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Verifying the red wines adulteration through isotopic and chromatographic investigations coupled with multivariate statistic interpretation of the data

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ABSTRACT

The assessment of wine traceability and authenticity is a major concern that has gained a lot of interest internationally since the wine has always been subjected to various fraudulent practices. Practiced since ancient times, wine fraud has become more sophisticated in the present day, taking many forms. Consumers, regulatory bodies and manufacturers are all interested to have reliable analytical tools and information to allow the authentication and detection of wine adulteration or incorrect labelling. This research study evaluates and proposes a possible strategy for the detection of adulterated sweet or medium sweet red table wines using appropriate chemical parameters which can reveal prohibited practices in the winemaking process. The work is performed on 29 table wine samples, bought from the market, packed in PET bottles. Exogenous addition of sugar and water in the counterfeited table red wines was detected by the measurement of stable isotopes content ($\delta^{13}C$ and $\delta^{18}O$) known as origin markers and supplementary confirmed by other classical parameters, as the alcoholic strength of the wines (‰ vol.) and the presence of 5-(hydroxymethyl)-2-furaldehyde (HMF) and of synthetic sweeteners or synthetic red dyes used to correct deficiencies of taste and colour. Additionally, the nature and profile of anthocyanins, as indicator of the red colour of wine, was investigated in the table wines and then compared with that of authentic wines obtained by microvinification of *Vitis vinifera*, in order to determine their authenticity.
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1. Introduction

Identity and authenticity of products are current topics in food science mainly due to the interest of consumers for what they eat, and for producers to protect their brands. Therefore the development of authenticity tests that can confirm the product description or detect fraudulent practices is a necessity (Cserháti, Forgács, Deyl, & Miksik, 2005).

The matrix wine is of particular importance within the authenticity testing of foods, because wine production and trade has always been associated with high costs, which made it vulnerable for adulteration (Ogrinc, Košir, Kocjančič & Kidrič, 2001). Wine counterfeiting or adulteration refers to the illegal techniques that have the goal to substitute one valuable component with a cheaper one, in order to increase profit, thus affecting the final product image by changing the wine chemical composition and sensorial properties (Holmberg, 2010).

Evaluation of the authenticity of wine involves checking several aspects, such as declaration of origin, vintage, variety and production method (Versari, Laurie, Ricci, Laghi, & Parpinello, 2014). In recent years wine control authorities have been confronted with an increasing number of cases of wines which were suspected not to be authentic or adulterated (Holmberg, 2010). In winemaking, typical adulteration are: non-authorized addition of natural or synthetic sweeteners, addition of ethanol, water dilution, addi-

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tion of synthetic substances used to hide various defects of the product and the usage of other prohibited ingredients or additives (Košir, Kocjančič, Ogrinc, & Kidrič, 2001; Vicol, 2009). Enrichment with sugars before or during fermentation and also the ethanol addition are misused to increase the natural ethanol content and thus the value of the wine, for which a higher market price might be achieved (Christoph et al., 2004). However, natural sweeteners addition is accepted when the grapes do not accumulate enough sugars due to inappropriate weather conditions. The legality of this procedure varies by country, region and even wine type (Regulation (EU) No 1308, 2013). On the other hand, the addition of an adulterant agent (distillate) to the wine is illegal because in this case the increase in the alcoholic strength of wine is not due the normal fermentation process of the grapes (Vicol, 2009).

Adulteration of wines by the addition of illegal substances like synthetic sweeteners and synthetic dyes, in order to correct deficiencies, have lately become a serious problem and are increasingly practiced. These prohibited practices in the winemaking process represent a real danger for human health (having carcinogenic action), which is why the EU Member States had to establish a system of regular surveys to monitor synthetic sweeteners and synthetic dyes. In Romanian wine industry, according to law no. 244/2002, the addition of synthetic sweeteners and synthetic dyes in wine in order to obtain sweet or medium sweet wine is prohibited.

The wine colour quality is a trait highly valued by the consumers. For red wines, the colour is produced by the phenolic compounds, the most important being the anthocyanins, which give the wine's blue–red (He et al., 2012). Anthocyanins profile, or fingerprint, has been used for many authors as markers for wine authenticity, especially to differentiate the wine varieties (Fraige, Pereira-Filho, & Carrilho, 2014). Since the colour is often a criterion of quality in food, the analysis of these pigments in wines has received considerable attention in the past years, being important tracers for the colour of red wines (Alberts, Stander, & de Villiers, 2012).

The classical authenticity assessment of food is usually based on the analysis of specific marker compounds, which are indicative of a certain property of the product and subsequent use of statistical methods to test for identity and authenticity. The marker has to be specific, its natural variation must be limited and well characterized, and be measured accurately (Primrose, Woolfe, & Rollinson, 2010). For all these, analytical expertise and knowledge of data interpretation is essential (Esslinger, Riedl, & Fauhl-Hassek, 2014).

