



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

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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.

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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.

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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; Zykwińska, 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 (SO₂) was added (30 ppm) into each bucket after crushing to prevent the growth of spoilage microorganisms. *Saccharomyces cerevisiae* 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 dH₂O 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

Table 1
The enzyme combinations used in this study (“Minus” mark refers to No, “Plus” mark refers to Yes). Untreated (U) with no added enzymes is used as negative control, Lafase HE Grand cru (Cru) is used as positive control; as contextualised in Gao et al. (2016).

Treatment	Endo-polygalacturonase (EPG) EC 3.2.1.15	Pectin methyl esterase EC 3.1.1.11	Pectin lyase EC 4.2.2.10	Endo-arabinanase EC 3.2.1.99	Endo-galactanase EC 3.2.1.89
U	–	–	–	–	–
EPG	+	–	–	–	–
EPM	+	+	–	–	–
PL	–	–	+	–	–
ARA	+	+	–	+	–
GAL	+	+	–	–	+
Cru	Unknown protein content. Description from manufacturer. Pectolytic enzyme preparation, purified in CE for the production red wines that are rich in colouring matter and structured tannins, destined for ageing (Laffort, France)				

Table 2
Information on the specific enzyme activities of enzyme preparations used in the study, substrates tested include polygalacturonic acid, arabinan, galactan and citrus pectin. Specific activity is expressed in nanokatal per gram of recombinant enzymes or commercial enzyme preparation, corresponding to 1 nmol reducing sugar formed per second in the defined conditions for polygalacturonase, arabinanase and galactanase, or to the variants of one absorbance unit in 1 min at 25 °C and at the selected pH for pectin lyase. Laffort HE Grand Cru was tested with polygalacturonic acid and citrus pectin for the data shown in this table.

Enzyme	Enzyme activity
Endo-polygalacturonase (EPG)	27806.83 ± 458
Pectin methyl esterase (PME)	6999.83 ± 656.9
Pectin lyase (PL)	3871.76 ± 116.49
Arabinanase (ARA)	5034.97 ± 349.28
Galactanase (GAL)	48722.3 ± 3559.722
Laffort HE Grand Cru	17327.39 ± 1670.73 (EPG) 2350.28 ± 235.52 (PL)

levels. The scanning was performed in duplicate per sample (with two technical repeats).

2.5. Comprehensive microarray polymer profiling (CoMPP) of cell wall samples

To analyse and compare the degradation of the cell wall polymers by virtue of changes in their epitope abundance, AIR sourced from fermented pomace from each of the fermentations was sequentially extracted first with CDTA (cyclo-hexane-diamino-tetra-acetic acid) and then with NaOH (described in Moller et al., 2007); to obtain pectin-rich and hemicellulose-rich fractions. After centrifugation, extracts were printed on nitrocellulose membranes and then probed with a series of monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs). The raw data generated was used for multivariate data analysis, and was also separately normalized for creating the microarray heatmaps, the highest signal was set as 100, and others were adjusted accordingly, a cut-off of 5 was applied.

2.6. Univariate and multivariate data analysis

The Statistica programme (Statsoft, Sandton, South Africa) was used to perform the univariate data analysis on the monosaccharide composition data (using ANOVA, $p=0.05$). Multivariate analysis was performed using the SIMCA 14 software package (MKS, Umea, Sweden) to perform the PCA (principal component analysis).

3. Results and discussion

To provide more insight into the manner in which grape berry cell wall polymers are organized and how these structures are unraveled (Zietsman, Moore, Fangel, Willats, and Vivier, 2015) and degraded (Gao et al., 2015) by carbohydrate enzyme mixtures a combinatorial design experiment was performed. High-

throughput cell wall profiling tools with multivariate tools allowed us to determine how each enzyme combination acted during the ferment. A number of factors were taken into consideration for the design; such as the fact that most commercial enzyme blends claim that the endo-polygalacturonase (EPG) is the core enzyme, thus a fermentation treatment with only purified recombinant EPG was included. Furthermore, considering that berry pectin is highly methyl-esterified (Gao et al., 2015), the combinations used included pectin methyl esterases, pectin lyases, arabinanases and galactanases; for the coding system EPG, PME, PL, ARA, GAL in different enzyme mixtures (see Section 2.1).

