

Unveiling Diversity in Amino Acid Stable Isotope Profiles for Classifying Italian Rice Varieties, Refining Types and Cultivation Methods

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1 Introduction

Approximately 50% of rice (*Oryza sativa* L.) in the European Union is produced in Italy, predominantly in the Northern regions following river Po. Highly valued Italian rice cultivars belong to the japonica variety and include Carnaroli, which is elastic, resistant and ideal for risotto dishes, Arborio, with wide grains and the ability to maintain large amounts of starch when cooking, and Baldo, which has an elongated grain and is the richest variety in minerals (Riso Delta Po PGI, 2020). Even though these possess different qualitative properties, their morphological differences are minimal [1], thus rendering them targets of fraudulent activities [2]. Therefore, it is important to develop methods that can distinguish between the different rice varieties.

The public perception of organic foods as healthier, as well as the transition towards organic rice agriculture in the Italian rice sector [3], makes the authentication of these products highly relevant. Moreover, the availability of market choice between white and brown rice can have further implications on the identification of authenticity markers. Brown rice includes the endosperm, embryo and bran and is nutritionally superior to white rice [4].

Studies employing isotope ratio mass spectrometry (IRMS), have examined the individual effects of refining type, variety and cultivation on the bulk stable isotope values of

different food products [4,5]. However, Compound-specific (CS) IRMS methods can prove more effective than bulk in the discrimination of organic and conventional food products, providing information on individual components (e.g. amino acids, fatty acids, nitrate) by the addition of a separation step prior to the isotope analysis [6]. In this work, we applied bulk and CS amino acids analysis by Elemental Analyser (EA)- IRMS and Gas Chromatography - Combustion (GC - C) - IRMS, respectively, with the aim to obtain the stable isotope profile of different Italian rice varieties (Carnaroli, Arborio, Baldo, S. Andrea, Rosa Marchetti), refining types (brown/ white) and cultivations (organic/ conventional). The findings of this research aid in the identification of robust stable isotope markers for the organic and, potentially, varietal authentication of cereals.

2 Materials & Methods

A total of thirty eight rice samples of different cultivation systems (organic/ conventional), types (brown/ white) and varieties (Carnaroli, Arborio, Baldo, Rosa Marchetti and S. Andrea) were collected from Northern Italian provinces (Lombardy and Piedmont) in 2022. The values of bulk and 9 amino-acid $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were analysed by EA and GC-C-IRMS. The results were evaluated by MANOVA, followed by an LDA classification and a decision tree model.

3 Results & Discussion

Brown rice was found to exhibit significantly lower $\delta^{13}\text{C}_{\text{val}}$ and $\delta^{13}\text{C}_{\text{leu}}$ values, but higher $\delta^{13}\text{C}_{\text{gly}}$ and $\delta^{13}\text{C}_{\text{phe}}$ values overall in the conventional samples ($p < 0.05$), while no significant differences were observed between the brown and white samples of organic cultivation ($p > 0.05$). Interestingly, bulk $\delta^{13}\text{C}$ values were not found to be significantly different between brown and white rice in neither organic nor conventional rice samples. Statistically significant separation ($p < 0.05$) was achieved between the brown organic and brown conventional samples based on the $\delta^{15}\text{N}$ values of ala, val, ile, leu, gly, pro, asx, glx and phe. Notably, these were significantly higher than the difference between the bulk values (+0.4 ‰).

The significantly lower $\delta^{15}\text{N}$ values noted for conventional brown rice compared to conventional white rice can be attributed to the prominent effect of the synthetic fertilizer in the nitrogen isotopic composition of the rice grain, especially in the outer layer, which is retained to a greater extent in the case of the former rather than the latter. On the other hand, organic brown and white rice exhibited more similar ranges, since the $\delta^{15}\text{N}$ profile of organic fertilizers is closer to the natural background levels, resulting in some degree of homogeneity throughout the grain.

The LDA model successfully separated the conventional from the organic Carnaroli rice, as well as the conventional Arborio from both the conventional and the organic Carnaroli samples. Generic rice was also clearly differentiated from all other classes. Lastly, $\delta^{13}\text{C}_{\text{AAAs}}$ and $\delta^{15}\text{N}_{\text{AAAs}}$ contributed significantly more to the LDs compared to the bulk values.

Decision tree analysis differentiated the Generic rice from all other classes based solely on its $\delta^{15}\text{N}_{\text{leu}}$ value, which was lower than 2.5 ‰, with a probability of 1.

Additionally, the $\delta^{13}\text{C}_{\text{bulk}}$ value proved to be a key differentiator between classes, with a value higher than or equal to -26 ‰ indicating Arborio_Conv, and a value between -27 and -26 ‰ indicating Carnaroli_Org, with a probability of 1, when the $\delta^{15}\text{N}_{\text{leu}}$ value was higher than or equal to 2.5 ‰.

Conclusions

This study highlights the added value of the extractable information from compound-specific IRMS analysis, compared to bulk analysis, being able to achieve separation both between premium rice varieties and cultivation methods.

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