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Oligosaccharides of Cabernet Sauvignon, Syrah and Monastrell red wines

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ABSTRACT

Wine oligosaccharides were recently characterized and their concentrations, their composition and their roles on different wines remain to be determined. The concentration and composition of oligosaccharides in Cabernet Sauvignon, Syrah and Monastrell wines was studied. Oligosaccharide fractions were isolated by high resolution size-exclusion chromatography. The neutral and acidic sugar composition was determined by gas chromatography. The MS spectra of the oligosaccharides were performed on an AccuTOF mass spectrometer. Molar-mass distributions were determined by coupling size exclusion chromatography with a multi-angle light scattering device (MALLS) and a differential refractive index detector. Results showed significant differences in the oligosaccharidic fraction from Cabernet Sauvignon, Syrah and Monastrell wines. This study shows the influence that the grape variety seems have on the quantity, composition and structure of oligosaccharides in the finished wine. To our knowledge, this is the first report to research the oligosaccharides composition of Cabernet Sauvignon, Syrah and Monastrell wines.

1. Introduction

Due to their importance for the properties of wines, polyphenols, proteins, and polysaccharides have been widely studied. However, the identification, quantification and composition of oligosaccharides in wine have only recently been the object of study (Bordiga et al., 2012; Ducasse, Williams, Meudec, Cheynier, & Doco, 2010; Ducasse et al., 2011).

Oligosaccharides contain between three and fifteen monosaccharide residues covalently linked through glycosidic bonds. Important medicinal, food and agricultural applications are associated with these molecules (Gibson & Roberfroid, 1995; Qiang, YongLie, & QianBing, 2009). Oligosaccharides are related to plant defense responses (Darvill & Albersheim, 1984). In addition, these molecules are well established as dietary antioxidants (Otaka, 2006) and are thought to provide various health benefits (Lama-Muñoz, Rodríguez-Gutiérrez, Rubio-Senent, & Fernández-Bolaños, 2012). In wine, oligosaccharides were identified for a long time as sucrose and various diholosides (Doco, Williams, Vidal, & Pellerin, 1997), and they are found too as short chain galacturonic acid (2–6 DP). These molecules are originated in the degradation of the smooth region of pectin (Pellerin & Cabanis, 1998). As regards wine quality, some oligosaccharides have physicochemical properties such as an ability to chelate cations (Cescutti & Rizzo, 2001). Besides, Quijada-Morín, Williams, Rivas-Gonzalo, Doco, and Escribano-Bailón (2014) reported than astringency perception is positively related to certain monosaccharides in the oligosaccharide fraction from Tempranillo wines. The first isolation and characterization of the oligosaccharide fractions in red wines was made by Ducasse et al. (2010). Recently, Bordiga et al. (2012) have isolated and characterized forty-five complex free oligosaccharides in red and white wines.

Monastrell, also known internationally as the French name of "Mourvèdre", is the main wine grape cultivar in southeastern Spain. Cabernet Sauvignon and Syrah, two of the most widely used varieties in the world, are also used in this region to complement Monastrell wines. However, to our knowledge there is no study comparing the oligosaccharide content of the wines obtained with these three grape cultivars.

The aim of the present work was to study the oligosaccharide fractions of Cabernet Sauvignon, Syrah and Monastrell wines.

2. Materials and methods

2.1. Grape materials

The raw materials used for this study were berries from *Vitis vinifera* cvs. Cabernet Sauvignon, Syrah and Monastrell grown in

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Murcia (southern Spain). The grapes were carefully harvested at commercial maturity with a total of soluble solids content between 24 and 26°Brix during the 2007 vintage.

2.2. Preparation of Trials

Three 90 kg lots of grapes from each variety (Cabernet Sauvignon, Syrah and Monastrell) were destemmed and crushed, and distributed into 100 L stainless steel tanks to yield triplicate control lots. At the same time, Potassium bisulfite (8 g/100 kg grape) was added.

2.3. Fermentation

All fermentations were started by adding commercial dry yeast (Levuline Gala, OenoFrance, Bordeaux, France) at 10 g/hL and were carried out in 100-L stainless steel tanks equipped with temperature control (25 °C) which enabled the fermentation kinetics to be regulated. Each lot was fermented to completion, and when alcoholic fermentation was finished (as attested by sugar analysis), the musts were pressed at 150 kPa in a 75-L tank membrane press (Hidro 80L, Ausavil, Spain). Free-run juice and press wines of each trial were combined and stored in 50-L tanks. One month later the wines were racked. After spontaneous malolactic fermentation, the wines were racked again and supplied with 25 mg/L sulfur dioxide. The wines were cold stabilized (−3 °C), bottled and stored in the experimental wine cellar at 18 °C until analysis.

