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Analytical Methods

Peptide fingerprint of high quality *Campania* white wines by MALDI-TOF mass spectrometry

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ABSTRACT

Food traceability is essential to preserve the identity of unique quality traits against frauds or commercial disputes. Therefore, there is a growing demand of new traceability systems for the collection of information related to units/batches of food ingredients and products.

A rapid method based on peptide profiles obtained from tryptic digests of whole wine proteins by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry is described. Reliable peptide fingerprints were obtained for high quality *Campania* white wines, providing a signature of the finished products. The MALDI spectra revealed the presence of common diagnostic ions, but also evidenced differences between wines. Furthermore, the MALDI-TOF spectral traces were converted into simulated images to obtain a graphical representation of spectra. The resulting "mass codes" constitute a simple tool to display differences between samples, suggesting their potential use as "biological bar codes" for food authenticity and traceability, probably applicable to other classes of certified food products.

1. Introduction

The EU General Food Law Regulation defines traceability as "the ability to trace and follow a food, feed, food-producing animal or substance, through all stages of production, processing and distribution". Food traceability is strictly connected with "food safety" and "food quality", as it can help to preserve the identity of unique quality traits, against frauds or commercial disputes (Raspor, 2005). In addition, traceability has a role to play in promotion of informed consumer choice because it offers the potential to verify label information on product and ingredient history (Pascal & Mahe, 2001; Peres, Barlet, Loiseau, & Montet, 2007). The European regulation 178/2002, from January 1st 2005, imposes rigorous requirements for food traceability.

An integrated traceability control system should be able to identify with accuracy materials (i.e. ingredients) and actions (i.e. processing and production methods) applied in food chain, with the aim to document the history of a product along the entire production chain from primary raw materials to the final consumable product (Peres et al., 2007). Therefore, the aim of these systems is not only limited to the ability to detect and trace batches of high-risk products, but also to support quality assurance processes for products, with regard to certify food quality and authenticity (Lupien, 2005). During the last years, these concepts have been connected to the specific food origin/region, as geographical or regional indicators often define the varieties of raw materials that can be used for a given certified product. In this regard, for several foods with clear origin and/or for traded products (e.g. wine, ham, cheese, oils, etc.), the identity preservation is of fundamental importance.

In this framework, there is a growing demand of new traceability systems for the collection and authentication of any information of units/batches of ingredients and products (Ammendrup & Barcos, 2006; Muller & Steinhart, 2007). In the past years, the development of biological identification technologies (i.e. DNA fingerprints, chemical and biochemical characterisation, etc.) has greatly contributed to support and check traceability systems. In parallel, computer technology provided many new and innovative tools for tracing products (Raspor, 2005). Simple hand-written or printed labels are being rapidly replaced or supplemented by computer-readable identification codes (e.g. bar codes, radio frequency tags). These electronic identifiers allow the simple and fast tracking of items by automatically gathering and storing information in computer databases.

Biological, analytical and informatics tools have been synergistically proposed and utilised for traceability in the wine industry (Pinder & Meredith, 2003). The world's great wines are produced from a relatively small number of evolutionary and closely related cultivars of a single grape species, *Vitis vinifera* L. Great efforts have been spent in the identification of winegrape cultivars. A key role in the viticulture and enology field has been played in the last years by DNA typing (Garcia-Beneytez, Moreno-Arribas, Borrego, Polo, & Ibanez, 2002; Siret, Gigaud, Rosec, & This, 2002). However,

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like the classical ampelographic techniques, although useful for grape identification, the DNA fingerprint can not be applied to the juice or wine. The biochemical characterisation of both volatile (including aroma compounds) and non-volatile compounds (e.g. pigments, tannins, polyphenols, anthocyanins, etc.) also greatly contributed to the characterisation of grapes, musts and wines (Carpentieri, Marino, & Amoresano, 2007). With the advancement of analytical techniques, together with the more conventional liquid chromatography approaches (including HPLC and FPLC), mass spectrometry (mainly GC/MS and ESI/MS) has been widely used for wine compounds characterisation, due to its high resolution and detection power (Flamini & De Rosso, 2006; Flamini & Panighel, 2006; Wang & Sporns, 1999). For example, Catharino and co-workers reported the characterisation of must samples derived from six grapes varieties by ESI–MS in negative ion-mode, a technique that is particularly suitable for ionisation and identification of acidic compounds such as phenols, flavonoids and sugars (Catharino et al., 2006). Furthermore, many studies have been recently focused on nitrogen-containing compounds, such as proteins and polypeptides, that, despite the low amount and the minimal contribution to nutritive values, seem to play important roles in various technological and enological issues (Ferreira, Picarra-Pereira, Monteiro, Loureiro, & Teixeira, 2002). A special attention has been paid to proteins for their potential use in varietal identification of grapes, juices, musts and wine (Sarmento, Oliveira, Slatner, & Boilton, 2001).

