Food Research International 129 (2020) 108889

Contents lists available at ScienceDirect

Food Research International

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

The impact of carbohydrate-active enzymes on mediating cell wall polysaccharide-tannin interactions in a wine-like matrix

Andrea Osete-Alcaraz[a], Encarna Gómez-Plaza[a,*], Pilar Martínez-Pérez[a], Florent Weiller[b], Julia Schückel[c,d], William G.T. Willats[e,d], John P. Moore[b], José M. Ros-García[a], Ana B. Bautista-Ortín[a]

[a] Department of Food Science and Technology, Faculty of Veterinary Science, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain
[b] Institute for Wine Biotechnology, Department of Viticulture and Oenology, Faculty of AgriSciences, Stellenbosch University, Matieland, 7602, South Africa
[c] Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, DK-1001, Denmark
[d] Glycospot, Thorvaldsensvej 40, B102, DK-1871 Frederiksberg C, Denmark[1]
[e] School of Agriculture, Food and Rural Development, Newcastle University, Newcastle-upon-Tyne, United Kingdom

ARTICLE INFO

Keywords:
Tannins
Wine
Cellulase
Polygalacturonase
Pectin lyase
Polysaccharides
Cell wall

ABSTRACT

Tannins are present in grape skins and seeds from where they are transferred into the must-wine matrix during the maceration stages of winemaking. However, tannin transfer is often incomplete. This could be due, among other reasons, to tannins becoming bound to grape cell wall polysaccharides, including soluble polymers, which are released during vinification and are present in high concentrations in the must/wine. The use of cell wall deconstructing enzymes offers the possibility of reducing these interactions, releasing more tannins into the final wine. The main aim of this study was to evaluate the optimal addition (individually, in combination or sequentially) of hydrolytic enzymes that would prevent tight polysaccharide-tannin associations. The use of comprehensive microarray polymer profiling (CoMPP) methodology provided key insights into how the enzyme treatments impacted the grape cell wall matrix and tannin binding. The results demonstrated that polygalacturonase + pectin-lyase promoted the highest release of tannins into solution.

1. Introduction

The quality of a red wine depends, among other factors, on its concentration of phenolic compounds, especially concerning anthocyanin and tannin content. During vinification, phenolic content, mainly anthocyanins and tannins can decrease, in part due to interactions between these components and grape cell walls present in suspension (Bindon, Smith, Holt, & Kennedy, 2010; Osete-Alcaraz, Bautista-Ortín, Ortega-Regules, & Gómez-Plaza, 2019). Hydrophobic interactions and hydrogen bonds have shown to drive the main associations that occur between tannins and cell wall polysaccharides and the strength of these interactions depends on the structure and conformation of both the tannins and the cell materials (Le Bourvellec, Bouchet, & Renard, 2005; McMannus et al., 1985). In the case of anthocyanins, a two-stage process is observed where initial binding to cell wall polysaccharides occurs via ionic and hydrophobic interactions in a rapid manner, followed by a delayed phase involving anthocyanins undergoing stacking upon the anthocyanin-cell wall layers (Padayachee et al., 2012). Moreover, during the interaction process, it has also been shown that the anthocyanins compete with tannins for binding to cell walls, and this increases the tannin content of released into the medium (Bautista-Ortín, Martínez-Hernández, Ruiz-García, Gil-Muñoz, & Gómez-Plaza, 2016).

Hydrolytic enzymes are often used during winemaking to degrade grape cell wall polysaccharide network and thereby facilitating the release of phenolic compounds present in the skin and seeds into the wine (Bautista-Ortín et al., 2013; Busse-Valverde, Gómez-Plaza, López-Roca, Gil-Muñoz, & Bautista-Ortin, 2011; Romero-Cascales, Fernández-Fernández, Ros-García, López-Roca, & Gómez-Plaza, 2008; Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2012). Previous studies carried out by our research group have reported that enzymes may limit the retention of tannins by grape cell walls through the removal of pectic polysaccharides which are rich in galacturonic acid (Bautista-Ortín, Ben Abdallah, Castro-López, Jiménez-Martínez, & Gómez-Plaza, 2016; Castro-Lopez, Gómez-Plaza, Ortega-Regules, Lozada, & Bautista-Ortin, 2016).

* Corresponding author.
E-mail address: encarnag@um.es (E. Gómez-Plaza).
[1] Present address.

https://doi.org/10.1016/j.foodres.2019.108889
Received 28 March 2019; Received in revised form 28 September 2019; Accepted 12 December 2019
Available online 14 December 2019
0963-9969/

These soluble polysaccharides sourced from the deconstruction of the polysaccharide network might also display a high affinity for the tannins (Bautista-Ortín, Ben Abdallah, et al., 2016) and that may affect the behaviour of tannins in solution. Studies have shown that the interaction between tannins and soluble polysaccharides is governed by both the tannin composition as well as the respective polysaccharide structures (Riou, Vernhet, & Doco, 2002; Watrelot, Le Bourvellec, Imberty, & Renard, 2014). These polysaccharide properties may thus affect tannin aggregation (Li, Wilkinson, Mierczynska-Vasilev, & Bindon, 2019). Riou et al. (2002) and Watrelot et al. (2014) have shown, for example, that in the case of low molecular tannins arabinogalactan proteins (AGPs) and rhamnogalacturonan II monomers do not seem to influence the mean-degree of polymerization necessary for tannin aggregation, whereas the rhamnogalacturonan II dimers, in contrast, do have an impact. Other studies showed that larger molecular weight polysaccharide such as mannoprotein are able to form large, high light-scattering aggregates, whereas arabinogalactan polymers exhibited only weak interactions with seed tannins (Li et al., 2019). In the case of higher molecular weight tannins, aggregation occurs in the presence of AGPs (Watrelot et al., 2014).

Commercial wine enzymes normally contain mainly pectolytic preparations (e.g. polygalacturonases, and to a lesser degree, pectin methylesterase and pectin-lyase activities), cellulases, and acid proteases (Romero-Cascales et al., 2008). In studies carried out by Zietsman, Moore, Fangel, Willats, and Vivier (2015) it is suggested that the deconstruction of the skin cell wall polysaccharide network would be more effective if different enzymatic activities are added sequentially rather than all at the same time as a single preparation. It was hypothesised that an initial enzymatic hydrolysis of the pectin layers would soften the core central network of cellulose-hemicelluloses. Then, the addition of cellulases with hemicellulases could partially eliminate the cellulose and xyloglucan cross-links or 'tethers' allowing greater access into of the cell wall matrix.

This, in turn, could allow a greater liberation of phenolic compounds from grape solids into the wine during alcoholic fermentation. It could also limit largely the adsorption of polyphenols onto insoluble cell walls during winemaking (Bindon, Kassandra, & Smith, 2016). However, another factor to take into consideration is that enzyme treatment may enhance the contribution of high molecular weight polysaccharides to wines while decreasing those of intermediate (40 kDa) size, suggesting changes in the polysaccharide composition introduced by the enzyme that potentially increase polysaccharide solubility (Kassara, Li, Smith, Blando, & Bindon, 2019).

