TRACKING GRAPEVINE VARIETAL COMPOSITION IN WINE USING HIGH RESOLUTION MELTING

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Introduction

Food fraudulent practices is a global concern, affecting several different products, among them wine is considered to be the 9th product most at risk of fraudulent practices (Szpylka, 2018). The wine sector belongs to an important worldwide market, with a value of 30.4 bn EUR in 2017 (OIV, 2018a). The fact that some wine are highly quoted in the market, make them more likely to be adulterated, since the return of such practice is more profitable. The development of different analytical tools to monitor and control such events have been pursued in the food area. In the wine sector some of the frauds that have been reported involve both geographical provenience and the varietal composition adulteration (Pereira et al., 2018). The identification of the varietal composition has been assessed using different technological approaches, however, the use of DNA-based markers have been considered to be a reliable mean to achieve this target, since it is independent of environmental and ecological practices (Pereira et al., 2018).

Still, DNA recovery from wine samples is very difficult and yields very fragmented DNA molecules, requiring the use DNA markers that are both highly informative and small sized. Single Nucleotide Polymorphism (SNP) markers have demonstrated to be highly recommended in forensic samples, and have been widely applied to grapevine varietal identification, joining the two requests necessary for the development of a DNA-based authenticity system (Pereira et al., 2018). High resolution melting (HRM) represents a novel approach that enables the genotyping of SNPs in a large number of samples and is highly reproducible between labs (Ganopoulos et al., 2013). HRM analysis is based on the generation of different melting-curve profiles as a result of sequence variation present in the double-stranded DNA (Ganopoulos et al., 2013; Pereira and Martins-Lopes, 2015; Pereira et al., 2017).

The aim of this study is to report on the existence of the HRM technology, based on SNP markers, that allows the identification of the grapevine variety in wine samples.

Methods overview

The methods applied to a wine authenticity scheme is resumed in Figure 1. Generally, SNP markers able to differentiate among grapevine varieties were screened in several genes involved in the anthocyanin biosynthetic pathway (*UFGT*- UDP-glucose: flavonoid 3- O-glucosyltransferase - Pereira and Martins-Lopes, 2015; *F3H*- flavanone 3-hydroxylase – Gomes et al., 2018). The leaf samples were collected from certified vineyards and grapevine varieties were validated using SSR markers and by the ampelography experts, according to the OIV descriptors (OIV, 2018b). The wines were prepared using certified grapes under controlled microvinification conditions (Pereira et al., 2017).

The High resolution melting (HRM) assays were prepared based on the SNP information, considering the most polymorphic regions. All the SNPs were validated using both multiple clones of the same grapevine variety, for stability, and after the HRM assay using Sanger sequence, for the confirmation of reliability and reproducibility (Pereira and Martins-Lopes, 2015 ; Pereira et al., 2017 ; Gomes et al., 2018).
The DNA samples were extracted from wines following the method described in Pereira et al. (2011). In each HRM assay, DNA samples from wine and corresponding leaf, use as positive control, were used to validate the assays. The methods applied to a wine authenticity scheme are resumed in Figure 1.

