Carbohydrate Polymers 152 (2016) 510–519

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Dissecting the polysaccharide-rich grape cell wall matrix using recombinant pectinases during winemaking

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 11 April 2016
Received in revised form 29 May 2016
Accepted 31 May 2016
Available online 13 June 2016

Keywords:
Recombinant pectinases
Grape pomace
Multivariate data analysis
Cell wall model
Pectin
Winemaking

ABSTRACT

The effectiveness of enzyme-mediated-maceration in red winemaking relies on the use of an optimum combination of specific enzymes. A lack of information on the relevant enzyme activities and the corresponding polysaccharide-rich berry cell wall structure is a major limitation. This study used different combinations of purified recombinant pectinases with cell wall profiling tools to follow the deconstruction process during winemaking. Multivariate data analysis of the glycan microarray (CoMPP) and gas chromatography (GC) results revealed that pectin lyase performed almost as effectively in de-pectination as certain commercial enzyme mixtures. Surprisingly the combination of *endo*-polygalacturonase and pectin-methyl-esterase only unraveled the cell walls without de-pectination. Datasets from the various combinations used confirmed pectin-rich and xyloglucan-rich layers within the grape pomace. These data support a proposed grape cell wall model which can serve as a foundation to evaluate testable hypotheses in future studies aimed at developing tailor-made enzymes for winemaking scenarios.

1. Introduction

Grape berries contain a number of nutritious and flavour enhancing (health beneficial, e.g. anthocyanins, tannins, stilbenes, aromatic terpenes, etc.) compounds which are shown to be mainly localized in the vacuole(s) of berry skin cells (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014; González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015). Release of these favourable compounds relies heavily on the efficiency and the control of the berry cell wall deconstruction process (Gao, Fangel, Willats, Vivier, & Moore, 2015; Zietsman, Moore, Fangel, Willats, Trygg et al., 2015). Maceration during fermentation is controlled by the winemaker to achieve optimal extraction of these metabolites and macromolecules from the pooled harvested berries into the alcoholic fermentation (AF) during of the conversion must into wine (Arnous & Meyer, 2010). The maceration process, mainly in red winemaking, involves fermenting berry skins (i.e. caps) with must (i.e. pomace and juice) being punched down several times a day during the AF.

Commercial enzyme preparations are added during the maceration process to aid cell wall degradation and the release of favourable compounds for many years (Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2012). However, the scientific understanding of how these enzymes (mainly produced from wood-rot fungi) act on grape berries is far from clear (Gao et al., 2015; Zietsman, Moore, Fangel, Willats, Trygg et al., 2015). There is much unknown about specific enzyme action, inferred from studies on other species and tissues (not grapes), in the context of winemaking. We for example do not have sufficient information on target grape cell wall polymers that polysaccharide-degrading enzymes act on, although this has been partially remedied with recent studies (e.g. Gao et al., 2015; Zietsman, Moore, Fangel, Willats, Trygg et al., 2015; Zietsman, Moore, Fangel, Willats, & Vivier, 2015). However, crude semi-purified enzyme preparations may still have unwanted side-activities which could negatively impact the wine processing and final quality (Fia, Canuti, & Rosi, 2014). Hence, more scientific knowledge of berry cell wall architecture would help the design of more customisable enzyme preparations; possibly even at the grape cultivar level, providing tailor-made solutions for winemakers, to achieve optimal macer-

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

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

http://dx.doi.org/10.1016/j.carbpol.2016.05.115
0144-8617/

ation, but also advancing our fundamental understanding of berry cell wall structure-function relationships at the polymer level.

There is generally a limited of understanding of the more intricate fundamental architectural nature of the grape cell wall. Several plant cell wall models have been proposed through data collected on various plant species including *Arabidopsis thaliana* (Somerville et al., 2004; Coenen, Bakx, Verhoef, Schols, & Voragen, 2007, Popper & Fry, 2008; Park & Cosgrove, 2012), however, these models are constantly undergoing re-evaluation as new data is generated challenging our previous ideas providing new hypotheses to test (Vincken et al., 2003; Zykwinska, Thibault, & Ralet, 2007; Park & Cosgrove, 2012). It is important to consider that cell wall structure and composition varies among the species (Carpita & Gibeaut, 1993) and within different plant organs and tissues of the same species (Somerville et al., 2004).

As limited studies have been performed on grape cell walls, it is very important to obtain more information on cell wall architecture particularly in the context of maceration and winemaking. Cell wall profiling approaches has been validated on grape leaves, grape berries and winemaking studies to directly probe changes in cell wall polymer organization and architecture (Moore, Fangel, Willats, & Vivier, 2014, Moore, Nguema-Ona et al., 2014; Gao et al., 2015; Zietsman, Moore, Fangel, Willats, Trygg et al., 2015; Zietsman, Moore, Fangel, Willats, & Vivier, 2015). The information provided from these studies not only confirmed the datasets acquired using classical techniques, but through the addition of CoMPP technology has generated a significant amount of new knowledge on subtle changes at the polymer epitope level. However these profiling and fractionation methods alone have not brought us to a fuller understanding of the role of carbohydrate active enzymes (and their synergistic effect) in disrupting and deconstructing grape cell wall architecture during the winemaking process. For this we need a more detailed combinatorial experimental design and study of enzyme action on grape cell walls.

