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FOOD INTEGRITY

5th FOODINTEGRITY CONFERENCE

Nantes, France

14-15 November 2018

Assuring the integrity of the food chain:
Delivering real world solutions



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APPLICABILITY OF HRM ANALYSIS FOR CARNAROLI RICE AUTHENTICATION BASED ON POLYMORPHISMS OF THE WAXY GENE

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Rice (Oryza sativa L.) is a staple food and one of the most important cereals in the worldwide. Italy, the leading rice producer in Europe, holds nearly 200 different varieties in the available germplasm [1]. The Carnaroli rice is a high quality and priced variety belonging to the group of japonica ecotype, produced mainly in Piedmont. It is considered one of the finest Italian rice varieties due to its excellent cooking resistance, given by a low tendency to lose starch and a good ability to absorb liquid while creaming, being, thus, ideal for the preparation of traditional risotto. Italian rice varieties have different characteristics, from which the starch composition is a highly relevant parameter. Together with amylopectin, amylose is the main component of starch, whose ratio is determinant for the rice cooking properties. After cooking, varieties with high amylose content have dry, firm and separate grains, while low amylose ones usually have tender, cohesive and glossy texture [2]. Amylose synthesis is catalysed by the granule bound starch synthase (GBSS) that is encoded by the Waxy gene (Wx), being located on the chromosome 6. Various nucleotide polymorphisms have been associated with the Wx gene, namely (CT)n repeats and several single nucleotide polymorphisms (SNP) [2]. The aim of this work was to propose a new method based on high resolution melting (HRM) analysis, exploiting those polymorphisms to differentiate Carnaroli rice from other closely related varieties.

The Italian rice varieties sold as Carnaroli (Carnaroli, Carnaval, Caravaggio, Koepe, Poseidone, Karnak, Carnise, L202 and L252), as Roma-Baldo (Roma, Baldo, Cammeo, Galileo and Casanova), as Arborio (Volano, Telemaco and Generale), as Thaibonnet (Gladio) and as Sant'Andrea (Sant'Andrea) were acquired from producers. DNA from rice grains was extracted with NucleoSpin food kit (Macherey-Nagel, Düren, Germany). In silico analysis was performed in the Wx gene to design primers targeting the (CT)n microsatellite and the G/T in the first intron. Two sets of primers were designed to amplify fragments of 183 bp and 341 bp for HRM method development and sequencing analysis, respectively. The sequencing results showed that all the varieties commercialised as Carnaroli have 17 CT repeats on the microsatellite, while the others have 18 and 20, in the case of Gladio. Additionally, the first intron SNP is G for Carnaroli, while is a T for all varieties except Gladio. The development of a real-time PCR assay targeting the 183 bp fragment with EvaGreen dye combined with HRM analysis enabled the differentiation of Carnaroli rice varieties in Cluster 1, Roma-Baldo, Arborio and Sant'Andrea varieties in cluster 2 and Gladio in cluster 3, with levels of confidence above

98% (Fig. 1). The results were well corroborated with the sequencing data. Therefore, the proposed new HRM method can be a simple, specific and high-throughput tool for the authentication of Carnaroli rice.

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