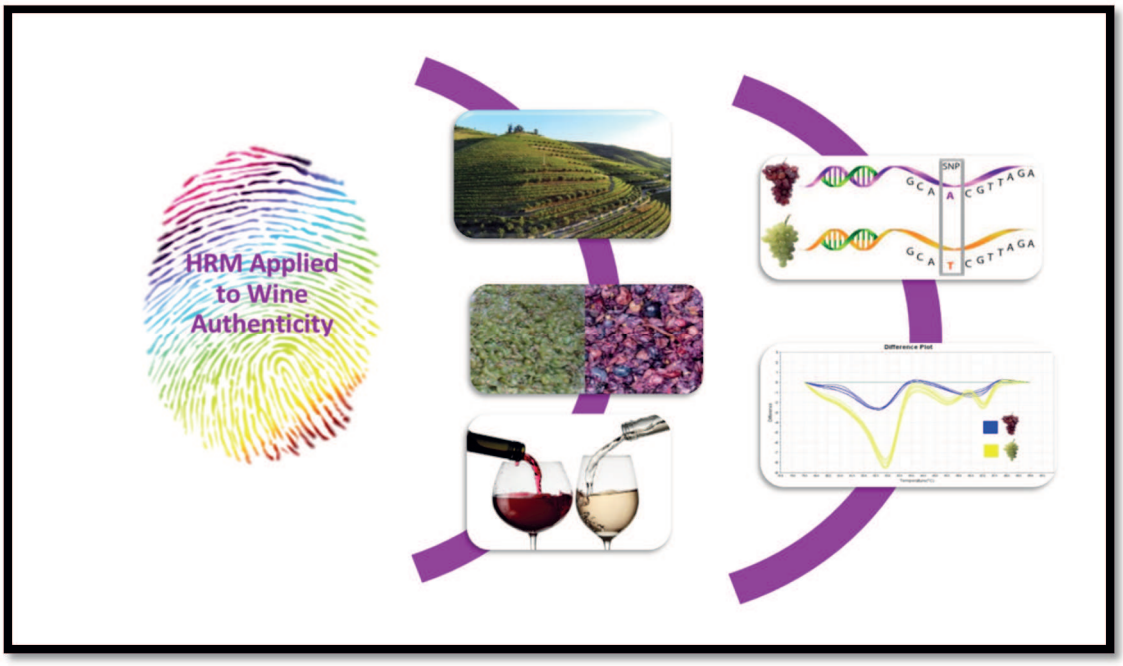

Figure 1. Overall methodological approach applied to wine authenticity using High Resolution Melting.

Results and Discussion

The use of SNP markers rely on a careful selection of polymorphic DNA regions, enabling the discrimination of different grapevine varieties. The strategy adopted consisted on the sequencing of several genes that belong to the anthocyanin pathway, since the grapevine varieties are differentiated by their phenolic compound composition. The genes that were considered were *UFGT and CHI* - chalcone isomerase (Pereira and Martins-Lopes, 2015), and *F3H* and *LDOX* - leucoanthocyanidin dioxygenase (Gomes et al., 2018). Although sequence information has been obtained for the four genes, the two genes that were more polymorphic, and therefore more informative, were *UFGT* and *F3H* (Table 1). Only the two most discriminative genes, *UFGT* and *F3H,* were selected to design HRM assays.

Table 1. List of genes from the anthocyanin sequenced in 21 grapevine varieties, the number of SNPs identified and the discriminating power of such genes among the grapevine analysed (adapted from Pereira and Martins-Lopes, 2015 and Gomes et al., 2018).

Gene	Number of SNPs/INDELs	Variety discrimination
UFGT	59	18/21
CHI	5	
F3H	12	5/21
LDOX	1	1/21

Several HRM assays were designed and tested in leaf samples Pereira and Martins-Lopes, 2015; Gomes et al., 2018) and were validated with different clones of the grapevine varieties under study. The designed assays were further tested against different matrices (leaf, must and wine), being clear that the HRM assays that comprised smaller size fragments were more adequate for wine samples (Pereira et al., 2017). A new HRM assay, based on the *F3H gene*, was designed comprising a fragment size of 375 bp, and differing in the case of Cabernet Sauvignon and Merlot on 1 position within the amplified fragment, namely 291, were Merlot is heterozygous in opposite to Cabernet Sauvignon is homozygous (Figure 2). The designed HRM assay revealed to be highly sensitive and efficient for grapevine identification in wine.