3.1. Infrared spectroscopy confirmed consistency of ferments

To ensure that standard wine parameters of the fermentations were broadly similar between treatments and controls before commencing the process of cell wall analysis, the final wines from the seven fermentations were analysed using the FOSS winescan. These quality control data are shown in Supplementary Table 2; whereas phenolic, colour and tannin analysis is provided in Supplementary Table 3. Generally, the wine parameters showed evidence of a consistent fermentation without any faults evident, such as excessive volatile acidity, albeit on an experimental scale. This confirmed that both the fermentations and the pomace were suitable for comparative analysis via high-throughput cell wall profiling tools.

3.2. Significant de-pectination observed by PL and Grand Cru as determined by monosaccharide composition analysis

Fig. 1 showed the concentration of nine main monosaccharides in AIR (mg/g in dry mass) from the 7 fermentations (3 biological repeats), in general, all the analyses revealed an abundance of GalA (ca. 180–250 mg/g), with the presence of Ara (ca. 40–50 mg/g), Gal (ca. 30–40 mg/g) and Rha (ca. 15–20 mg/g) representing the main pectin constituents. In addition; Xyl (ca. 25–40 mg/g), Man (ca. 60–80 mg/g) and Glc (ca. 40–60 mg/g) concentrations reflected hemicellulosic polymers, such as xyloglucan and mannans. These compositions are similar to recent studies on fermented berry cell walls (Gao et al., 2015), and Pinotage skins (Zietsman, Moore, Fangel, Willats, Trygg et al., 2015).

By comparing the GC data, the statistically significant decrease of GalA in PL and Grand Cru treated pomace is clearly observable (Fig. 1), which strongly suggests that de-pectination occurred more effectively with these two enzyme preparations. A number of previous studies have stated that the grape berry cell wall has high degree of esterification (more than 50%) (Nunan, Davies, Robinson, & Fincher, 2001; Vicens et al., 2009; Gao et al., 2015). We confirmed esterification was fairly similar (ca. 50%, see Supplementary Table 4) between enzyme-treated and untreated grape AIR confirming our assertion in a previous study on wine enzymes (Gao, Fangel, Willats, Vivier, & Moore, 2016). Esterification can prevent certain enzymes, such as endo-polygalacturonases (EPG) and