Hence, the main aim of this study was to determine the optimal combination and form of addition (individually, set-mixtures or sequential) of purified carbohydrate-active hydrolytic enzymes in deconstructing grape cell wall matrices under wine-like conditions. Connecting enzyme action and grape cell wall deconstruction with tannin release into the wine-like solution, in the context of tannin-polysaccharide interactions, is a major objective of this research approach. Methods employed in this regard include CoMPP (which stands for comprehensive microarray polymer profiling) glycan microarrays. Zietsman et al. (2015) and Gao, Zietsman, Vivier, and Moore (2019) also reported on the usefulness of the CoMPP technique in evaluating the effectiveness of the different type of enzymes and their combinations in both disrupting and deconstructing the glycan-rich grape cell wall architecture during wine fermentation. In addition, the soluble polysaccharides released from cell walls after the action of the enzymes were measured by size exclusion chromatography (SEC), whereas the tannin content was assessed using phoroglucinolysis and SEC methodology.

2. Material and methods

2.1. Isolation of grape cell walls

Purified cell walls (CW) were prepared from the fresh skins of *Vitis vinifera* L. cv. Monastrell grapes. Cell walls were obtained by the method of De Vries, Voragen, Rombouts, and Pilnik (1981) adapted by Apolinar-Valiente, Romero-Cascales, López-Roca, Gómez-Plaza, and Ros-García (2010). Briefly, the skins of 500 berries were cleaned and washed with milli-Q water. The peeled skins were ground using liquid nitrogen to obtain a fine powder and then the powder immersed in boiling milli-Q water for 5 min to inactivate enzymes. Processed samples were then homogenized (mixed at 10,000 rpm) for 1 min and centrifuged (10 min at 18,000g) to recover the solid grape skin residue. The solid residues were then washed several times with 70% ethanol (30 min at 30 °C), to remove alcohol soluble solids and phenols, and finally with absolute ethanol and acetone to obtain dry cell wall powders (alcohol insoluble residues, AIR).

2.2. Interaction assays for tannins, enzymes and grape cell walls

The interaction reactions were performed in 3 mL glass tubes. Briefly, 32.5 mg of CW isolated from Monastrell grape skins were mixed with 5 mg of a commercial tannin previously redissolved in 2.5 mL of model solution (ethanol 12% and pH 3.6 adjusted with trifluoroacetic acid) in presence and absence of purified enzyme preparations. Each tube had a final concentration of 2000 mg/L tannin and 13 mg/mL of cell wall material. Tubes were agitated in an orbital shaker for 90 min (200 rpm) at room temperature. A blank without tannin and a blank without the cell wall were included, the latter to control the tannin desorption from the AIR. During the agitation phase it is presumed the binding between the tannins and cell walls occurs. After incubation, the samples were centrifuged (13,000 rpm), the supernatants were concentrated and dried under vacuum at 35 °C (CentriVap Vacuum concentrator, Labconco, MO).

The purified enzymes used included; cellulase (EC 3.2.1.4, Cel, 100 mg/L) from *Aspergillus niger*, pectinmethylesterase (EC 3.1.1.11, PME, 25 mg/L) on orange peel, *endo*-polygalacturonase (EC 3.2.1.15, PG, 100 mg/L) from *Rhizopus sp*., pectin-lyase plus polygalacturonase (EC4.2.2.10 and EC 3.2.1.15, PL + PG, 100 mg/L) from *Aspergillus japonicus* and a xylanase (EC 3.2.1.8, Xyl, 50 mg/L) from *Thermomyces lanuginosus*. These were supplied from Sigma-Aldrich (St. Louis, MO, USA). Their specific activity (expressed in nanokatal per gram of commercial enzyme preparation, corresponding to 1 nmol reducing sugar formed per second in the defined conditions) was 18333.3 for cellulose, 1489666.6 for pectinmethylesterase, 10,000 for *endo*-polygalacturonase, 2500/ 2500 for pectin lyase plus polygalacturonase and 41666.6 for xylanase. Enzymes were added either singly, in a single combination set (ET) or sequentially (ESEQ and ESEQI). In sequential experiments the time between enzyme additions were of 20 min intervals, except for cellulase which was added after 30 min. The various addition methods are shown in Table 1.

Six replicates were performed for each experimental treatment. The supernatants from three replicates were concentrated and then dissolved in 0.250 mL of methanol. The methanol extracts were analysed

Table 1
The enzyme combinations and forms of addition used in this study.

	Enzymatic Activities
ET1	Cel + PME + PG + Xyl
ET2	Cel + PME + [PG + PL] + Xyl
ESQ1	1° Cel, 2°PME, 3° PG, 4° Xyl
ESQ2	1° Cel, 2°PME, 3° [PG + PL], 4° Xyl
ESQI1	1° PME, 2°Cel, 3° PG, 4° Xyl
ESQI2	1° PME, 2°Cel, 3° [PG + PL], 4° Xyl

for proanthocyanidin content by using phloroglucinolysis and size exclusion chromatography (SEC) analysis. The samples were measured for polysaccharide content by dissolving analytes in 0.25 mL milliQ water and then processed via SEC. The tannin-cell wall complexes were precipitated in 70% ethanol, followed by absolute ethanol and acetone to obtain AIR powders.

2.3. Analysis of tannins by phloroglucinolysis

The concentration and composition of tannins were analysed using the phloroglucinol reagent according to the method described by Kennedy and Jones (2001) with some modifications. Briefly, a solution of 0.2 M HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid, was prepared (termed the phloroglucinolysis reagent). The methanolic extract was left to react with the phloroglucinolysis reagent (1:1) in a water bath for 20 min at 50 °C and then combined with 2 volumes of 200 mM aqueous sodium acetate to stop the reaction. HPLC analysis followed the conditions described by Busse-Valverde et al. (2010). The HPLC apparatus was a Waters 2695 system (Waters, Milford, MA) equipped with an autosampler system, a Waters 2996 photodiode array detector. Samples (10 μL injection volume) were injected onto an Atlantis dC18 column (250 × 4.6 mm, 5 μm packing) protected with a guard column of the same material (20 mm × 4.6 mm, 5 μm packing) (Waters, Milford, MA). The elution conditions were as follows: 0.8 mL/min flow rate; oven temperature, 30 °C; solvent A, water/formic acid (98:2, v/v), and solvent B, acetonitrile/solvent A (80:20 v/v). Elution began with 0% B for 5 min, then a linear gradient from 0% to 10% B in 30 min and then a gradient from 10% to 20% in 30 min, followed by washing and equilibration of the column.

Tannin cleavage products were identified based upon their response factors at 280 nm relative to (+)-catechin, which was used as the quantitative standard. These analyses allowed determination of the recovery by mass of the total tannin content, the apparent mean degree of polymerization (mDP) and the percentage of each constitutive unit. The mDP was calculated as the sum of all subunits (i.e. flavan-3-ol monomer and phloroglucinol adducts, in mole units) divided by the sum of all flavan-3-ol monomers (in mole units).

