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Analytical Methods

Discrimination of varietal wines according to their volatiles

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ABSTRACT

A method is being proposed in order to discriminate bottled wines of different varieties when no other information is known. The advantages of the method consist in the fact that anyone who wants to certify the variety, which is written on the label or the area of origin, can use such a technique to achieve the conformity. Additionally, the method can be easily applied by laboratories equipped with a GC. The differentiation has been achieved by using only seven of the total extracted volatiles, mainly higher alcohols and higher alcohol esters, namely 3-methyl-1-butanol, 2,3-butanediol, ethyl lactate, 3-methyl-1-butyl acetate, 2-phenylethanol, phenyl ethyl acetate and p-hydroxy phenyl ethanol. These key compounds are not relevant to a single variety. The proposed method does not take into account variables such as the year of vintage and fermentation procedures (agitation, temperature).

1. Introduction

Traceability and origin identification become increasingly important when referring to foodstuffs. As far as wine is concerned, vine variety and origin are, among other factors, criteria that determine quality and commercial added value.

Various methods have been proposed in order to identify the origin of the various wines. Discrimination of Spanish wines according to their geographical origin has been achieved using Stepwise Discriminant Analysis (Huerta-Diaz-Reganon, Salinas, & Masoud, 1997). In a similar way, French red wines have been classified using multivariate analysis based on chemical data (acids, alcohols, esters, total phenols, pH, and colour) (Sivertsen, Hollen, Nicolaysen, & Risvik, 1999). Wines of Ribeira Sacra Certified Brand of Origin (CBO) have been differentiated from wines of two others CBOs in Galicia, using multivariate chemometric techniques and trace elements analysis data (Latorre, García-Jares, Mèdina, & Herrero, 1994). Other authors investigated the composition and concentration of volatiles (at the germplasm level) in 42 grape cultivars belonging to seven genotypic groups using headspace solid-phase microextraction with GC–MS in order to improve the fruit quality by understanding effects of fruit aroma (Yang et al., 2009). Determination of volatile compounds from wines made with seven clones of Monastrell grapes was performed using ultrasound extraction of the compounds and Linear Discriminant Analysis (Gómez-Plaza, Gil-Muñoz, Carreño-Espín, Fernández-López, & Martínez-Cutillas, 1999).

In wines, production of higher alcohols is influenced by the amino acid composition of the grapes and the yeast strain. As claimed by Rapp and Versini (1995), there is a strong correlation between the amino acid spectrum in must and the absolute and relative levels of higher alcohols in wine. The variation of amino acid profiles in must depend on variety, fertilisation, composition of soil and other factors related to ecological and environmental conditions (Rapp & Versini, 1995). Previous attempts to discriminate wines based only on amino acids (glutamic, aspartic, proline, leucine, alanine and serine) were not successful (Rapp & Versini, 1995).

According to Riberéau-Gaynon, Dubourdieu, Donèche, and Lonvaud (1998), 10% of higher alcohols come from corresponding amino acids through transamination, decarboxylation and hydrogenation. Another 25% are derived from the sugar skeleton and the remaining 65% from other amino acids. Based on this, it has been suggested that the composition of higher alcohols is close

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related to the amino acid pattern of must or grapes. The higher alcohols used for discrimination of wines, come mainly from three amino acids; leucine is the main precursor of 3-methyl-1-butanol and its acetate, phenylalanine of 2-phenylethanol and tyrosine of p-hydroxy phenyl ethanol (=tyrosol) (Dall'Asta et al., 2011; Dickinson, 2008; Yang et al., 2009).

Regarding the relationships of higher alcohols to amino acids, linearity was assessed for 3-methyl-1-butanol and total free amino acids in must and found to be significant (>95%) (Rapp & Versini, 1995). Rapp and Versini (1995) observed the same significance positive linear correlation between 3-methyl-1-butyl acetate and total free amino acids. However, this correlation is observed only within the same vintage year. In the case of 2-phenylethanol, the increase in free amino acids concentration in must leads to a decrease in 2-phenylethanol with the correlation significant at more than 95% (Rapp & Versini, 1995).

Alcohols and polyols of 93 red wines produced from the grape varieties Cabernet Sauvignon, Tempranillo, Monastrell and Bobal were analysed by Discriminant Analysis (Aleixandre, Lizama, Álvarez, & García, 2000). These authors showed that isoamyl alcohol (Cabernet Sauvignon), cis-3-hexenol and isobutyl alcohol (Tempranillo), methanol and cis-3-hexenol (Monastrell), and 2,3-butanediol (Bobal) were the most important components in differentiation of the varieties.