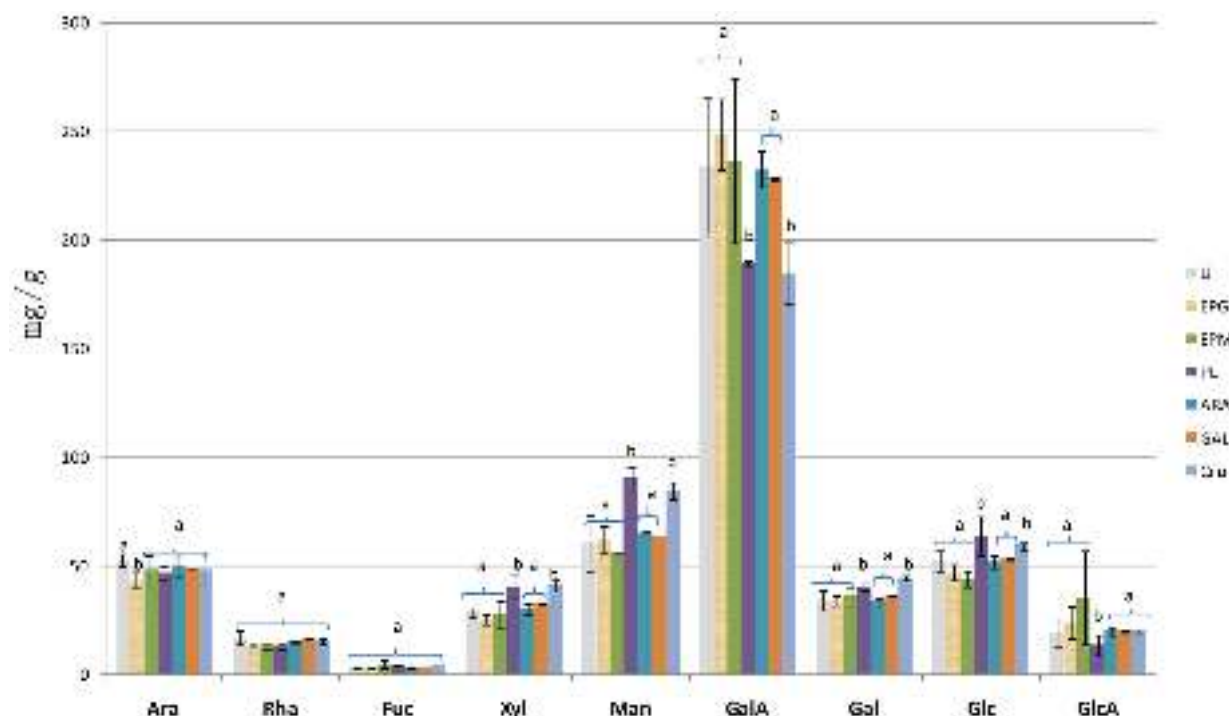


Fig. 1. The monosaccharide compositions of AIR sourced from fermented pomace of the seven treatments (Legend codes: Untreated represented as (U), Endopolygalacturonase as (EPG), EPG + Pectin Methyl Esterase (PME) as (EPM), Pectin Lyase as (PL), EPG + PME + Arabinanase as (ARA), EPG + PME + Galactanase as (GAL), Laffort HE Grand Cru as (Cru)). The composition is expressed in mg/g (dry weight of AIR samples), the monosaccharide abbreviations are: Ara: arabinose; Rha: rhamnose; Fuc: fucose; Xyl: xylose; GalA: galacturonic acid; Man: mannose; Gal: galactose; Glc: glucose; GlcA: glucuronic acid. Error bars represent the standard deviation of the mean value of three biological repeats; different letters indicate a statistically significant difference between treatments for a specific monosaccharide, (95% confidence level, ANOVA, $p=0.05$).

pectate lyases (Bonnin, Garnier & Ralet, 2014) from cleaving the pectin main chain (Van Alebeek, Christensen, Schols, Mikkelsen, & Voragen, 2002). This may explain why the cell wall structure did not undergo effective degradation by applying only EPG to the ferments. Pectin methyl esterases were added in 3 combinations (EPM, GAL, ARA) to help with de-methyl-esterification to open up the pectin main chain for the EPG to act on the presumably de-esterified polymers. All enzymes were tested and showed clear enzyme activity—see Table 2. To acquire more detailed information on the actions of these enzymes at polymer epitope level, CoMPP was therefore performed.

3.3. De-pectination and de-esterification is revealed by CoMPP and multivariate data analysis

CoMPP employs a number of well characterised monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) (Fig. 2C) which target specific epitopes associated with cell wall polymers (e.g. HG, RGI, AGPs, extensins, etc.) (Moller et al., 2007)—see Supplementary Table 5. By combining this technique with enzyme treatment we aimed to enhance our understanding of how pectinases are able to degrade and unravel the multi-layer structure of the grape pectin-rich cell wall. The raw data generated from CoMPP was analysed using multivariate data analysis (SIMCA) for creating the PCA models (scores and loadings) and was processed to generate the associated heatmaps (Fig. 2A–C).

PL and Cru sit very close to each other and the major variables (loading plot in Fig. 2B) which correlates with first component; PC1 at 51.7% of the variance in the dataset. This first component derives mainly from a diminished abundance of pectin epitopes, which is further supported by the monosaccharide compositional analysis dataset (i.e. statistically significant decrease of GalA, Fig. 1), suggesting effective de-pectination by these enzymes. PC2 explains 18.8% of the variation in the scores plot (Fig. 2B) and separates EPM,

ARA and GAL treatments from the U and EPG samples. The corresponding loading plot (Fig. 2B) suggests loading variables mAbs LM18 and JIM5 (reflecting HG with a low degree of DE) act as the main contributor to separation of these samples, which would infer, de-esterification in the samples containing PME (i.e. EPM, ARA and GAL). U and EPG cluster together in the score plot (Fig. 2A) with the corresponding loading variables, indicating an abundance of HG (high DE), RGI, XyG, AGP and extensin epitopes (Fig. 2B). The raw data was also scaled to generate a heatmap (Fig. 2C) of the relative epitope abundance from the mAbs and CBMs in the different treatments relative to the controls. From the signal abundance, the heatmap confirmed the GC dataset (Fig. 1) showing de-pectination occurred (i.e. HG and RGI) while xyloglucan and cellulose epitopes increased in abundance (Gao et al., 2015; Zietsman, Moore, Fangel, Willats, Trygg et al., 2015). It is clear that PL and Grand Cru worked more effectively at de-pectination (decrease of HG polymers and RGI epitopes) polymers, again confirming the GC data (Fig. 1).