2.4. Analysis of tannins by SEC

An adaptation of the method described by Kennedy and Taylor (2003) was followed for size exclusion chromatography (SEC) analysis. The method involved connecting two PL (Polymer Labs, Amherst, MA, USA) gel columns; (1) (300 × 7.5 mm, 5 μm) 500 Å column (effective molecular mass range of up to 4000 using polystyrene standards) followed by (2) a 100 Å column (effective molecular mass range of 500–30,000 using polystyrene standards) connected in series. A guard column containing the same PL material (50 × 7.5 mm, 5 lm) was used to protect the apparatus. An isocratic mixture was used consisting of a mobile phase of N,N-dimethylformamide containing 1% glacial acetic acid, 5% water and 0.15 M lithium chloride at a flow-rate of 1 mL/min. The injection loop volume mixed 10 μL of sample diluted (1:5, v/v) with mobile phase. The column temperature was 60 °C and elution monitored at 280 nm.

2.5. Cell wall profiling using glycan microarrays

Each sample of the skin cell walls before and after enzymatic treatment was analysed in triplicate using the CoMPP glycan microarray techniques. CoMPP employs probes (monoclonal antibodies mAbs and carbohydrate binding modules CBMs specific for cell wall polymer epitopes) which detect polysaccharide polymers directly by virtue of their epitope regions/moieties. CoMPP entails the sequential extraction of pectin- and hemicellulose-rich fractions (using chelating and alkali extractants) from 10 mg of CW. First the chelating agent diamino-*cyclo*-hexane-tetra-acetic acid (CDTA) is used to enrich for pectins and thereafter 4 M NaOH (with $NaBH_4$ 1% v/v) is used to extract more tightly bound neutral sugar polymers (Moller et al., 2008; Moore et al., 2014). The two fraction classes were printed on nitrocellulose membranes and the probed individually with 26 different monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) as described in Zietsman et al. (2015). A mean spot signal was calculated and normalized to the highest signal in the dataset being set to 100. A signal cut-off value of 5 was imposed.

2.6. Polysaccharides solubilized from cell walls analysed using SEC

For analysis of polysaccharide molecular mass distribution, the supernatant solutions were concentrated and then dissolved in milliQ water. 20 μL of these concentrated preparations were injected onto a system composed of two serially connected Shodex OHPAK KB-803 and KB-805 columns (0.8 × 30 cm; Showa Denko K.K., Tokyo, Japan). Detection of eluents were performed using a Waters 2414 refractive index detector and operated at a flow rate of 1 mL/min in 0.1 M $LiNO_3$ mobile phase. Column calibration was carried out using Shodex P-82 Pullulan Standards (Phenomenex, Lane Cove, NSW, Australia) ranging from 5 kDa to 800 kDa. A logarithmic equation was determined using the cumulative mass distribution values measured at the 50% mark for each standard in order to determine the molecular masses present in solution.

2.7. Statistical analysis

The properties of the tannins remaining in solution were analyzed with an analysis of variance and Tukey's range test ($p \leq 0.05$). Analyses were carried out using the statistical package StatGraphics Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

3. Results and discussion

3.1. Determination of the degree of interaction between tannins and cell walls

The capacity of tannins to interact with grape cell wall polysaccharides have been reported previously (Bautista-Ortín, Cano-Lechuga, Ruiz-García, & Gómez-Plaza, 2014; Bindon et al., 2010). Studies using commercially available model polysaccharides have demonstrated that tannins interact preferentially with pectins followed by xyloglucan, showing the least affinity for cellulose (Le Bourvellec et al., 2005). Therefore, the use of enzymes that degrade the polysaccharide network of grape cell walls (composed of pectins, cellulose, and hemicelluloses) has the potential to reduce the adsorbing of tannins in wine ferments.

In this study, the degree of interaction between a commercial seed tannin and Monastrell grape skin cell walls, in the presence and absence of enzymes was determined. One question that may arise is whether the tannins could somehow inhibit the action of the enzyme (due to the reactivity between proteins and tannins). However, the results of Castro-Lopez et al. (2016) in a very similar experiment demonstrated that enzymes acted very rapidly, indicating that the presence of tannins in the solution did not interfere with the enzymes.

The degree of interaction was followed by observing the changes in the tannins that remained in solution compared with control treatments (Table 2 and Figs. S1–S2). The reaction of CWs with tannin in solution led to a 54.5% decrease in the tannin content of the solution. The presence of enzymes in solution (individual, combined set and sequential) lowered the adsorption of tannins onto CWs between 32.2 and 46.4% when the enzymes were used individually, between 45.0 and 46.8% when they were added sequentially and between 44.6 and 51.3% when used in a combined set addition. The lowest adsorption of tannins was obtained with the presence of PG + PL followed of PG, Cel and PME, although no significant differences were found between these

Table 2
Concentration and composition of tannins that remained in solution in presence and absence of enzymes and percentage of retained tannins, measured by phloroglucinolysis and SEC.

Samples	TT[a] (mg/L)	RT[b] (mg/L)	%Adsorption[c]	%Adsorption[d]	mDP[e]	Ecatg[f] (μM)
Tannin	1788.4f				2.70b	831.4e
CW + Tannin	814.1 a		54.5	57.2	2.44a	321.7a
CW + Tannin + Cel	1042.3cd	228.2	41.7	45.4	2.36a	399.8bc
CW + Tannin + PME	1012.3cd	198.2	43.4	50.6	2.38a	392.1bc
CW + Tannin + PG	1055.9d	246.8	41.0	47.7	2.38a	412.4c
CW + Tannin + [PG + PL]	1213.0e	398.9	32.2	29.3	2.43a	497.5d
CW + Tannin + Xyl	958.3bc	144.2	46.4	53.4	2.39a	370.4b
CW + Tannin + ET1	990.9cd	176.8	44.6	52.0	2.41a	391.3bc
CW + Tannin + ET2	871.1ab	57.0	51.3	53.4	2.40a	331.2a
CW + Tannin + ESEQ1	968.9cd	154.8	45.8	56.4	2.40a	376.2bc
CW + Tannin + ESEQ2	951.6bc	137.5	46.8	48.7	2.39a	370.1b
CW + Tannin + ESEQI1	978.9cd	164.8	45.3	43.2	2.66b	397.0bc
CW + Tannin + ESEQI2	983.0cd	168.9	45.0	58.2	2.40a	380.0bc

Different letters in the same column indicate statistically significant differences according to a Tukey test ($P < 0.05$).
[a] TT: total tannins measured by the phloroglucinolysis method.
[b] RT: retained tannins.
[c] %Adsorption measured by phloroglucinolysis.
[d] %Adsorption measured by SEC.
[e] mDP: mean degree of polymerization.
[f] Ecatg: epicatechin-gallate.

latter treatments. Notably, the ET2 and Xyl additions were the less efficient in reducing adsorption. A lower retention capacity of tannins with CWs in the presence of PG, Cel and a maceration enzyme composed of polygalacturonase, pectin lyase and pectin methylesterase has been previously observed by Bautista-Ortín, Ben Abdallah, et al. (2016).

Castro-Lopez et al. (2016) using Monastrell grape skin cell walls and the same commercial enzyme also observed a lower tannin adsorption onto the cell walls when enzyme was added, all the studies pointing to a certain effect of pectolytic enzymes and cellulase in promoting a higher content of tannins in solution, lowering the adsorption onto cell walls. Ruiz-García, Smith, and Bindon (2014) showed that more than 54% of cell wall-bound proanthocyanidins were found within the pectic fraction. Removal of pectic polysaccharides from cell walls therefore significantly reduced the adsorption of proanthocyanidins.

