Carbohydrate Polymers 133 (2015) 567–577

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Dissecting the polysaccharide-rich grape cell wall changes during winemaking using combined high-throughput and fractionation methods

Yu Gao [a], Jonatan U. Fangel [b], William G.T. Willats [b], Melané A. Vivier [a], John P. Moore [a,*]

[a] Institute for Wine Biotechnology, Department of Viticulture and Oenology, Faculty of AgriSciences, Stellenbosch University, Matieland 7602, South Africa
[b] Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, DK-1001, Denmark

ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 3 July 2015
Accepted 9 July 2015
Available online 22 July 2015

Keywords:
Cell wall
Fractionation
Grape pomace
Profiling
Pectin
Xyloglucan
Winemaking

ABSTRACT

Limited information is available on grape wall-derived polymeric structure/composition and how this changes during fermentation. Commercial winemaking operations use enzymes that target the polysaccharide-rich polymers of the cell walls of grape tissues to clarify musts and extract pigments during the fermentations. In this study, we have assessed changes in polysaccharide composition/turnover throughout the winemaking process by applying recently developed cell wall profiling approaches for monosaccharide composition (GC–MS), infra-red (IR) spectroscopy and comprehensive microarray polymer profiling (CoMPP). CoMPP performed on the concentrated soluble wine polysaccharides showed a fraction rich in rhamnogalacturonan I (RGI), homogalacturonan (HG) and arabinogalactan proteins (AGPs). We also used chemical and enzymatic fractionation techniques in addition to CoMPP to understand the berry deconstruction process more in-depth. CoMPP and gravimetric analysis of the fractionated pomace used aqueous buffers and CDTA solutions to obtain a pectin-rich fraction (pulp tightly-bound to skins) containing HG, RGI and AGPs; and then alkali (sodium carbonate and potassium hydroxide), liberating a xyloglucan-rich fraction (mainly skins). Interestingly this fraction was found to include pectins consisting of tightly associated and highly methyl-esterified HG and RGI networks. This was supported by enzymatic fractionation targeting pectin and xyloglucan polymers. A unique aspect is datasets suggesting that enzyme-resistant pectin polymers 'coat' the inner xyloglucan-rich skin cells. This data has important implications for developing effective strategies for efficient release of favorable compounds (pigments, tannins, aromatics, etc.) from the berry tissues during winemaking. This study provides a framework to understand the complex interactions between the grape matrix and carbohydrate-active enzymes to produce wine of desired quality and consistency.

1. Introduction

Grape berry composition has a direct influence on final wine quality (Hernández-Hierro et al., 2014). The physical breaking of the grape berries during the crush and the subsequent degradation of berry tissues through chemical and enzymatic reactions form the must (grape juice), the cap (mostly skins and pulp, which we define as de-seeded pomace) and sediment (mostly pulp and seeds) (González-Neves et al., 2010), which provides the fermentation matrix for wine production. The release of grape-derived compounds (particularly from the skins) contributes to the specific wine-style and the perceived quality of the wine (Busse-Valverde, Gómez-Plaza, López-Roca, Gil-Muñoz, & Bautista-Ortín, 2011). Wine industries apply commercial enzyme blends (mainly crude enzyme mixtures from fungi such as *Aspergillus* spp.) during alcoholic fermentation to aid the winemaking process and enhance quality parameters. For example, in red wine fermentations, enzyme mixes are included to support maceration (e.g. leaching of phenolic compounds from berry skin into must) (Canal-Llauberes, 1993). Enzymes are added to both red and white wine fermentations for the purpose of clarifying the wine, decreasing viscous polymers and improving free-run juice/wine volumes (Canal-Llauberes, 1993).

Abbreviations: AIR, alcohol insoluble residue; AGP, arabinogalactan protein; CoMPP, comprehensive microarray polymer profiling; CBM, carbohydrate binding module; CDTA, cyclohexanediamine-tetra-acetic acid; EPG, endo-polygalacturonase; FT-IR, Fourier transform-infrared spectroscopy; GC, gas chromatography; HG, homogalacturonan; mAb, monoclonal antibody; PME, pectin methyl esterase; RG, rhamnogalacturonan.