The methodologies applied for wine protein characterisation and identification included electrophoretic methods (native-PAGE, SDS–PAGE, IEF and CE, 2D-PAGE), chromatographic methods (FPLC, SE–LC, RP–LC) and mass spectrometry techniques (Moreno-Arribas, Pueyo, & Polo, 2002). A detailed characterisation of wine proteins revealed that they mainly derive from grape and autolyzed yeast (Ferreira et al., 2002). The profile of soluble proteins found in the juice of ripe grapes and wine also appears surprisingly simple, with a predominance of the abundant pathogenesis-related (PR) proteins, including chitinases (50% of the soluble proteins in the grape berries) and thaumatin-like proteins (Van Loon, 1985). These proteins constitute a defence system of plants against fungal attack.

In the present work we propose the application of peptide profiles derived from whole wine protein tryptic digests to obtain a signature of high quality *Campania* white wines by MALDI-TOF mass spectrometry. To this purpose, the MALDI-TOF spectral traces have been converted into simulated images to obtain a graphical representation of the peak lists. The obtained "mass codes" constitute a simple but effective tool to display differences between samples, suggesting their potential use as "biological bar codes" for food authenticity and traceability.

2. Materials and methods

2.1. Materials

Alpha-cyano-4-hydroxycinnamic (HCCA), trifluoroacetic acid, trypsin and other analytical grade reagents were purchased from Sigma Chemical Co. (Milan, Italy). Methanol and chloroform were from Carlo Erba (Milan, Italy).

2.2. Methods

2.2.1. Wine sampling

High quality *Campania* region white wines produced from different *V. vinifera* cultivars (Fiano, Greco di Tufo, Falanghina) were used for the analysis as reported in Table 1. All selected wines were certified products, with the designations of "Denomination of Origin Certified" (DOC) or "Denomination of Origin Certified and Guaranteed" (DOCG), with the exception of the Fiagre, having a "Typical Geographical Indication" (IGT) brand. The latter has been included in the analysis as it constitute a good sample to validate the proposed method, due to its combined composition (Fiano and Greco grapes, 70:30, v:v). All varieties were cultivated in South Italy and all wines belong to the same vintage (2006). To account for the variance due to individual viticultural and enologic practices, only commercially produced wines from different winemakers were selected for the analysis. The Fiano, Fiagre and Greco wines from Caggiano winemaker were kindly provided by Mr. Antonio Caggiano.

Table 1
Wines, grape varieties and winemakers under investigation

Sample	Wine	Tag	Variety/cultivar	Winemaker
#1	Fiano di Avellino DOCG[a]	FIc	Fiano 100%	Antonio Caggiano
#2	Greco di Tufo DOCG	GR	Greco 100%	Antonio Caggiano
#3	Fiagre Vino da Tavola IGT	FR	Fiano 70%, Greco 30%	Antonio Caggiano
#4	Fiano Antiche Terre DOCG	FIat	Fiano 100%	D'Antiche Terre
#5	Falanghina Campi Flegrei DOC	FCF	Falanghina 100%	Mustilli
#6	Falanghina Sant'Agata dei Goti DOC	FSA	Falanghina 100%	Cantine Farro
#7	Falanghina Taburno DOC	FT	Falanghina 100%	Fontanavecchia

All wines were produced in *Campania* region and belong to the same vintage (2006). The DOC, DOCG and IGT labels referred to "Denomination of Origin Certified", "Denomination of Origin Certified and Guaranteed" and "Typical Geographical Indication", respectively.

[a] Two different bottles from independent batches were analysed for this sample.

2.2.2. Wine protein extraction

Wine aliquots (20 mL) were filtered with 0.22 μm pore size cellulose acetate membrane filter (Millipore Co, MA, USA). Wine proteins were extracted with a cold mixture of 2:1 chloroform/methanol (1:1, v/v) for 30 min in ice. Samples were centrifuged at 7000*g* for 5 min at 4 °C and the protein interphase recovered by carefully removing both upper and lower phases. The protein pellets were finally washed with cold acetone and resuspended in loading buffer for SDS–PAGE analysis, performed as previously described. Alternatively, protein pellets were resuspended in ammonium carbonate buffer for tryptic digestion. Method reproducibility was addressed by performing the extraction on four aliquots of the same wine sample in parallel.

2.2.3. Amino acid sequencing by Edman degradation

For N-terminal sequencing, wine proteins, separated by SDS–PAGE, were transferred onto a Prosorb cartridge (Applera Italia, Monza, Italy) and directly subjected to Edman degradation on a Procise Model 491 sequencer (Applera Italia, Monza, Italy) (Chambery et al., 2006a). Protein identification has been performed by a similarity search, using the NCBI BLASTP software (version 2.2.16), available on www.expasy.ch web site.

2.2.4. Proteolytic cleavage

The extracted wine proteins were resuspended in 100 μL of 50 mM NH_4HCO_3 containing 10% CH_3CN and sonicated into an ultrasonic bath for 10 min. Tryptic digestion was performed at 2, 16 and 18 h at 37 °C by three subsequent additions of 50 ng TPCK-treated trypsin (Sigma, Milan, Italy). After digestion, samples were centrifuged at 12000*g* for 10 min. Insoluble material, was dissolved in formic acid and added to the supernatants in 1:10 ratio. Mixtures were analysed by SDS–PAGE to evaluate the complete-