The observation that enzymes added in a combined set or sequentially do not achieve a lower retention of tannins (versus added individually) may be due to an increase in cell wall porosity brought on by pectin elimination due to the enzymes action. Enzymes induced porosity may enhance the encapsulation of tannins within the cell wall network (Le Bourvellec et al., 2005). However, another hypothesis is possible, that a substantial release of soluble polysaccharides from the cell wall network due to the enzymes action, could also lead to their interaction with the tannins present in solution. The tannin-polysaccharides complexes may also be soluble in the medium but also may precipitate out of solution, as described by Bindon et al. (2016) and may not be measurable by phloroglucinolysis. This hypothesis will be addressed later on.

Mean degree of polymerization (mDP) values for the tannins remaining in solution decreased in presence of the grape cell walls. This would indicate that higher molecular weight tannins have a greater capacity for interaction with cell walls which has also been observed previously (Bautista-Ortín et al., 2014; Bindon, Madani, Pendleton, Smith, & Kennedy, 2014). Tannins show multiple sites suitable for simultaneous binding to various regions of polysaccharides (Le Bourvellec et al., 2005). It is thought that larger tannins would have more binding sites for these type of interactions. With the presence of enzymes, the mDP values did not change significantly, except with ESEQI1. The mDP values of the tannins remaining in solution after the action of ESEQI1 were very close to those shown by the original tannins. We think that this enzyme addition modified the grape cell walls architecture in these samples reducing their interaction with high molecular weight tannins in some manner. In this way, Bautista-Ortín, Ben Abdallah, et al. (2016) reported a reduced interaction between polymer tannins and cell walls when Cel was combined with a commercial enzyme. On the other hand, Castro-Lopez et al. (2016) observed no effect on polymeric tannin solution concentration when the commercial enzyme was used on its own.

(−)-Epicatechin-3-O-gallate decreased from 831.4 to 321.7 μM after reaction with cell walls. Tang, Covington, and Hancock (2003) showed that both the molecular weight and number of galloyl groups positively correlated with the strength of binding. With enzyme addition, a lower interaction between cell walls and galloylated tannins was observed especially with PG + PL and then followed by PG, while the presence of ET2 did not produce an effect on galloylated tannin content. Other authors found that the galloylation percentage did not change when only the commercial enzyme was added (Bautista-Ortín, Ben Abdallah, et al., 2016).

As phloroglucinolysis alone only provides partial information on tannin composition and complexes, size exclusion chromatography (SEC) was also carried out. This methodology provides mass distribution profiles of the samples and measures the degree of oxidised tannins that remain in solution. These oxidised tannins cannot be depolymerised in acidic medium and therefore analysed by phloroglucinolysis. With SEC, the polymeric tannins are easily eluted (presenting lower retention times) compared to those of lower molecular mass. The chromatograms obtained by SEC (Supporting Information S1 and S2) generally mirrored the tannins measured in acidic medium. In general, a higher adsorption percentage of tannins were observed (see Table 1) when oxidized tannins are also taken into account. Oxidised tannins have shown a greater capacity to bind CW polysaccharides (Bautista-Ortín et al., 2014). Only when PL and SEQI1 were used, did the retention percentage, measured by SEC, drop to a lower level than that obtained when analyzing tannins by phloroglucinolysis, indicating that these enzymes seem to limit also the adsorption of oxidized tannins onto cell walls.

Grape cell walls appear to interact mainly with polymeric and oligomeric tannins in solution (see Figs. S1 and S2 in Supporting material). Enzyme addition decreased these interactions with oligomeric tannins, followed by polymeric and monomeric tannins. Interestingly, no changes were observed using mDP values obtained by phloroglucinolysis that correlated with these observations. It is possible that the presence of a higher amount of oxidised tannins present in the samples where PG + PL and SEQI1 were added appeared to explain the effect since these tannins are not measurable by phloroglucinolysis and,

Table 3
Heatmap of epitope abundance (0–100) found in the pectin (CDTA) and hemicellulose (NaOH) fractions extracted from the cell walls of fresh (CW′) and treated (CW, after interaction with a tannin in absence or presence of enzymes added individually, in set (ET) and sequentially (EQ)) grape skins.

Fractions	Samples	HG partially/de-esterifies(mAb JIM5)	HG partially esterifies(mAb JIM7)	HG partially/de-esterifies(mAb LM8)	HG partially/de-esterifies(mAb LM19)	HG partially esterifies(mAb LM20)	HG Ca2+ crosslinked (mAb 2F4)	Xylogalacturonan (mAb LM8)	Backbone of rhamnogalacturonan I (mAb INRA-RU1)	Backbone of rhamnogalacturonan I (mAb INRA-RU2)	(1→4)-β-D-galactan (mAb LM5)	Feruloylated (1→4)-β-D-galactan (mAb LM9)	Linearised (1→5)-α-L-arabinan (mAb LM13)	(1→4)-β-D-mannan (mAb LM21)	(1→4)-β-D-mannan/galactomannan (mAb LM22)	(1→3)-β-D-glucan (mAb BS-400-2)	Xyloglucan (XXXG motif) (mAb LM15)	Xyloglucan (mAb LM25)	(1→4)-β-D-xylan (mAb LM10)	(1→4)-β-D-xylan/arabinoxylan (mAb LM11)	Cellulose (cristalline<9 (mAb CBM3a)	Extensin (mAb LM1)	Extensin (mAb JIM11)	Extensin (mAb JIM20)	AGP (mAb JIM8)	AGP (mAb JIM13)	AGP (mAb JIM14)	AGP, β-linked GlcA (mAb LM2)
CDTA	CW'	41	63	18	9	48	0	0	13	24	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
	CW	64	73	42	26	61	6	0	23	22	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0
	CW + Tan	68	69	46	28	60	7	0	22	26	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	CW + Tan + Cel	55	59	38	23	50	3	0	21	29	8	0	0	0	0	0	0	3	0	0	0	0	0	0	0	7	0	0	
	CW + Tan + PME	55	67	42	23	49	4	0	21	23	4	0	0	0	0	0	3	5	0	0	0	0	0	0	0	6	0	0	
	CW + Tan + PG	43	74	26	12	44	0	0	18	25	7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	7	0	0	
	CW + Tan+ [PG+PL]	4	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	CW + Tan + Xyl	55	70	35	18	51	2	0	18	23	7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	6	0	0	
	CW + Tan + ET1	41	62	22	9	37	0	0	21	27	8	0	0	0	0	0	0	3	0	0	0	0	0	0	0	7	0	0	
	CW + Tan + ET2	26	8	17	7	0	0	0	14	20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	
	CW + Tan + ESEQ1	35	54	20	9	27	0	0	19	24	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	
	CW + Tan + ESEQ2	32	13	17	4	0	0	0	13	21	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	
	CW + Tan + ESEQI1	27	50	14	5	24	0	0	17	24	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	
	CW + Tan + ESEQI2	27	11	17	7	0	0	0	13	24	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	
NaOH	CW'	0	0	0	0	0	0	0	16	21	15	0	0	0	0	0	70	72	0	0	0	0	0	0	0	0	0	0	
	CW	0	0	0	0	0	0	0	8	8	11	0	0	0	0	0	71	88	0	0	0	0	5	2	0	7	0	0	
	CW + Tan	0	0	0	0	0	0	0	6	11	15	0	0	0	0	0	75	93	0	0	0	0	7	0	0	7	0	0	
	CW + Tan + Cel	0	0	0	0	0	0	0	9	26	16	0	0	0	0	0	73	95	0	0	0	0	7	0	3	8	0	0	
	CW + Tan + PME	0	0	0	0	0	0	0	8	12	11	0	0	0	0	0	75	96	0	0	0	0	7	0	0	9	0	0	
	CW + Tan + PG	0	0	0	0	0	0	0	11	11	20	0	0	0	0	0	72	89	0	0	0	0	7	6	0	9	0	0	
	CW + Tan+ [PG+PL]	0	0	6	3	0	0	0	26	54	58	3	0	0	0	0	74	91	0	0	0	0	3	0	0	9	0	0	
	CW + Tan + Xyl	0	0	0	0	0	0	0	11	10	18	0	0	0	0	0	75	93	0	0	0	0	3	0	0	8	0	0	
	CW + Tan + ET1	0	0	0	0	0	0	0	16	25	20	0	0	0	0	0	73	92	0	0	0	0	3	0	0	11	0	0	
	CW + Tan + ET2	0	0	6	0	0	0	0	22	54	56	0	0	0	0	0	70	83	0	0	0	0	0	6	3	9	0	0	
	CW + Tan + ESEQ1	0	0	0	0	0	0	0	13	20	24	0	0	0	0	0	65	79	0	0	0	0	5	5	0	8	0	0	
	CW + Tan + ESEQ2	0	0	0	0	0	0	0	19	47	50	0	0	0	0	0	68	83	0	0	0	0	3	0	3	8	0	0	
	CW + Tan + ESEQI1	0	0	0	0	0	0	0	13	25	20	0	0	0	0	0	71	83	0	0	0	0	3	0	0	10	0	0	
	CW + Tan + ESEQI2	0	0	6	6	0	0	0	37	68	71	3	0	0	0	0	69	82	0	0	0	0	6	8	6	11	0	0	