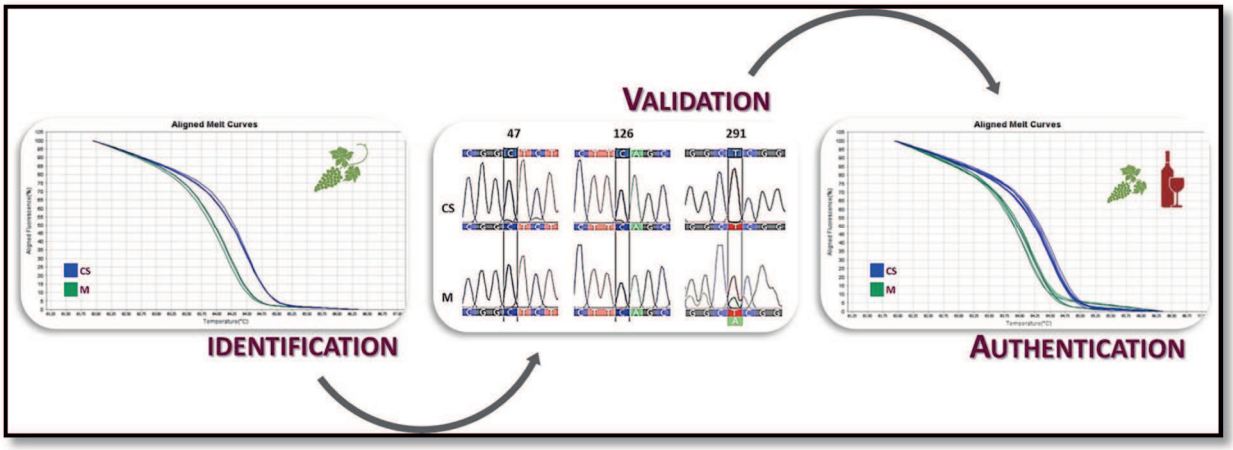

Figure 2. Discrimination between two grapevine varieties, Cabernet Sauvignon and Merlot, using HRM technology. The identification of each grapevine variety was based on HRM assay of the F3H gene. The amplicons were validated using Sanger sequence, were it was possible to identify a difference at the 291 bp position of the F3H gene. The authentication consisted on the use of DNA samples from leaf and controlled monovarietal wines using the designed HRM assay, producing a coincident melting profile between the two matrices of the same grapevine variety.

The use of smaller fragments in processed food products and wine is highly recommended when designing a PCR assay (Pereira et al., 2017; Pereira et al., 2018; Vietina et al., 2013). On the other hand the use of HRM technology, based on MeltDoctor™ chemistry, allows to develop consistent and reproducible results in cost-effective way (Pereira et al. 2017; Pereira et al., 2018; Madesis et al., 2014).

Conclusion and Future Perspectives

The definition of a reliable and robust wine authenticity scheme is imperative considering the worldwide wine market, so fairness of trade maybe achieved. The consumers are more aware of fraudulent practices and they need to be assured that the product is authentic in all its dimensions. In the wine industry although there are registered controls in the Denomination of Origins, they are not sufficient to prevent frauds. Here we report on a methodology that is able to verify the presence of a given grapevine variety in a wine using a DNA-based approach, HRM. The HRM assay developed is able to identify the variety present, using only 5 mL of wine. The HRM assay is highly reproducible and sensitive, being therefore reliable for such a system. This research sets the basis for a wide DNA-based authenticity scheme. Nevertheless, in order for it to be applied to all the grapevine varieties there is the requirement that a set of discriminative SNP markers, such as the ones described by Cabezas et al. (2011), maybe used to design HRM assays for such purpose.

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Abstract

Wine authenticity is a major concern worldwide, with around 5% of the wines being mislabelled and sold in secondary markets. Thus, methods suitable to combat some fraudulent practices in the wine sector are imperative. DNA-based methodologies are a reliable mean of tracking wine varietal composition. However, DNA recovery from wine samples is very difficult and yields very fragmented DNA molecules, requiring the use DNA markers that are both highly informative and small sized. Single Nucleotide Polymorphism (SNP) markers have demonstrated to be highly recommended in forensic samples, and have been widely applied to grapevine varietal identification, joining the two requests necessary for the development of a DNA-based authenticity system. High resolution melting (HRM) represents a method that enables the genotyping of SNPs in a large number of samples and is highly reproducible between labs. HRM analysis is based on the generation of different melting-curve profiles as a result of sequence variation present in the double-stranded DNA. The HRM assay designed was able to discriminate among two wine samples that only differ on a single position. This sets the basis for the implementation of a grapevine varietal identification scheme in wine samples.