, Ziai, & Badi, 2006; Khar, Banerjee, Jadhav, & Lawande, 2011; Martins, Petropoulos, & Ferreira, 2016; Montano, Manuel Beato, Mansilla, & Orgaz, 2011). In spite of the increasing attention of consumers for the origin of food and high reputation of products with a distinct geographical identity, a relatively small number of investigations regarding traceability of garlic can be found in the scientific literature. Classification of garlic according to the cultivars and/or the geographical origin was attempted using compositional data provided by ^{1}H high resolution magic angle spinning-nuclear magnetic resonance (HRMAS-NMR) spectroscopy (Ritota et al., 2012), Fourier transform infrared spectroscopy (Lu, Ross, Powers, Aston, & Rasco, 2011), high resolution mass spectrometry (Hrbek, Rektorisova, Chmelarova, Ovesna, & Hajslova, 2017), high performance liquid chromatography (Montano et al., 2011) and electronic nose (Trirongjitmoah, Juengmunkong, Srikulnath, & Somboon, 2015). The above studies proved the fingerprint ability of the organo-sulfur compounds and other metabolome components, including organic acids, sugars, fatty acids and amino acids.

Analysis of the multi-element composition by means of atomic spectroscopy techniques is potentially useful for tracing plant-based food (D'Archivio, Giannitto, Incani, & Nisi, 2014; Drivelos & Georgiou, 2012; Giacomo, Del Signore, & Giaccio, 2007; Valentin & Watling, 2013), as the elemental composition of plants reflects the soil from

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https://doi.org/10.1016/j.foodchem.2018.09.088
Received 11 May 2018; Received in revised form 11 September 2018; Accepted 13 September 2018

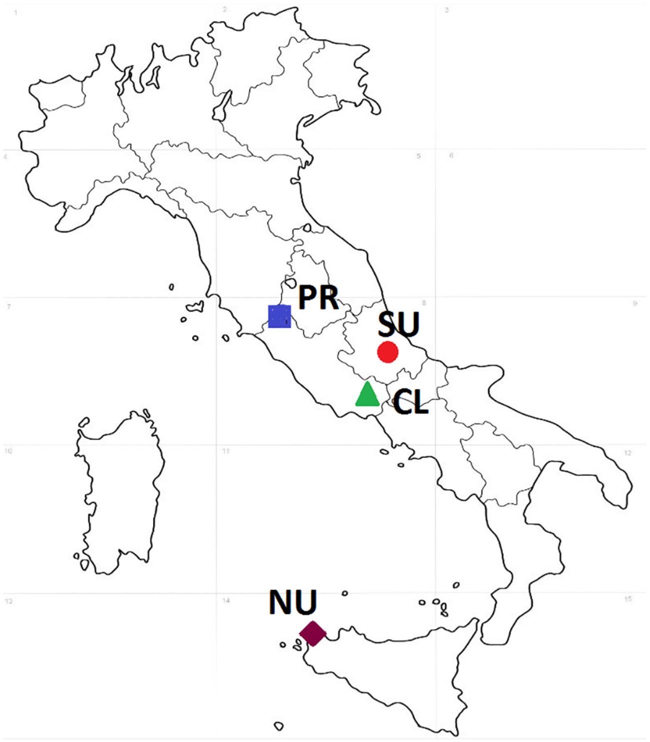

Fig. 1. Geographical origin of the Italian red garlic samples: Sulmona (SU), Proceno (PR), Castelliri (CL) and Nubia (NU). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where these are grown. In a previous investigation (Smith, 2005), garlic samples cultivated in different countries were differentiated with good accuracy by comparing the trace metal profile of the sample to a database of authentic reference samples. This study also revealed that the mineral composition of garlic matches the country where is cultivated rather than the origin of the bulb, which supports the close connection between garlic composition and that of the growing soil. However, although trace metal data seems useful to establish the country where garlic is grown in order to enforce existing importation laws and regulations, the discriminatory power of mineral composition for tracing local garlic varieties cultivated in specific regions was not evaluated before.