10 20 30 40 50 60 70 80 90 100

therefore, do not participate in the calculated mDP values.

3.2. Characterization of the polysaccharides present in the cell walls and those liberated during treatment with enzymes

In order to provide a rationale for tannin-cell wall interactions or lack of interaction it is necessary to investigate grape cell wall polysaccharide architecture and how it is affected by the enzyme action. To achieve this objective the CoMPP technique was used to investigate the enzymatic deconstruction of grape cell walls in wine-like scenarios by virtue of polysaccharide epitopes (Gao et al., 2019) and soluble polysaccharides liberated from enzyme addition were investigated using SEC analysis.

The study of the glycan polysaccharide composition using CoMPP analysis (see Table 3) enhanced general understanding of enzymatic degradation and unravelling of the multi-layered structure of wine grape pectin-rich cell walls. The CoMPP results are depicted in heat map format and indicates the relative abundance of specific cell wall polysaccharides presented as a percentage of the whole dataset. Overall, the analysis showed that the CDTA fraction of the control samples (without enzyme contact) contained mostly pectin specific polymers, but there was also xyloglucan (mAb LM25) and arabinogalactan proteins (AGPs) present.

The NaOH extraction enriches for hemicelluloses but also shows unbranched RG-I and galactan (mAb LM5) and contains xyloglucan and some strongly associated pectin (highly esterified HG and RGI) as a coating layer (Gao, Fangel, Willats, Vivier, & Moore, 2016). Overall, the presence of ethanol in samples promoted an increase in polymers extracted and dissolved in CDTA and NaOH probed using CoMPP. The presence of tannins bound to CWs did not seem to influence the signals detected from different epitopes.

In PG + PL treated samples and for the CDTA fraction, there was a drastic decrease in the values for homogalacturonan (HG) epitopes (mAbs JIM5 and JIM7, LM20, LM19, LM18, 2F4, LM8, INRA-RU1, INRA-RU2) compared to untreated controls. This decrease in HG epitopes observed with PG + PL treatment could be due to the diffusion of relatively small oligosaccharides, from within the cell wall particles, into the model wine-like solution and also demonstrates the effective action of the enzymes, ruling out the possible inhibition in the presence of tannins

Interestingly, the effect of PG + PL on removing these epitopes was reduced when the PG + PL was combined with other enzymes. In the corresponding NaOH extracts, there was a higher extraction of unbranched RG-I and galactan polymers versus the control samples. These results appear due to the unravelling effect of enzyme hydrolysis on cell wall structure. In contrast, a strong decrease in RG1 and side chain epitopes (mAbs INRA-RU1, INRA-RU2) but not on hemicellulose epitopes (mAbs LM15, CBM3a) was found when PG + PL was used on

Cabernet Sauvignon grape matrices during winemaking (Gao et al., 2016), although these data are not completely comparable as the Gao et al. (2016) study was performed in a winemaking matrix during fermentation. A diminished abundance of pectin epitopes (HG polymers and RGI epitopes) was also observed by this same author in the cell wall Cabernet Sauvignon grape pomaces isolated after treatment with PG + PL and the combination of PG + PL plus PME during the vinification process. This indicates that these enzymes worked more effectively at wine grape de-pectination during wine fermentation (Gao et al., 2016, 2019).

A less marked decrease in HG epitope signals was shown in PG treated samples. Interestingly, PG combined with other enzymes seemed to increase its capacity to degrade the pectins. This was noted when they were added sequentially as in the case of ESEQI samples. The cell walls of Monastrell grapes are characterized as having a high degree of esterification (more than 50%) (Ortega-Regules, Ros-García, Bautista-Ortín, López-Roca, & Gómez-Plaza, 2008). This would prevent such as PG (Bonnin, Garnier, & Ralet, 2014) from cleaving the heavily esterified pectin main chains (Van Alebeek, Christensen, Schols, Mikkelsen, & Voragen, 2002) requiring the combined action of other enzymes, such as PME.

No differences were observed between the polymers extracted with CDTA from cell walls treated with Cel, PME and Xyl. The NaOH extracts showed very little in the way of treatment effects and the results proved difficult to interpret only showing RG-1 coating layer disruption with no xyloglucan changes observable. Zietsman et al. (2015) observed a marked decrease in xyloglucan signals in Pinotage cell walls using an *endo*-1,4-β-glucanase and a pectinase mixture under enzyme buffered conditions not model wine solutions.

As indicated previously, the liberation of soluble polysaccharides from cell walls due to the effect of enzyme treatments was also studied since this information could help us to clarify some of our observations. Figs. 1 and 2 and Table 4 give the molecular weight distributions of polymeric, oligomeric and monomeric polysaccharides that were degraded and moved into the solution in the samples that were treated with enzymes, in comparison with their untreated controls. A cell wall degradation effect is already observed in the model solution which appears to release mainly high molecular weight polysaccharides. This result coincides with that obtained with CoMPP analyses. With the addition of enzymes, a different mass distribution profile is observed. Enzyme treatments produced a higher yield of polysaccharides in solution with a concomitant reduction in their average size.

When enzymes were added individually, PG + PL produced the greatest release of cell wall polysaccharides which were extensively degraded. It is clear that PG + PL treatment is very effective at removing pectin-coating layers (Gao et al., 2016, 2019) providing easier access to the inner hemicellulose-rich cell wall polysaccharides of the grape tissue.