2.4. Isolation of oligosaccharide fractions

The oligosaccharide fractions were isolated as previously described (Ducasse et al., 2010, 2011). Cabernet Sauvignon, Syrah and Monastrell wines (5 mL) were partially depigmented by decolourization on a column of MN Polyamide SC6 (5 × 1 cm) previously equilibrated with 1 M NaCl. The wine oligosaccharides not retained on the polyamide column were eluted by 2 bed volumes of 1 M NaCl (Brillouet, Moutounet, & Escudier, 1989). High-resolution size exclusion chromatography was performed by loading 2 mL of the previously concentrated fraction on a Superdex 30-HR column (60 × 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 × 4 cm), equilibrated at 1 mL/min in 30 mM ammonium formiate pH 5.6. The elution of polysaccharides was followed with an Erma-ERC 7512 (Erma, Japan) refractive index detector combined with a Waters Baseline 810-software. One fraction was collected between 60 and 93 min. The isolated fraction was freeze-dried, re-dissolved in water, and freeze-dried again four times to completely remove the ammonium salt. The resulting fraction corresponded to the Cabernet Sauvignon, Syrah and Monastrell oligosaccharide fraction.

2.5. Sugar composition as trimethylsilyl derivatives

The monosaccharide composition was determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 16 h), by gas chromatography (GC) of their per-*O*-trimethylsilylated methyl glycoside derivatives (Doco, O'Neill, & Pellerin, 2001). The separation of the TMS derivatives was previously described (Apolinar-Valiente et al., 2014).

2.6. Glycosyl-linkage determination

The glycosyl-linkages composition was determined by GC–MS of the partially methylated alditol acetates. One milligram of polysaccharides in 0.5 mL dimethylsulfoxide was methylated using methyl sulfinyl carbanion and methyl iodide (Hakomori, 1964). The methylated materials were then treated with 2 M TFA (1.15 h at 120 °C). The released methylated monosaccharides were converted to their corresponding alditols by treatment with $NaDH_4$ and then acetylated (Harris, Henri, Blakeney, & Stone, 1984). Partially methylated alditol acetates were analyzed by GC–EI–MS using a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film); temperature programming 135 °C for 10 min, then 1.2 °C/min to 180 °C, coupled to a HP5973 MSD (Vidal, Williams, O'Neill, & Pellerin, 2001).

2.7. ESI mass spectrometry

Wine oligosaccharide samples (50 μg) in 1:1 MeOH–water (5 μL) were injected directly into an AccuTOF (AccuTOF™ JMS-T100 LC, Jeol, Japan) mass spectrometer equipped with an ESI source and a time-of-flight (TOF) mass analyzer in negative ion modes. The source voltage was set at −2000 V (negative ESI), the orifice voltage at −45 V (negative ESI), the desolvating chamber temperature at 250 °C and the orifice temperature at 80 °C, the mass ranging from 200 to 4000 Da. ESI-TOF spectra were obtained and extracted as ASCII files.

2.8. Determination of molar mass

Molar-mass distributions were determined at 25 °C by coupling size exclusion chromatography with a multi-angle light scattering device (MALLS) and a differential refractive index detector. SEC elution was performed on OHPAK guard column followed by four serial Shodex Ohpak KB-803, KB-804, KB-805 and KB-806 columns (0.8 × 30 cm; Shodex Showa Denkko, Japan) at 1 mL/min flow rate in 0.1 M $LiNO_3$ after filtration through 0.1 μm filter unit. The MALLS photometer, a DAWN-HELEOS from Wyatt Technology Inc. (Wyatt Technology Corporation, Santa Barbara, CA, USA), was equipped with a GA–AS laser (λ = 658 nm). The concentration of each eluted oligosaccharide was determined using the differential refractive index detector (Optilab TrEX, Wyatt Technology Inc. USA). All collected data were analyzed using Astra V 6.0.6 software with the zimm plot (order 1) technique for molar-mass estimation and a dn/dc of 0.145 mL/g (Redgwell, Schmitt, Beaulieu, & Curti, 2005).

2.9. Statistical data treatment

Average values, standard deviation and statistical significance were calculated and performed with the Statgraphics Plus 5.1. Package.

3. Results and discussion

3.1. Cabernet Sauvignon, Syrah and Monastrell wine oligosaccharide fractions: quantification and characterization

The fraction eluted on Superdex 30-HR column contained a complex mixture of small sugars, was taken to represent the oligosaccharide fraction of the studied wines and were distributed similarly as described previously in Carignan, Merlot and Monastrell wines (Apolinar-Valiente et al., 2014; Ducasse et al., 2010).

When the glycosyl residue composition of Cabernet Sauvignon, Syrah and Monastrell oligosaccharides was analyzed (Table 1), all the wine oligosaccharides included rhamnose, arabinose, galactose, xylose and galacturonic and glucuronic acids coming from the pecto-cellulosic cell walls of grape berries. Other sugars such as mannose and glucose were released from the yeast polysaccharides. The glycosyl residue composition (Table 1) showed most of the sugars known to form part of the composition of complex carbohydrates in wine (Ayestarán, Guadalupe, & León, 2004; Doco,