In a recent study by Gao et al. (2015) chemical fractionation combined with CoMPP characterised the wine polysaccharides and bulk pomace polymers released during a standard red wine fermentation (using a clarification enzyme) from Cabernet Sauvignon grapes. A second study by Gao et al. (2016) demonstrated how commercial enzymes are able to reduce intra-vineyard variation of grape berry cell walls via de-pectination and improve extractability of colour and tannins whilst not appearing to influence pectin acetylation. However further information is needed using various combinations of purified recombinant pectinase enzymes (Novozymes, Denmark), with a commercial enzyme preparation (for maceration) as a control, in the context of winemaking. The aim was to evaluate the successive steps necessary to break down the grape berry cell wall in a wine matrix, evaluating the efficacy of different enzymes.

2. Experimental

2.1. Vinification and maceration

Grape berries (*Vitis vinifera* cv. Cabernet Sauvignon) were harvested from the Welgevallen experimental vineyard (33°56′42″S, 18°51′44″E, Department of Viticulture and Oenology), Stellenbosch University, South Africa. The Brix° level for the harvest was ca. 24 (sugar content approx. 275 g/l), assessed using standard viticulture sampling approach. The harvested berries were pooled over the whole vineyard, in order to emulate a typical commercial harvest and winemaking procedure, and then split into separate buckets (5 kg each), and then de-stemmed crushed separately before individual fermentations. Sodium bisulfate (SO_2) was added (30 ppm) into each bucket after crushing to prevent the growth of spoilage microorganisms. *Sacchromyces cereviseae* commercial strain VIN13 (Anchor Yeast, Cape Town, South Africa) at 0.2 g/l (rehydrated and prepared following the manufacturer's directions) was inoculated into each bucket. To each of the buckets were added different combinations of recombinant enzyme(s) (sourced from Novozymes, Denmark); buckets were inoculated in triplicate for statistical reproducibility. Information on mode of action of the enzymes is listed in Table 1. As stated all recombinant enzymes are from Novozymes (Denmark) and the dosage of enzyme added was according to the manufacturer's instructions (i.e. overdosed). The purified nature of the enzymes and activities are provided in Tables 1 and 2 ; and Supplementary Table 1. The wine was fermented at 25 °C for approx. 10 days until the sugar level approached zero (<5 g/l), and then pressed to separate the fermented skins and pulp (pomace) from the free-run wine. The pomace samples were selected to be representative by a composite sampling approach from each bucket following the *Theory of Sampling* (described in Petersen, Minkkinen, & Esbensen, 2005), while the wine was stored at −4 °C until further analysis.

2.2. Cell wall preparation from experimental pomace

The pomace samples after fermentation were de-seeded, and then milled in liquid nitrogen using a Retsch Mixer Mill (30 rounds/min, 30 s, Retsch, Haan, Germany). The resulting powder was incubated in 80% v/v ethanol at 95 °C for 15 min to deactivate any endogenous enzymes, thereafter the pellets were washed by a series of organic solvents (methanol, chloroform, acetone, described in Gao et al. (2015)), following solvent treatment the pelleted material was resuspended in dH_2O and freeze-dried to yield an alcohol insoluble residue (AIR) powder. The use of methanol, chloroform and acetone was validated in the PhD thesis of Fangel (2013), particularly with respect to CoMPP technology, as the optimal combination of solvents.

2.3. Monosaccharide composition analysis using gas chromatography

To analyse and compare the bulk chemical degradation of the cell walls; AIR sourced from pomace of each fermentation was analysed using gas chromatography coupled with mass spectrometer (GC–MS) to determine their cell wall monosaccharides as described in Gao et al. (2015). The AIR samples were hydrolyzed using 2 M TFA (2 h, 110 °C) to monosaccharides, which were then converted to their methoxy derivatives using methanol/methanol HCl (16 h, 80 °C), followed by the silylation with HMDS/TMCS/pyridine (3:1:9, Sylon HTP kit, Sigma-Aldrich, MO, USA). The separation and analysis of each of these derivatives were performed using a gas chromatograph (Agilent 6890 N, Agilent Technologies, CA, USA) coupled to an Agilent 5975 MS mass spectrometry fitted with a polar (95% dimethylpolysiloxane) ZB-Semivolatiles Guardian GC column (30 m, 0.25 mm ID, 0.25 μm film thickness). The nine major cell wall monosaccharides analysed were: arabinose (Ara), fucose (Fuc), rhamnose (Rha), xylose (Xyl), mannose (Man), galacturonic acid (GalA), galactose (Gal), glucose (Glc) and glucuronic acid (GlcA).

2.4. Infra-Red (IR) spectroscopy for wines parameters

A calibrated spectroscopic method was used on all experimental wines to confirm the consistency of all fermentations. To analyse the main oenological parameters, wines (50 ml in triplicate from each fermentation) were analysed using Fourier transform infrared (FT-IR) spectroscopy with a WineScan FT120 Basic instrument (Foss Analytical, Hillerød, Denmark). The oenological parameters tested were: pH, volatile acidity, total acid, glucose, fructose and ethanol