3.4. Impact of PL and Grand cru on berry cell wall degradation

To further simplify the interpretations, the models were repeated but with the inclusion of only the PL and Cru treatments versus the U controls (see Fig. 3). Fig. 3A shows a very distinct separation of PL and Cru from the U samples and this appears primarily due to the PC1 component which explains 68.5% of the variance in the data. Inspection of the corresponding heatmap (Fig. 3C) supports the PCA scores (Fig. 3A) and loading (Fig. 3B) plots. PL and Cru show a significant decrease in abundance of all the HG and RGI side chain epitopes compared to the U samples (see Fig. 3C boxed), and the differences in the PC2 direction between PL and Cru appear to be driven due to PL showing a higher abundance of HG epitopes with low DE (LM18, LM19, 2F4, JIM5) than Cru (Fig. 3C). It is also important to notice that the epitope signal of RGI abundance also showed a significant decrease (Fig. 3C).

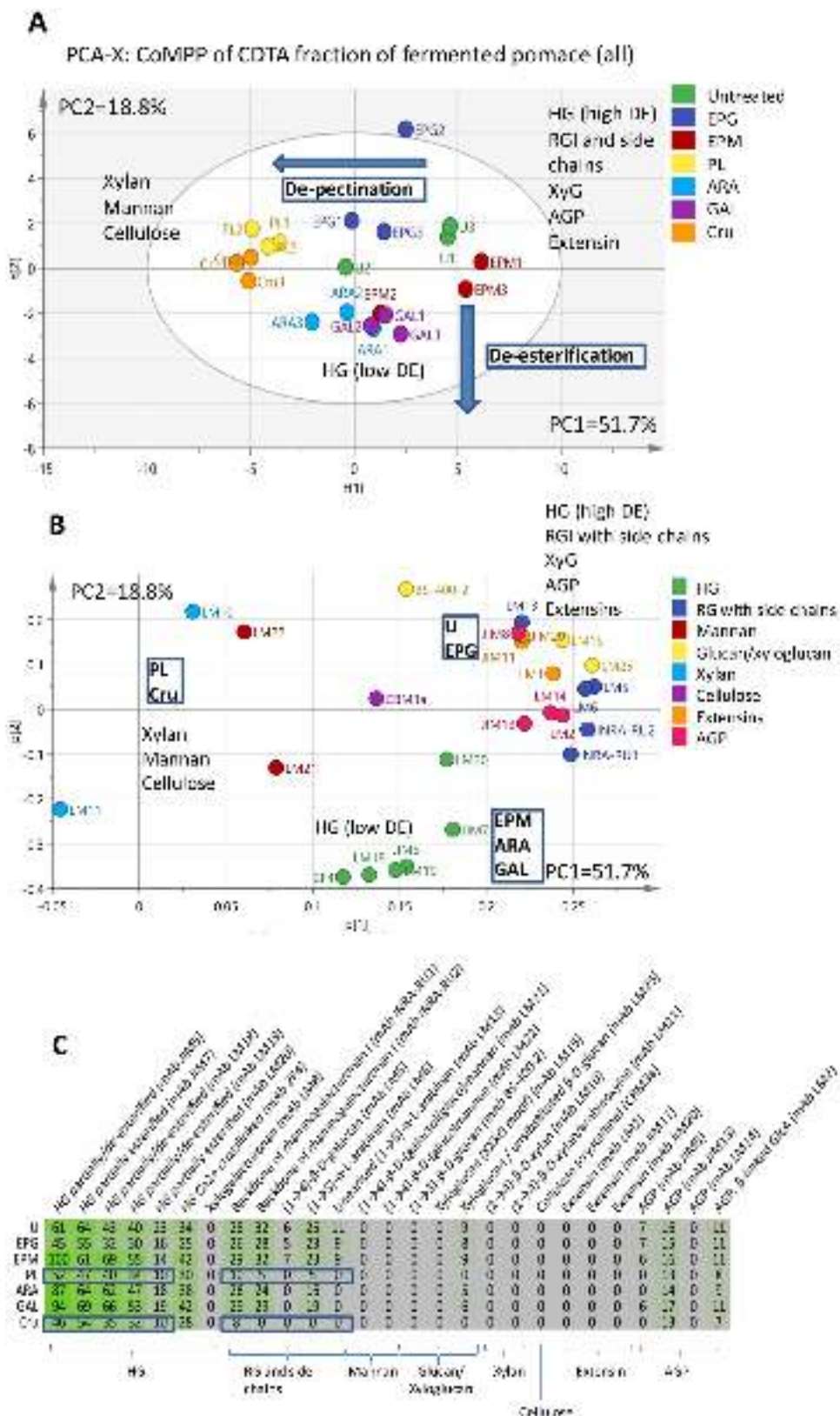
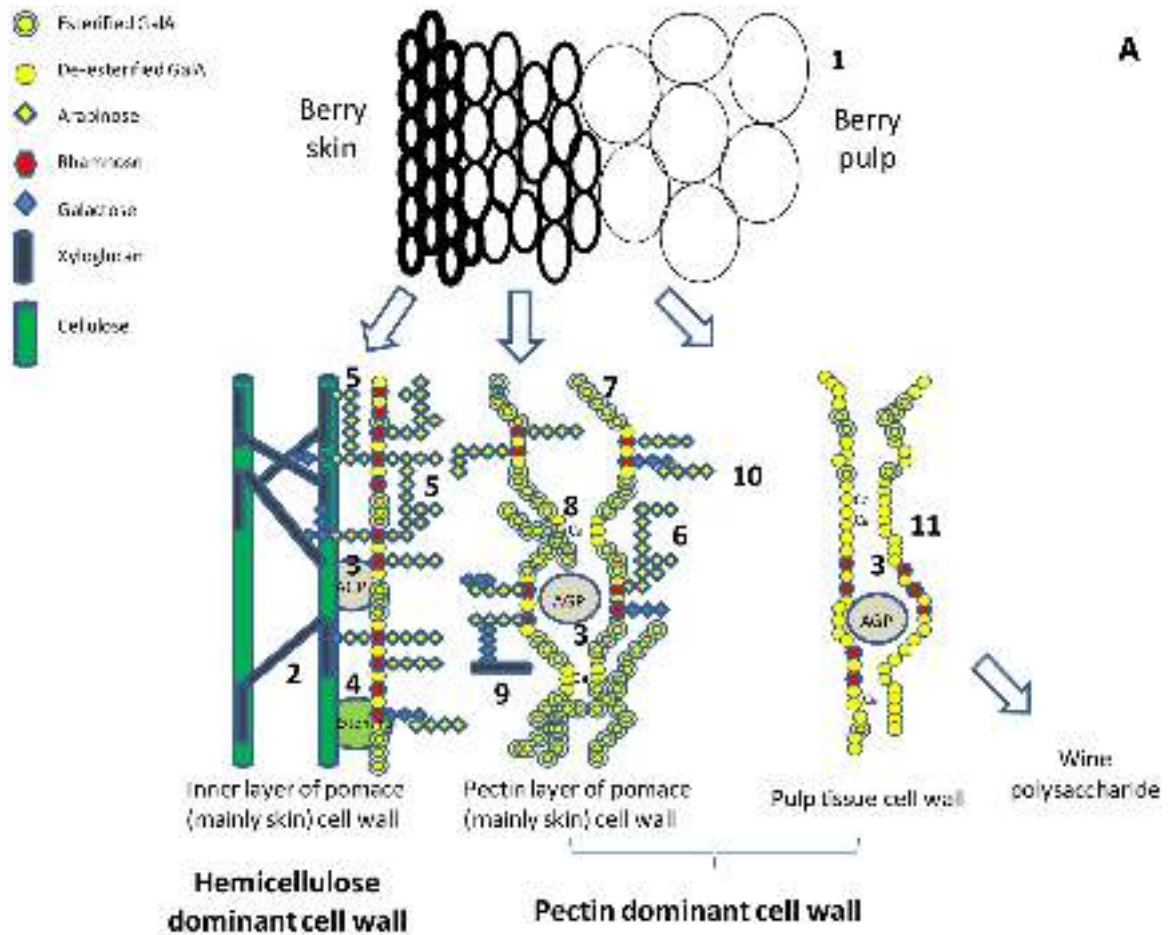