In the present work, we investigated the usefulness of mineral composition in the geographical identification of garlic cultivated in relatively close sites of Italy (Fig. 1). In particular, we characterized by means of inductively coupled plasma optical emission spectrometry (ICP-OES) the red garlic varieties coming from Sulmona (SU), Castelliri (CS), Nubia (NU) and Proceno (PR). In each of the four investigated areas the garlic farmers are associated in consortia and, apart from sharing the agronomic practices and marketing policy, breed local bulbs, which should ensure specificity and homogeneity of the red garlic variety produced in that zone. Differentiation of red garlic coming from SU, CS and PR and a white variety cultivated in Piacenza (Italy) was the subject of a previous investigation (Ritota et al., 2012) that, however, did not consider the mineral composition but was based on the metabolomic profile determined by HRMAS-NMR. Apart from usual classification approach, here performed using linear discriminant analysis (LDA), we applied for the first time a class modelling method to the metal trace data collected from the four garlic varieties. As compared with the classification methods that evaluate simultaneously the data samples belonging to at least two well represented classes and identify delimiters between the classes, class-modelling can be used to treat a single class or applied to multi-class problems but the classes are treated individually (Callao & Ruisánchez, 2018; Forina, Oliveri, Lanteri, & Casale, 2008). Once a class model has been generated by building an enclosed space around the class samples, application of modelling rules permits to make a decision on whether a new sample really belongs or not to that class, which is the question to be answered in the verification of food compliance with given specifications, including geographical origin.

2. Experimental

2.1. Garlic samples and chemicals

Red garlic samples cultivated in 2017 were directly acquired from the consortia *Aglio rosso di Sulmona* (SU), *Aglio rosso di Nubia* (NU), *Aglio rosso di Proceno* (PC) and *Cooperativa Aglio Rosso di Castelliri* (CL) collecting the producers working in the respective territories. Suprapure nitric acid (65%) from Merck KGaA (Darmstadt, Germany) was used to digest the samples. Ultrapure water (18.2 MΩ cm resistivity at 25 °C) was obtained from a water purification system Milli Q (Millipore, Germany). A multi-element stock solution containing all the analysed elements (Ba, Cu, Mg, Mn, Sr, Zn at a concentration of 10 µg/mL and Ca, Na, Fe 100 µg/mL in 10% w/w HNO_3) was purchased from Fluka Analytical, (Sigma-Aldrich, MO, USA). Standard solutions in 2% nitric acid were prepared using 100 mL PMP volumetric flasks. Polypropylene vials were used for sample storage and analysis.

2.2. Sample preparation

The individual samples analysed by means of ICP-OES consist of cloves extracted from different garlic bulbs. A clove was sliced and dried in a Büchi TO-50 (Büchi Labortechnik AG, Flawil, Switzerland) drying tube at 70 °C under moderate vacuum until constant weight and successively ground. 0.4 g of garlic powder was introduced into a Teflon digestion vessel together with 10 mL of nitric acid and 10 mL of ultrapure water. The mineralization was carried out in a microwave oven Ethos One (Milestone, Bergamo, Italy) at constant power (1000 W). The sample temperature was increased to 200° C in 10 min and was kept at 200 °C for 40 min. The digested samples, cooled at room temperature, were transferred to a volumetric flask and diluted to 50 mL with ultrapure water.

2.3. ICP-OES analysis

Concentrations of Ba, Ca, Cu, Fe, Mg, Mn, Na, Sr and Zn in garlic digested samples were determined using an Iris Intrepid ER/S Thermo-Elemental (ThermoScientific, USA) inductively coupled plasma optical emission spectrometer equipped with an Echelle grating optical system and a charge injection device (CID) solid-state detector. A Timberline IIL (ThermoScientific) autosampler, connected to a peristaltic pump, was part of the sample introduction system. The sample was introduced at a flow rate of 1.85 mL min^{-1} into the argon plasma through a cross-flow pneumatic nebulizer and a polytetrafluoroethylene spray chamber. The torch-reading configuration was radial and the operating conditions were those recommended by the manufacturer: radiofrequency power of 1.2 kW, coolant gas flow of 12 L min^{-1}, nebulizer gas flow of 0.4 L min^{-1} and auxiliary gas flow of 0.5 L min^{-1}. The elements were detected at the wavelengths (given in Table 1) providing the maximum signal intensity and minimum spectral overlap. The background correction of all the emission lines was performed manually. The calibration curves were built by analysing in triplicate nine standard solutions containing all the target metals within two different concentration ranges: from 5 µg/L to 1 mg/L for Ba, Cu, Mn, Mg, Sr, Zn and from 50 µg/L to 10 mg/L for Ca, Fe, Na.