* Corresponding author.
E-mail address: moorejp@sun.ac.za (J.P. Moore).

http://dx.doi.org/10.1016/j.carbpol.2015.07.026
0144-8617/

In essence, these enzymes are added to "work on" the berry tissues to disrupt and/or weaken the cell walls that contain the compounds of interest in the various cells of the berry skin and pulp tissues. These enzyme blends therefore contain a number of carbohydrate active enzymes (these include amongst others endo-polygalacturonase (EPG), pectin-lyase (PL), arabinanase, galactanase, rhamnogalacturonase (RGase), endo-glucanase (EG), xyloglucanase (XEG) and cellulases) which actively assist the breakdown of the grape cell walls of berry tissues, favoring the release of important compounds such as sugars, organic acids, anthocyanins, tannins and monoterpenes (Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2012). The structure(s) of the grape tissue cell walls are complex and still poorly understood at the polymer architectural level. Currently, we know that the grape berry skin cell walls consist of cellulose, hemicellulose and are particularly rich in pectin (Lecas & Brillouet, 1994; Moore, Fangel, Willats, & Vivier, 2014; Saulnier & Thibault, 1987; Vidal et al., 2003; Zietsman et al., 2015). This pectin component contains a number of polymers (homogalacturonan (HG), rhamnogalacturonan I (RGI), side chains such as arabinans and galactans, rhamnogalacturonan II RGII and arabinogalactan-proteins (AGPs)) (Arnous & Meyer, 2009; Moore, Fangel, et al., 2014; Ralet et al., 2010) and was proposed to be associated with other cell wall polymers (cellulose and hemicellulose) (Doco, Williams, Pauly, O'Neill, & Pellerin, 2003). The HG and RG1 backbones of plant pectins are often acetylated and methylesterified to various degrees while the RG1 side chains include various lengths of linear and branched galactans, arabinans and arabinogalactans (Fry, 2011). These different cell wall components undergo compositional changes during berry development (ripening) with pectin and hemicelluloses both showing degrees of depolymerisation (Moore, Fangel, et al., 2014; Nunan et al., 1998). The ripeness levels of harvested grapes correlated with a greater degree of depolymerisation with overripe (27 Brix°) versus ripe (23 Brix°) in a study performed on Pinotage (Zietsman et al., 2015). It was found that wine maceration enzymes were useful in ripe grapes where skin cell walls were much more intact than in overripe grapes where significant tissue polymer degradation had already taken place (Zietsman et al., 2015).

Recent work on grape-and wine-derived cell wall polysaccharides almost exclusively relies on monosaccharide compositional analysis (i.e. total polysaccharide hydrolysis followed by chromatography of the main monomeric sugars released) (Arnous & Meyer, 2009; Bindon, Bacic, & Kennedy, 2012; Mendes, Prozil, Evtuguina, & Cruz Lopes, 2013). The advantage of using the CoMPP (Comprehensive Microarray Polymer Profiling) methodology (Moller et al., 2007) is that polysaccharides are directly identified by virtue of their epitope abundance, albeit semi-quantitatively in a profiling approach (Persson, Sørensen, Moller, Willats, & Markus, 2011). Using monosaccharide composition analysis (York, Darvill, McNeil, Stevenson, & Albersheim, 1985) and CoMPP (Moller et al., 2007) in conjunction with ATR-FT-MIR spectroscopy (Moore, Nguema-Ona, et al., 2014) allows for a more complete picture to be obtained of cell wall composition and turnover. These higher-throughput methods have been successfully applied in a comparative grape ripening study (Moore, Fangel, et al., 2014) and to monitor the effects of enzyme addition on grape skin cells walls during wine fermentation (Zietsman et al., 2015). For example, in the work by Zietsman et al. (2015) it was observed that partial unraveling of skin cell walls occurred during fermentation demonstrating the value of the approach in detecting more subtle cell wall structural alterations than that achievable using a complete acid hydrolysis approach (Arnous & Meyer, 2009).