Fig. 2. CoMPP results showing a PCA score (A) and loading (B) plot of the CDTA extract (pectin-rich) of seven treatments. (C) CoMPP heatmap of epitope abundance in the pomace CDTA extracts, the values of variables are average of three biological replicates. A cut-off (<5) was applied. The plots are coloured according to the treatments in (A) and to the polymer category in (B).

3.5. Hemicellulose-rich fraction (NaOH extract)

To break down the grape berry cell wall during wine fermentations, the enzymes need to get through the pectin-rich fraction

to reach the hemicellulose-rich fraction which mainly contains xyloglucan and some strongly associated pectin (highly esterified HG and RGI) as a coating layer (Gao et al., 2015). NaOH was used



B

No.	Description	Reference
1	Tissue differences in grape berry	Nyman et al., 1998
2	Tethering mode of hemicellulose-dominant fraction	This study
3	Parallel form of cellulose and associated xyloglucan	Guerrieri et al., 2010; Brant and Waldron, 1996; Cumming et al., 2005
4	AGPs are associated with all fractions of cell wall	Moore et al., 2014a; Gao et al., 2015; Ziemann et al., 2015; this study
5	Extensins are mainly associated with pomace cell walls	Jackson et al., 2001; Ribeiro et al., 2006; this study
6	RG1-dominant pectin layer coating hemicellulose fraction	Gao et al., 2015 and this study
7	Branched arabinan on inner side chain of RG1	This study
8	High esterification levels of HG in pectin-rich fraction	Gao et al., 2015; this study
9	Ca ²⁺ cross-linking in pectin-rich fraction, masked by esterified HG	This study
10	Low amounts of xyloglucan are associated with pectin	Moore et al., 2014a; Gao et al., 2015
11	Unusual linkage 'Ara-Gal-Rha' in pectin-rich fraction	This study
12	De-esterified HG, RG1 and AGP in pulp cell walls and wine polysaccharides	Gao et al., 2015

Fig. 5. (A) A proposed model of the grape berry cell wall. (B) The numbers in the figure reflect important studies on grape cell wall tissue and cell structure (Jackson et al., 2001; Ribeiro et al., 2006).

mAbs INRA-RU1, INRA-RU2, LM5, LM6, LM13), but not the hemicellulose epitopes (e.g. mAb LM15, CBM3a etc.). This again confirmed the suggested two layers present in the grape pomace cell walls (Gao et al., 2015). Thus, PL and Cru penetrated more effectively and caused significant degradation of the highly esterified pectin-rich fraction (Fig. 4) that coats the xyloglucan-cellulose rich cell layers (Gao et al., 2015).

The HG in this coating layer (Fig. 4C) was mainly in the de-esterified form, due to the treatment with NaOH, a saponification reagent (Gao et al., 2015). By comparing these enzyme treated fermentations, our hypothesis of two fractions of grape berry pomace cell walls appear well supported (Gao et al., 2015). A number of points indicate this; Fig. 4C showed only PL and Cru performed effective de-pectination (Figs. 1, 2C and 3C) while other treatments did not show significant effects. Interestingly, even though there

is a marked decrease of epitopes associated with RGI and its side chain arabinans (mAbs LM6 and LM13), the galactan abundance is fairly constant with PL or Cru treatments (Fig. 4C). The removal of pectin also increased the exposure of hemicellulose, including cellulose epitopes, again probably due to epitope masking effects (Marcus et al., 2008), evidenced by the increase in signals for mAbs BS-400-2, LM15 and LM25 observed after PL and Cru treatment.