Concerning statistical analysis of the results Principal Component Analysis (PCA) has been also used to study other foodstuffs besides wines, discriminating Robusta and Arabica coffees (Casal, Oliveira, Alves, & Ferreira, 2000) as well as different blends (Bicchi, Panero, Pellegrino, & Vanni, 1997). Also, PCA and discrimination analysis (DA) were used to identify olive oil adulteration with various other seed oils, discrimination of animal fats from vegetable oils, and animal fat adulteration with seed oils as well as olive oils on the basis of their geographical origin (Dourtoglou, Dourtoglou, Diamadopoulou, & Lalas, 2013).

In this work a method is being proposed to allow discrimination of bottled wines made from different grape varieties when no other information is known, based on the volatiles present in the wine. The driving idea was to use extracted volatiles instead of amino acids (which are influenced by many factors) in must or in wine. The method was applied to two varietal wines, Agiorgitiko and Moschofilero (one red and one white), which are cultivated mainly in the Peloponnese (southern Greece) in delimited area. The volatiles used were higher alcohols and associated esters. The dataset from GC analysis was subjected to PCA and DA. Additional samples from other individual wine varieties (Xinomavro, Cabernet Sauvignon, Chardonnay), a mixed wine (made of Roditis, Savattiano, Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, Robola and Vilana varieties), and ferment model solutions were used for comparison in order to test the discrimination potential of the proposed method.

2. Materials and methods

2.1. Wine samples

Commercial bottled wines were purchased from a local shop, which was able to confirm variety and geographical origin. A1–A15 were different wines of the Agiorgitiko variety (Group 1 – Agiorgitiko) while M1–M11 were various wines of the Moschofilero variety (Group 2 – Moschofilero). The brands and the vintages of all wines used are presented in Table 1.

Additionally, an experimental wine (MIX) was prepared in order to test the discrimination ability of the method. It was made of 57% of Roditis (white Greek variety), 38% of Savattiano (white Greek variety) and 5% of Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, Robola (Greek variety) and Vilana (Greek variety). In MIX no extraction of compounds from the grape skins has taken place (produced as white wine). For the production of MIX, after pressing the grapes, the must was transferred to a stainless steel tank. All fermentation procedures were carried out under controlled temperature ranging from 16 °C to 17 °C. The must was fermented by its native strains (no addition of commercial *Saccharomyces cerevisiae* strains). The MIX, Xinomavro (XIN) (a red variety originated from the northern part of Greece) and Cabernet Sauvignon (S1) made up a third group (Group 3 – Other). A fourth group (Group 4 – Chardonnay) was made up of Chardonnay wines (CT, CA, CW, CJ, CP).

Table 1
Brand names of wines.

Code	Name	Vintage
M1	Mantinia Spyropoulos	1997
M2	Mantinia Megapanos	1997
M3	Mantinia Megapanos	1998
M4	Mantinia Cavino	1997
M5	Mantinia Vinifera	1998
M6	Mega Oinos Skouras	1998
M7	Paraskevopoulos	1999
M8	Mantinia Antonopoulos	1997
M9	Mantinia Spyropoulos	1998
M10	Mantinia Tselepos	1997
M11	Boutaris	1998
A1	Paraskevopoulos 14%vol	1999
A2	Paraskevopoulos 12.8%vol	1999
A3	Paraskevopoulos rose	1999
A4	Paraskevopoulos native strain	1999
A5	Skouras	1997
A6	Epilogi	1996
A7	Leontios Oinos	1995
A8	Nemea Reserve Cambas	1994
A9	Kouros	1996
A10	Kourtakis	1998
A11	Chateau Gaia	1997
A12	Chateau Gaia	1998
A13	Chateau Gaia	1999
A14	Paraskevopoulos	2000
A15	Paraskevopoulos	2000
XIN	Naousa Boutari	1998
S1	Cabernet Sauvignon	1997
CT	Tselepos Chardonnay	1999
CA	Antonopoulos Chardonnay	1999
CW	Wente Chardonnay California	1999
CJ	Jacob's Creek Chardonnay Australia	1999
CP	Papantoniou Chardonnay	1999

Three *S. cerevisiae* (SC) strains from Mantinia and Nemea Region (production areas of Moschofilero and Agiorgitiko wines, respectively) were isolated, purified and cultivated using standard procedures. These strains were used to ferment model solutions (sugar solutions) containing 20 g/L sucrose, 1% meat peptone and 1% yeast extract. Each SC strain fermented two identical solutions, and the volatiles produced were analysed by GC.

All procedures were conducted at the experimental winery of the Technological Educational Institution of Athens (Greece), at the Faculty of Food Science, Department of Oenology and Beverage Technology.

2.2. Extraction method

For the extraction of volatiles from wines or SC-fermented sugar solutions the following procedure was used: 20 g of NaCl were added into 100 g of wine, which was then extracted twice, using a mixture of 100 mL of pentane and 100 mL of diethyl ether. Where an emulsion was created during the extraction, 10 mL of saturated solution of NaCl was added. The organic layer was dried with Na_2SO_4, filtered through paper and, finally, the solvent was removed