Cel treatment, by contrast, produced high molecular weight polysaccharides and a decreasing molecular size trend was observed for PG, Xyl and PME treatments. Coincident with these results, Bautista-Ortín, Ben Abdallah, et al. (2016) observed that the loss of pectic material was facilitated by cellulase activity rather than PG action and Bindon et al. (2016) observed an increase in the lower molecular mass fractions in grape cell walls treated with a polygalacturonase-rich commercial enzyme preparation. Notably, the low molecular peak observed was within the range for the rhamnogalacturonan (RG) II monomer.

When the enzymes were added as a combined set or sequentially, they also promoted a release of polysaccharides, although this was marked only in in the case where the enzymes were added sequentially. The most marked polysaccharide degradation and polymeric size reduction in solution was when PG + PL was added, either alone or in combination with other enzymes. Interestingly, no great differences found between ESEQ2 and ESEQI2 treatments.

Regarding the effects of PG, and as it was described previously, it appeared most effective when it was added together with other enzymatic activities, as in the case of combined set ET1. The use of this enzyme in combinations appeared very important for the degradation of the released polymers in solution. No major differences were shown for ESEQ1 and ESEQI1 applications.

When tannins were present in the solution, a reduction in the soluble polysaccharide content, especially those of higher degree of polymerization, was observed. This would indicate that tannins not only interact with cell wall-associated polysaccharides, but also with

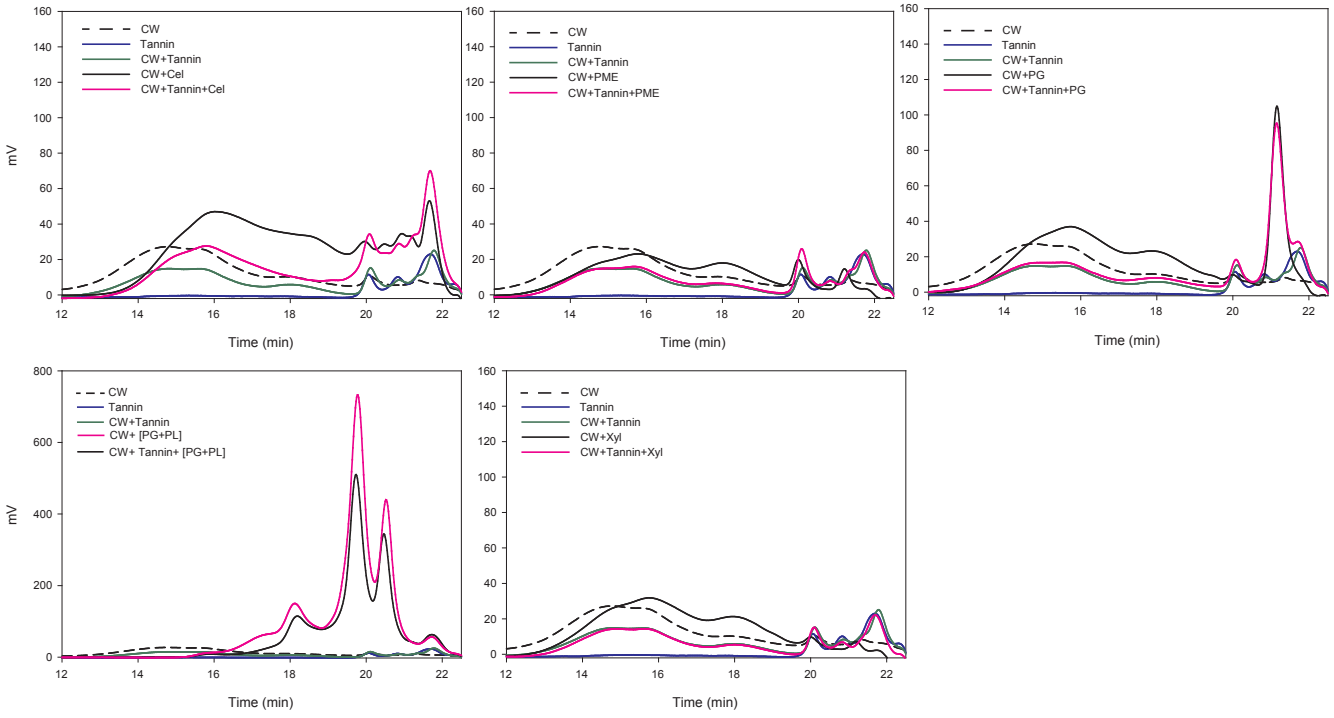

Fig. 1. Comparison of the size exclusion chromatograms of polysaccharides released from the skin cell walls in presence and absence of enzymes added individually.

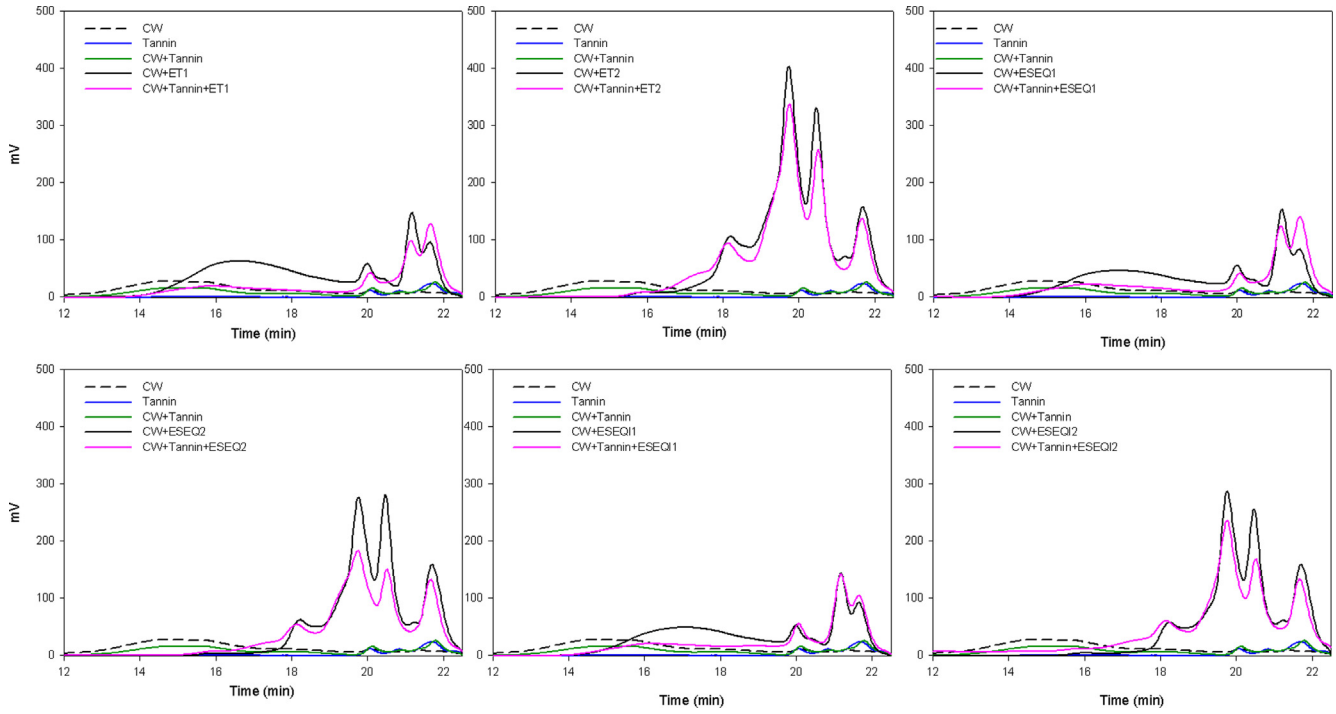

Fig. 2. Comparison of the size exclusion chromatograms of released polysaccharides from the skin cell walls in the presence and absence of enzymes added in combined set (ET) and sequentially (EQ) by SEC.

