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Characterization and valorisation of the Italian walnut (*Juglans regia* L.): a first application of stable isotope ratio analysis to determine walnut geographical origin

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Summary: *The isotope ratio mass spectrometry (IRMS) approach has been successfully used for geographic traceability of agricultural products. Here we preliminarily evaluate the IRMS ability in defining the geographical origin of the Italian walnuts. O and H stable isotope ratios are potentially successful in discriminating between Italian and international provenances.*

Keywords: *Food traceability, Italian walnut production, isotope ratio mass spectrometry (IRMS)*

Introduction

Juglans regia (L.), commonly named Persian or English walnut, is cultivated for nut production and is one of the oldest food sources known. Native to the mountain valleys of Central Asia, *J. regia* are grown worldwide in temperate areas. Walnut world production keeps increasing, exceeding three million tons since 2011, with China, USA and Iran as leading producing countries [1].

In Italy, despite the constantly growing demand due to walnuts high nutritional value, walnut cultivation went through a strong decline in the last decades. This may be due to the low agronomic standard care, the shortage of producers co-operation and association, which would allow farmers to act jointly reducing production costs, the use of plants grown from seed, causing the absence of product homogeneity, and the lack of selected and locally adapted varieties. As a consequence, Italy is among the top five net importing countries of in-shell walnut since the mid 1970s [1]. However, the climate and environmental conditions of the Italian peninsula are well suited to walnut cultivation, and still few local unique Italian varieties and ecotypes have survived. This is the case of the 'Bleggiana' variety, present in a restricted area – Bleggio – of the mountainous region Trentino, and still propagated by grafting. Important Italian ecotypes are Feltrina, present only in the mountainous area of Feltre in the Veneto region, and Sorrento, which is represented by at least eight different ecotypes distributed across the Sorrento peninsula and Campania region [2]. Additionally, international commercial varieties, such as Lara, Franquette and Chandler, showed to adapt well to the Italian variegated landscape and have already been introduced also in regions previously not dedicated to the walnut culture. The current context is therefore favourable to reintroduce the walnut cultivation on the Italian territory heading towards a local, high quality production. In this respect, tools capable of tracing the origin of specific products can help this process, especially protecting the local origin of the product.

The exploratory analysis here presented investigates the capability of H, O, C, N, and S stable isotope ratios to determine the geographical origin of walnut varieties locally cultivated in Italy. It is performed within a multidisciplinary project that aims to a complete characterization of the local Italian walnut accessions through the definition of a unique and typical profile by means of i) genetic profiling using microsatellite molecular markers; ii) H, O, C, N, and S stable isotope analysis; iii) metabolic compounds analysis, particularly lipids and phenols; and iv) sensory analysis.

Experimental

Dried samples of in-shell walnuts, from orchards located mainly in North-Eastern Italy, were provided by producers during the fruit harvest in 2017, each sample consisted of 10 to 15 walnuts from the same orchard. A total of 22 Italian samples were retrieved from four regions: Veneto (16), Trentino (3), Piedmont (1), Emilia (1) and Campania (1). As very preliminary test to discriminate between Italian and international samples, one sample from four foreign countries (Australia, California, Chile and France) was introduced in the analysis. All nuts of each sample were manually cracked and

shelled, and the seed kernels were frozen at -80°C; subsamples of 50 g on average were subsequently ball-milled using Mixer Mill MM400 homogenizer (Retsch GmbH, Haan, Germany) and stored at -20°C until lyophilisation. Around 4 g of grounded and lyophilised nut kernels were extracted with 25 mL mixture of petroleum ether:ether (2:1) homogenising with an Ultraturrax device (model X-620, Staufen, Germany; 11,500 rpm for 3 min) and using a centrifuge (ALC PK 131R, Thermo Electron Corporation, Germany; 4100 rpm for 5 min) to separate the ether from the residue. The residue was re-extracted twice. The ether was evaporated using a model R210-A rotary evaporator (Büchi, Flawil, Switzerland) at 37 °C. The defatted kernel was washed with deionised water twice with 30 mL of water using a centrifuge (4100 rpm for 3 min) to separate the water. The solid residue was lyophilised and conserved at room temperature up the analysis. The $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ stable isotope ratios of defatted powder and $^{13}C/^{12}C$ of oil fraction were measured using an Isotope Ratio Mass Spectrometer (Isoprime, Manchester, UK) after combustion of the sample (Isotope Vario, Elementar, Bremen, Germany). Measurement of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ of both the defatted powder and oil fraction was carried out with a Delta Plus XP IRMS (Thermo Fisher Scientific, Bremen, Germany) connected to a TC/EA pyrolyzer (Thermo Fisher Scietific).

Results

The preliminary investigation performed shows that H and O stable isotopes ratios seem to better discriminate different geographical origin (Fig. 1), with defatted kernel powder and walnut oil fraction providing similar trends. Specifically, the Italian samples cluster together, with the only exception of one sample from the Campania region. All the international samples fall out the Italian cluster; this is evident mainly for Chile and Australia. The sample from California and France, although not clustering with the Italian samples, are less distant. The analysis of stable isotope ratios for C, N and S, does not result in a clear distinction of samples according to their geographical origin. Distinctive values of S34 were detected only in four samples: the one from Australia and three from Italy (10.1‰, sample from Piedmont; -5.9‰ sample from Veneto; 11.4, sample from Trentino).

Conclusion

To the best of our knowledge these exploratory analyses are the first application of the isotope ratio mass spectrometry (IRMS) in assessing the geographical origin of walnuts. The preliminary results here presented, although revealing the need for an improvement in our data collection, in order to cover homogeneously the areas of walnut production, suggest that at least H and O stable isotope ratios could provide a successful approach to determine walnut origin and to detect potentially commercial frauds.

References

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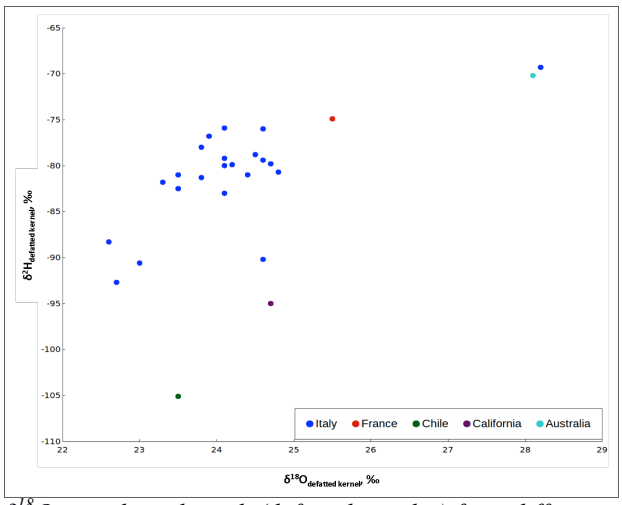

Fig. 1 Score plot of δ^2H versus $\delta^{18}O$ in walnuts kernel (defatted powder) from different geographical origin (different colours represent different provenances, see legend)