In food chemistry, measurement of the stable isotope ratios of the biologically important elements (H, C and O) and chemometric processing of the data were used in order to discriminate products according to geographical origin (Capron, Smeyersverbeke, & Massart, 2007; Dordevic, Wehrens, Postma, Buydens, & Camin, 2012; Košir et al., 2001; Scampicchio et al., 2012) or to detect adulterations (Capici, Mimmo, Kerschbaumer, Cesco, & Scampicchio, 2015; Gremaud, Quaile, Piantini, Pfammatter, & Corvi, 2004; Košir et al., 2001; Ogrinc et al., 2001; Reid, O'Donnell, & Downey, 2006; Wachter, Christoph, & Seifert, 2009). Application of multivariate statistics in wine authentication using stable isotope ratios was also conducted to differentiate the year of production (Ogrinc et al., 2001) and grape variety (Magdas, Cuna, Cristea, Ionete, & Costinel, 2012).

The official method adopted by the European Union (EU) and by the International Organisation of Vine and Wine (OIV), able to detect the addition of water and sugar to grape products, is represented by the isotopic determinations. The method requires reporting the isotopic pattern of the sample to a reference database with isotopic parameters for authentic wine samples (Regulation (EU) No 1308, 2013). In the case of the carbon stable isotopes in plant material, the main factor influencing the δ^{13}C values is the biosynthetic pathway of carbon dioxide fixation, used by plant species, represented by C3 pathway (Calvin plants, such as grapes, many other fruits, sugar beet, potato, which incorporate less ^{13}C than C4 plants, δ^{13}C from −22 to −28‰ in the carbohydrates) or the C4 pathway (Hatch–Slack plants such as cane, maize; δ^{13}C values from −10 to −14‰ in the carbohydrates) including the CAM plants (such as pineapple, vanilla that use both types of metabolic paths) (Primrose et al., 2010).

The most common wine frauds on the market are related with everyday consumption – table wine that is offered in multilayer packaging (PET), under different names, and less with quality wines.

This paper aims to highlight suitable techniques for testing the authenticity and traceability of wines followed by a critical discussion of the results. For that purpose, 29 table wines bought from the market and packed in PET bottles were investigated for fraudulent addition of both sugar and water and for the detection of synthetic sweeteners and colouring agents. Additionally, 23 authentic red wines obtained by microvinification at Murfatlar vineyard (South-East Romania) were investigated in this study. At first, the isotopic ratios of oxygen and carbon (^{18}O/^{16}O and ^{13}C/^{12}C) were determined in all the wine samples (commercial and authentic) to perform a first quality classification by comparing the measured isotopic data with that of the reference wine databank owned by the National R&D Institute for Cryogenics and Isotopic Technologies – ICSI Rm. Valcea (Romania). Subsequently, the nature and profile of anthocyanins, as an indicator of red colour of the wine, was investigated in commercial table wines in order to assess whether measuring certain anthocyanins ratios may provide reliable information on the naturalness of table wines.

2. Materials and methods

2.1. Wines samples

A set of 29 commercial sweet and medium sweet red wines purchased from the Romanian market were subject of this study. The table wines, bottled in polyethylene terephthalate (PET) containers, have the declared origin Romania (17 samples), Bulgaria (3 samples) and Republic of Moldova (7 samples), European Union (EU) (2 sample) and years of harvest 2011 and 2012.

Additionally, we characterized 23 authentic red wine samples, young and aged (vintages from 2009 to 2013). Authentic wine samples were produced on a small scale (batches of 150–200 kg) from grapes of known variety (Cabernet Sauvignon, Merlot, Feteasca Neagra, Pinot Noir and Mamaia), applying a traditional technology for obtaining quality dry red wines, without using any selected yeasts, nutrients or enzymes, complying with (Commission Regulation (EC) No 555, 2008), at the Laboratory of Processing Technology of Grapes and Wine Chemistry, in the Microvinification department of the Research Station for Viticulture and Oenology, Murfatlar, Romania.

All the wine samples were analysed in two replications. The isotopic analyses were performed immediately after opening the bottle. The rest of the wine was transferred to amber bottles in order to protect them against sunlight and was stored in the refrigerator until chromatographic determinations.

2.2. Isotopic analysis

The determination of $^{18}O/^{16}O_{VSMOW}$ (‰) isotopic ratio from the water extracted from wine and of $^{13}C/^{12}C_{PDB}$ (‰) isotopic ratio in the ethanol extracted from wine was performed. Prior to analysis, the wine samples were subject to a preparation procedure on an Automated Distillation Control System (ADCS Eurofins, France) in

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