Table 4
Total area measured in the size exclusion chromatography analysis for the polysaccharides liberated from cell walls and the area corresponding to high and medium molecular mass polysaccharides (those eluting from 12 to 18 min) and low to medium molecular mass polysaccharides (eluting from 18.01 to 22.5) when the experiment was conducted in presence and absence of enzymes.

Samples	Area (12–22.5 min)	Area (12–18 min)	Area (18.01–22.5 min)
CW	128.1	98.3	29.8
Tannin	18.5	−5.1	23.7
CW + Tannin	79.2	47.2	31.9
CW + Cel	267.0	144.4	122.6
CW + Tannin + Cel	173.3	75.7	97.6
CW + PME	113.6	77.8	35.8
CW + Tannin + PME	84.2	48.0	36.2
CW + PG	196.6	119.1	77.5
CW + Tannin + PG	130.0	56.1	73.9
CW + [PG + PL]	888.4	103.5	784.8
CW + Tannin + [PG + PL]	639.8	47.8	592.0
CW + Xyl	137.3	105.8	31.5
CW + Tannin + Xyl	67.9	41.9	26.0
CW + ET1	359.2	165.6	193.6
CW + Tannin + ET1	205.2	50.0	155.2
CW + ET2	677.9	39.8	638.1
CW + Tannin + ET2	607.5	65.1	542.4
CW + ESEQ1	296.0	115.1	180.9
CW + Tannin + ESEQ1	233.9	59.5	174.3
CW + ESEQ2	493.1	10.9	482.1
CW + Tannin + ESEQ2	402.3	39.9	362.3
CW + ESEQI1	298.1	115.9	182.2
CW + Tannin + ESEQI1	225.3	52.1	173.2
CW + ESEQI2	476.0	10.7	465.3
CW + Tannin + ESEQI2	467.6	71.6	396.0

those individual polysaccharides now free in solution and liberated by the action of ethanol and/or enzymes. Bautista-Ortín, Ben Abdallah, et al. (2016) and Bindon et al. (2016) found that interactions between solubilized tannins and polysaccharides in solution are probably higher than with those occurring with insoluble grape cell walls in the matrix. Renard, Baron, Guyot, and Drilleau (2001) reported that the presence in solution of soluble polysaccharides, which is increased with enzyme addition, promoted the desorption of tannins bound to cell walls. This would indicate a 'competitive' high affinity of these soluble polysaccharides for tannins compared with cell wall-associated 'particulate' insoluble polysaccharides. The highly polymerized polysaccharides showed the greatest interaction potential. Lower molecular weight polysaccharides displayed a markedly reduced interaction capacity and in this way, a high recovery of free tannins in solution was observed after interactions with supernatants rich in low molecular polysaccharides from PG + PL treated skin cell walls. The role of high molecular weight polysaccharides in binding tannins was reported by Bindon et al. (2016). When PG + PL was combined with other enzymatic activities and then added sequentially, the effect was reduced in comparison to PG + PL alone. This could be due to the conditions (pH and ethanol content) used in this wine-like matrix or to the presence of tannins, which may limit the capacity of these enzyme combinations to degrade polysaccharides effectively into smaller oligosaccharides. Another possibility is that an increased presence of cell-wall associated proteins could occur due to the action of the different enzymes acting together. In particular, PR proteins, which have shown to have a high capacity for tannin interactions and whose concentration is enhanced by enzyme actions (Bindon et al., 2016), could compete with solubilized polysaccharides and increase their content in solution.

4. Conclusions

The results showed that only when the enzymes were applied individually, and particularly for the PG + PL enzyme, a significant increase in the content of free tannins in solution was obtained. The PG + PL enzyme showed a marked efficacy in the de-pectination of cell walls. PG + PL treatment increased the exposure of grape hemicelluloses and released the highest quantity of soluble polysaccharides of all treatments. Moreover, due the low molecular weight of the polysaccharides released by PG + PL into solution (compared to the other enzymes and combinations tested) they showed the lowest capacity to interact with tannins. The use of multiple enzymes added as a combined set or sequentially was also, to a certain degree effective,

especially if PG + PL was included in the combination. Enzyme combinations tended to favour cell wall degradation, enhancing pore formation and favouring the release of grape tannins. However, the high molecular weight polysaccharides they released had a high capacity to interact with tannins, reducing their content in the wine-like solution. Thus, the increase in cell wall degradation necessary for the release of grape tannin must be balanced with need for a more effective breakdown of the polysaccharides in solution if the aim is to enhance tannin content in the final wines and the use of PG + PL seems to be the most promising approach.

Funding

This work was supported by the Ministerio de Economía y Competitividad from the Spanish government [Project AGL2015-65974-R] and FEDER funds. Grants to J.P.M. from the National Research Foundation (South Africa) as well as the Wine Industry Network of Expertise and Technology of South Africa (Winetech; Grant Nos. IWBT P14/03 and IWBT P14/04) and the Technology and Human Resources for Industry Programme (THRIP; Grant No. TP 13081327560) funded this research. The funders were not involved in the design of the study; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The Central Analytical Facility (CAF) of Stellenbosch University is thanked for providing technical support to J.P.M.

Authors' contributions

E.G.P and A.B.B.O. and J.M. conceived and designed the experiments and wrote the paper, A.O.A. and A.B.B.O. performed the experiments and the analysis of the data. F.W. helped performed the experiments and collect the data. J.P.M. helped interpret the results and draft the manuscript. J.S. and W.G.T.W. performed the CoMPP (glycan microarray) analysis. All authors read and approved the final manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108889.

References

Apolinar-Valiente, R., Romero-Cascales, I., López-Roca, J. M., Gómez-Plaza, E., & Ros-García, J. M. (2010). Application and comparison of four selected procedures for the isolation of cell-wall material from the skin of grapes cv. Monastrell. *Analytica Chimica Acta, 660*(1–2), 206–210.

Bautista-Ortín, A. B., Ben Abdallah, R., Castro-López, L., Jiménez-Martínez, M., & Gómez-Plaza, E. (2016). Technological implications of modifying the extent of cell wall-proanthocyanidin interactions using enzymes. *International Journal of Molecular Sciences, 17*(1), 123–128.

Bautista-Ortín, A. B., Cano-Lechuga, M., Ruiz-García, Y., & Gómez-Plaza, E. (2014). Interactions between grape skin cell wall material and commercial enological tannins. Practical implications. *Food Chemistry, 152*, 558–565.