3.6. A proposal for a hypothetical evidence-based grape berry cell wall model

Based on the datasets generated from this study, and the data presented in Gao et al. (2015), a hypothesis for a grape berry cell wall can be proposed. From these data and associated literature references, a simplified diagrammatic model was designed to incorporate the major findings to date on grape berry cell wall structure (Fig. 5A). The model is annotated using numbers to indicate tissue and cell wall polymer associations, from this study and previous research literature (Fig. 5B), for ease of inspection. To summarize the major important points, the diagrammatic grape berry cell wall model shows that the pomace contains 2 main fractions these being pectin-rich and hemicellulose-rich, respectively. The pectin-rich fraction also contained low amounts of RGI which has more branched arabinans as side chains, however, other side chains (i.e. galactans) are also present. The cell wall proteins, such as AGPs, are also associated with the pectin-rich fraction. The hemicellulose fraction is strongly associated with an RGI-dominant pectin layer, and coated with a multilayer highly-esterified HG-dominant pectin-rich fraction.

The model presented (Fig. 5) fits the enzyme data, although it is also important to note that the cell wall is different at the tissue level, thus, the model we suggest is based mainly on pomace studies (Gao et al., 2015). The pomace data reflects the thick skins of wine grapes with tightly-bound pulp layers. As grapes ripen the pulp layer probably starts to depolymerize after veraison (Creasy and Creasy, 2009), resulting in larger cells with thinner more fragile cell walls at more mature developmental stages (Nunan, Sims, & Bacic, 1998). When the berries are crushed, during winemaking, the pulp cell walls are presumably easily broken down and solubilised into the wine, thus generating the complex wine polysaccharide composition reported on in Gao et al. (2015), mainly composed of de-esterified HGs and RGI polymers, with the presence of AGPs; and unexpectedly some XyGs. We speculate the de-esterification process starts from pulp tissue, and progresses into skin tissue (arrow in Fig. 5). The diagrammatic model provides a useful basis for understanding berry deconstruction and the action of maceration enzymes on wine grapes; particularly during red winemaking.

4. Conclusion

In this study, we have applied purified (recombinant) pectinases to the winemaking process (Cabernet Sauvignon), coupled with validated high-throughput cell wall profiling tools, in order to reconstruct the berry cell wall structure from degradation data from different enzyme combinations. Glycan microarray technology (CoMPP) generated a number of interesting datasets by probing the specific monoclonal antibodies and carbohydrate binding modules epitopes within the degraded pomace.

Firstly, these datasets confirmed the high degree of esterification in pomace cell walls, this esterified HG, alternating with RGI, which forms the pectin backbone and acts as a barrier preventing enzymes (e.g. endo-polygalacturonases) from penetrating. This layer appears to be only accessible after de-esterification occurred, or to specific enzymes (i.e. pectin lyases). Secondly, several masking effects were observed, which would influence the efficiency

of enzymes to act as desired; for example due to possible blocking action by branched arabinans towards EPG or PL enzymes from reaching their target polymers. Thirdly, based on the data observed in mixtures containing arabinases and galactanases, the berry cell wall may not contain only “Gal-Ara-Rha” linkages as found in type I arabinogalactan, but also unusual linkages such as “Ara-Gal-Rha” in its RGI chain. This study also confirmed that the inner hemicellulose-rich layer is coated with a layer of RGI-rich pectin. In addition, we propose that less esterified HG that would more likely describe the cell wall structure of berry pulp tissues, however further work is necessary to validate this.

Nevertheless, the structural information generated from this study has potential impacts in a number of fields. Firstly, it provides a clearer target for designing maceration and clarification enzyme preparations, in order to achieve the most efficient cell wall deconstruction. Secondly, it provides baseline reference datasets for future transcriptomic studies on berry ripening in the context of cell wall remodeling and biosynthesis events. Thirdly, improved understanding of grape berry cell wall structure is very useful when considering plant-pathogen interactions with grapevines, particularly the mode(s) of infection of pathogenic fungi and the fungal enzymes needed to penetrate into berry cell walls. Hence this work provides a valuable foundation for a number of future studies and applications within the agricultural and industrial contexts of the grape and wine sciences.

Authors' contribution

Y. Gao, J. Moore and M. Vivier designed the study. Y. Gao performed the experiments and collected the data. Y. Gao interpreted the results and drafted the manuscript. J. Moore and M. Vivier advised with data interpretation and manuscript preparation. J. Fangel and W. Willats performed the CoMPP analysis. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2016.05.115>.

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