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Analysis of phenolic compounds in Muscatel wines produced in Portugal

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Abstract

A liquid chromatography method associated with mass spectrometry and diode array, fluorescence and electrochemical detectors was used in order to study phenolic composition of Muscatel sweet wines from Setúbal region in Portugal. Samples were collected during winemaking production at different representative producers of this region. Total phenolic contents of samples were also determined using the Folin–Ciocalteu method. Mass spectrometry results show that atmospheric pressure chemical ionisation (APCI) in negative mode presents higher sensitivity for the majority of the compounds studied. Some phenolic acids, stilbenes as resveratrol and piceid, and flavonols as quercetin and quercetin glycosides were identified in these Muscatel wines. For resveratrol, piceid, gallic acid, protocatechuic acid, catechin and quercetin, fluorescence and electrochemical properties were used as complementary or alternative methods of detection. Differences in phenolic composition and total phenolic contents were found among samples collected.

Keywords: Muscatel wine; Phenolic compounds; Fluorescence detector; Electrochemical detector; Mass spectrometry; Folin–Ciocalteu

1. Introduction

Phenolic compounds play an important role in colour and flavour of foods and beverages and its regular consumption on a diet has been associated with beneficial effects for human health [1]. Some phenolic compounds found in wines are antioxidants contributing to a reduction in the risk of cardiovascular diseases, others such as resveratrol, gallic acid and quercetin have been claimed to have activity against allergies, inflammation, hypertension, arthritis and carcinogens [2–6]. White wines, with a lower phenolic content than red wines, have lower antioxidant activity, although some phenolic compounds present in these wines are more effective in the in vitro inhibition of LDL oxidation process [7].

The type and concentration of phenolic compounds in wines is influenced by the chemical composition of the raw materials (grapes) which are influenced by the variety, ripening stage, atmospheric conditions during ripening and type of soil. The techniques used during the winemaking process of the wine and ageing conditions [8–10] are also important. Phenolic aldehydes (e.g. vanillin), benzoic acids (e.g. gallic acid), hydroxycinnamic acids (e.g. caffeic, ferulic and *p*-coumaric acids) and their esters obtained by condensation with tartaric acid (hydroxycinnamoyltartaric acids), flavanols, flavonols (e.g. quercetin) and anthocyanins are extracted from grapes during the winemaking process. Also flavan-3-ols as catechins present in the grape as monomers or polymerized to form proanthocyanidins and hydrolysable tannins [11,12] and stilbenes, as resveratrol or its glycoside form (piceid) occur in wine [13,14].

Some phenolic compounds can be extracted from wood during the ageing stage and oxidation reactions may also occur increasing the stability of the wine and its pleasant sensorial characteristics.

Phenolic compounds have been analysed by liquid chromatography (LC) with diode array (DAD), fluorescence (FD) [10,14,15] and electrochemical (ED) detection [16,17]. Liquid chromatography with mass spectrometry (MS) using atmospheric pressure ionisation (electrospray or chemical ionisation) has also been used [18] in order to identify their chemical structures.

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In Portugal, there are significant productions of dessert Muscatel wines in Setúbal and Douro regions. These wines are produced from white or red grapes of Muscatel vine varieties: the strong and characteristic bouquet of these grapes is usually much appreciated by consumers.

After harvest, Muscatel grapes are fermented and when sugar content of the must is about 90–100 g L^{-1}, spirit is added in order to stop the fermentation process. Flavour and phenolic compounds from grape pulp and skin are extracted in a maceration process lasting for several months. The wine is then separated from the pomace and the liquid from pressing the pomace is added to the wine; afterwards the wine is transferred into wooden barrels where it stays for at least 24 months [19].

The aim of this work was to identify compounds and monitor the changes in the phenolic composition during the winemaking process of Muscatel wines from different producers in the Setúbal region. Some phenolic compounds detected in the samples were identified by LC–MS^n and the analysis were also carried out with a LC-DAD-FD-ED system. Results obtained from chromatographic profiles were compared with the total phenolic content measured by Folin–Ciocalteu method.