Bautista-Ortín, A. B., Jiménez-Pascual, E., Busse-Valverde, N., López-Roca, J. M., Ros-García, J. M., & Gómez-Plaza, E. (2013). Effect of wine maceration enzymes on the extraction of grape seed proanthocyanidins. *Food and Bioprocess Technology, 6*(8), 2207–2212.

Bautista-Ortín, A. B., Martínez-Hernández, A., Ruiz-García, Y., Gil-Muñoz, R., & Gómez-Plaza, E. (2016). Anthocyanins influence tannin-cell wall interactions. *Food Chemistry, 206*, 239–248.

Bindon, K., Kassandra, S., & Smith, P. (2016). Retention of proanthocyanidin in wine like solution is conferred by a dynamic interaction between soluble and insoluble grape cell wall components. *Journal of Agricultural and Food Chemistry, 64*(44), 8406–8419.

Bindon, K., Madani, S., Pendleton, P., Smith, P., & Kennedy, J. (2014). Factors affecting skin tannin extractability in ripening grapes. *Journal of Agricultural and Food Chemistry, 62*(5), 1130–1141.

Bindon, K., Smith, P., Holt, H., & Kennedy, J. (2010). Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. *Journal of Agricultural and Food Chemistry, 58*(19), 10736–10746.

Bonnin, E., Garnier, C., & Ralet, M. (2014). Pectin-modifying enzymes and pectin-derived materials: Applications and impacts. *Applied Microbiology and Biotechnology, 98*(2), 519–532.

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., & Bautista-Ortin, A. B. (2011). The extraction of anthocyanins and proanthocyanidins from grapes to wine during fermentative maceration is affected by the enological technique. *Journal of Agricultural and Food Chemistry, 59*(10), 5450–5455.

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I., & Bautista-Ortín, A. B. (2010). Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. *Journal of Agricultural and Food Chemistry, 58*(21), 11333–11339.

Castro-Lopez, L. R., Gómez-Plaza, E., Ortega-Regules, A., Lozada, D., & Bautista-Ortin, A. B. (2016). Role of cell wall deconstructing enzymes in the proanthocyanidin–cell wall adsorption–desorption phenomena. *Food Chemistry, 196*, 526–532.

De Vries, J., Voragen, A. G. L., Rombouts, M., & Pilnik, W. (1981). Extraction and purification of pectins from alcohol insoluble solids from ripe and unripe apples. *Carbohydrate Polymers, 1*(2), 117–127.

Gao, Y., Fangel, J. U., Willats, W. G., Vivier, M. A., & Moore, J. P. (2016). Dissecting the polysaccharide-rich grape cell wall matrix using recombinant pectinases during winemaking. *Carbohydrate Polymers, 152*, 510–519.

Gao, Y., Zietsman, J. J., Vivier, M., & Moore, J. (2019). Deconstructing wine grape cell walls with enzymes during winemaking: New insights from glycan microarray technology. *Molecules, 24*(1), 165–174.

Kassara, S., Li, S., Smith, P., Blando, F., & Bindon, K. (2019). Pectolytic enzyme reduces the concentration of colloidal particles in wine due to changes in polysaccharide structure and aggregation properties. *International Journal of Biological Macromolecules, 140*, 546–555.

Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry, 49*(4), 1740–1746.

Kennedy, J. A., & Taylor, A. W. (2003). Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography A, 995*(1–2), 99–107.

Le Bourvellec, C., Bouchet, B., & Renard, C. M. G. C. (2005). Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1725*(1), 10–18.

Li, S., Wilkinson, K. L., Mierczynska-Vasilev, A., & Bindon, K. A. (2019). Applying nanoparticle tracking analysis to characterize the polydispersity of aggregates resulting from tannin–polysaccharide interactions in wine-like media. *Molecules, 24*(11), 2100.

McMannus, J. P., Davis, K. G., Beart, J. E., Gaffney, S. H., Lilley, S. H., & Haslam, E. (1985). Polyphenol interactions. I. Introduction: Some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *Journal of the Chemical Society, Perkin Transactions 2, 9*, 1429–1438.

Moller, I., Marcus, S. E., Haeger, A., Verhertbruggen, Y., Verhoef, R., Schols, H., ... Willats, W. (2008). High-throughput screening of monoclonal antibodies against plant cell wall glycans by hierarchical clustering of their carbohydrate microarray binding profiles. *Glycoconjugate Journal, 25*(1), 37–48.

Moore, J. P., Nguema-Ona, E., Fangel, J. U., Willats, W. G., Hugo, A., & Vivier, M. A. (2014). Profiling the main cell wall polysaccharides of grapevine leaves using high-throughput and fractionation methods. *Carbohydrate Polymers, 99*, 190–198.

Ortega-Regules, A., Ros-García, J. M., Bautista-Ortín, A. B., López-Roca, J. M., & Gómez-Plaza, E. (2008). Differences in morphology and composition of skin and pulp cell walls from grapes (Vitis vinifera L.): Technological implications. *European Food Research and Technology, 227*(1), 223–231.

Osete-Alcaraz, A., Bautista-Ortín, A. B., Ortega-Regules, A., & Gómez-Plaza, E. (2019). Elimination of suspended cell wall material in musts improves the phenolic content and color of red wines. *American Journal of Enology and Viticulture, 70*(2), 201–204.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., & Gidley, M. (2012). Binding of polyphenols to plant cell wall analogues Part I. Anthocyanins. *Food Chemistry, 134*(1), 155–161.

Renard, C. M., Baron, A., Guyot, S., & Drilleau, F. (2001). Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences. *International Journal of Biological Macromolecules, 29*(2), 115–125.

Riou, C., Vernhet, A., & Doco, T. (2002). Aggregation of grape seed tannins in model wine. Effect of wine polysaccharides. *Food Hydrocolloids, 16*(1), 17–23.

Romero-Cascales, I., Fernández-Fernández, J. I., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2008). Characterisation of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. *International Journal of Food Science and Technology, 43*(7), 1295–1305.

Romero-Cascales, I., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2012). The effect of a commercial pectolytic enzyme on grape skin cell wall degradation and colour evolution during the maceration process. *Food Chemistry, 130*(3), 626–631.

Ruiz-García, Y., Smith, P., & Bindon, K. (2014). Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydrate Polymers,*

114, 102–114.
Tang, H., Covington, A., & Hancock, R. (2003). Structure-activity relationships in the hydrophobic interactions of polyphenols with cellulose and collagen. *Biopolymers, 70*(3), 403–413.
Van Alebeek, G., Christensen, T., Schols, H., Mikkelsen, J., & Voragen, A. G. L. (2002). Mode of action of pectin lyase A of Aspergillus Niger on differently C6-substituted oligogalacturonides. *Journal of Biological Chemistry, 277*(29), 25929–25936.
Watrelot, A., Le Bourvellec, C., Imberty, A., & Renard, C. M. (2014). Neutral sugar side chains of pectins limit interactions with procyanidins. *Carbohydrate Polymers, 99*, 527–536.
Zietsman, J. J., Moore, J., Fangel, J., Willats, W., & Vivier, M. (2015). Profiling the Hydrolysis of isolated grape berry skin cell walls by purified enzymes. *Journal of Agricultural and Food Chemistry, 63*(37), 8267–8274.