The aim of this study was to characterise the different matrices obtained from Cabernet Sauvignon grapes (Moore, Fangel, et al., 2014) undergoing a standard red winemaking procedure by applying these higher-throughput methods (Moore, Nguema-Ona, et al., 2014). These matrices include the de-seeded pomace (processed to alcohol insoluble residue, AIR) at three different stages of fermentation (Brix° 24, 12 and 0) and the final wine (concentrated to obtain the main polysaccharide constituents) obtained. Further to this, a unique aspect of the study is the combination of profiling methods (Moore, Nguema-Ona, et al., 2014) with chemical and enzymatic fractionation protocols applied to the de-seeded pomace AIR in order to obtain an in-depth characterisation of the grape berry polysaccharide matrix as it underwent enzyme-assisted maceration and alcoholic fermentation.

2. Experimental

2.1. Grape sampling and processing during winemaking

The grape samples (*Vitis vinifera* cv. Cabernet Sauvignon) used in this study came from the Welgevallen experimental vineyard of the Department of Viticulture and Oenology, Stellenbosch University, South Africa. The vineyard is situated (33°56′ 42″ S, 18°51′44″ E), close to the Eerste River and composed of alluvial soils with light to medium texture and is arranged in a north–south row orientation. Vines are trained on a seven-wire vertical trellis system and are drip-irrigated. The berries have been harvested when the average Brix° was around 24 (sugar content 275 g/l), ca. 150 kg of berries were harvested from 80 vines in total. Red wine was fermented followed the procedure in Zietsman et al. (2015). Briefly, the berries (3 biological repeats, 50 kg each in 50 l buckets) were de-stalked and pressed and the must inoculated with *Sacchromyces cereviseae* VIN13 (Anchor Yeast, Cape Town, South Africa) at 0.2 g/l (following the manufacturer's suggestions). The DSM Rapidase pectinolytic clarification enzyme blend (mainly containing endo-polygalacturonases according to the product data sheets, http:\\www.dsm-foodspecialities.com) was added, following the recommended dosage for easier filtration and clarification at the end of fermentation. The fermentation ran for 8 days at 25 °C until the dry (sugar level determined by using the hydrometer, CDS Vintec, Germany). The residual sugar was lower than 5 g/l. The de-seeded pomace (grape residues) was sampled at 3 fermentation stages, i.e. at the start (Brix° 24, alcohol ca. 0% v/v), middle (Brix° 12, alcohol ca. 7% v/v) and end (Brix° 0, alcohol ca. 14% v/v) of fermentation. Pomace samples were collected by separating the cap from the seeds and must/wine. Then flash-frozen in liquid nitrogen and stored at −80 °C for later analysis, whereas the wine was racked and stored at −4 °C until the wine polysaccharides were concentrated and analysed.

2.2. Wine concentration and polysaccharide enrichment

The wine samples (alcohol ca. 14% w/w) were filtered using an Amicon ultrafiltration system (Millipore, South Africa) with a 5 kDa cut-off filtration membrane (Sigma–Aldrich, South Africa) to reduce ca. 80% of the original volume. Ice-cold absolute ethanol was then slowly added to the concentrated wine to precipitate the soluble polysaccharide polymers overnight; the precipitated residue was subsequently washed in organic solvents according to Moore, Nguema-Ona, et al. (2014).

2.3. Cell wall material isolation and fractionation from pomace

Pomace (de-seeded) from each of the three stages was milled in liquid nitrogen using a Retsch Mixer Mill (30 round/min, 30 s, Retsch, Haan, Germany). The liquid nitrogen cooled milled material was plunged into boiling 80% v/v aqueous ethanol for 15 min and then cooled, followed by centrifugation at 4000 rpm for 5 min. The pellets were sequentially washed in ethanol, methanol, chloroform