2. Experimental

2.1. Reagents

Acetonitrile (LC–MS and gradient grade) and methanol (HPLC grade) used were from Lab-Scan (Dublin, Ireland). The *o*-phosphoric acid 85% and formic acid (analytical reagent grade) were respectively from Riedel-deHaën (Seelze, Germany) and Panreac (Barcelona, Spain).

Deionised water with 0.050 μS cm^{-1} conductivity, prepared with a Mili-Q system (Millipore, Molsheim, France), was used in all experiments.

The standards as gallic acid, 5-hydroxymethylfurfural (5-HMF), protocatechuic acid, furfural, *p*-hydroxybenzoic acid, catechin, caffeic acid, vanillin, ferulic acid, *trans*-piceid, *trans*-resveratrol were obtained from Aldrich (Steinheim, Germany) and epicatechin, quercetin-3-glucoside and quercetin were obtained from Extrasynthése (Genay, France).

Standard stock solutions (at 10.0 g L^{-1}) were prepared by dissolving the compounds in methanol and stored in the darkness at 4 °C. Working solutions were prepared by dilution of the standard stock solutions with deionised water.

Folin–Ciocalteu reagent was obtained from Sigma (Steinheim, Germany) and sodium carbonate from Riedel-de Haën (Seelze, Germany).

2.2. Sampling

Muscatel Setúbal wines from the 2004 vintage were produced in three wineries identified as producers A, B and C. After spirit was added to stop fermentation, samples were collected during the maceration process (M) corresponding to wine in contact with the seeds and the skins: for 7 months ($1M_A$–$7M_A$) for producer A, for 5 months ($1M_B$–$5M_B$) for producer B and for 8 months ($2M_C$–$9M_C$) for producer C. The pomace was than pressed, the liquid obtained (LP) was added to the wine and a maturation stage (ST) was started. Samples were collected monthly: for 4 months ($1ST_A$–$4ST_A$) for producer A, 6 months ($1ST_B$–$6ST_B$) for producer B and 2 months for producer C ($1ST_C$–$2ST_C$).

2.3. Sample preparation

Ten millilitre of wine were extracted four times with 7 mL of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under vacuum. The residue was redissolved in 250 μL of methanol/water (6:4, v/v) [13] and analysed by liquid chromatography with diode array and mass spectrometry.

Wines were filtered with Acrodisc® Syringe Filter 0.45 (m HT Tuffryn® Membrane from Pall Corporation (Ann Arbor, USA) and analysed by liquid chromatography with diode array, fluorescence and electrochemical detection.

One millilitre of wine was diluted with 4 mL of deionised water for determination of the total phenolic content using the Folin–Ciolcalteu method.

2.4. Equipments and conditions of analysis

2.4.1. Liquid chromatography with mass spectrometry

Analyses by LC were performed with a Surveyor equipment from Thermo Finnigan. The mass spectrometry system was an LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources. The LC–MS system was run by Xcalibur version 1.3 software (Thermo Finnigan—Surveyor, San Jose, USA).

Separations were performed at 35 °C with a LiChrospher C18 (5 μm, 250 mm × 4 mm i.d.) column from Merck with a guard column of the same type. The samples were injected using a 20 μL loop.

The separations were carried out with a flow rate of 700 μL min^{-1} and the mobile phase consisted of a gradient mixture of eluent A (formic acid 0.5%) and eluent B (formic acid–acetonitrile–water 5:400:595, v/v/v). The following gradient of eluents was used: 0–15 min from 0 until 20% eluent B; 10 min with 20% eluent B; 25–70 min, from 20 until 70% eluent B; 70–75 min, with 70% eluent B; 75–85 min from 70 until 100% eluent B; 85–90 min, with 100% eluent B.

The following conditions were used for the mass spectrometer experiments:

- *ESI source*: temperature of the heated capillary 280 °C; electrospray voltage 3.7 kV in positive mode and 3.0 kV in negative mode;
- *APCI source*: vaporizer temperature 465 °C; discharge current 5 μA; temperature of the heated capillary 250 °C.

Nitrogen was used as sheath gas and auxiliary gas in the experiments performed by ESI and APCI. The sheath and auxiliary gas flow rates were 80 and 20 arbitrary units, respectively.