New knowledge on grape cell wall structure and the effects of maceration enzymes

One of the major limitations in understanding the impact of commercial maceration enzyme preparations has been the limited knowledge of the grape cell wall. Whilst many studies have looked at components of the cell wall, the methods for testing the individual components has been limited. Recent work by Gao *et al*. (2016) proposed a new cell wall structure based on a newly-developed method to gain a better understanding of the individual components in-situ. **Alana Seabrook**, from Laffort Company Australia, **Julie Barthoux**, enzyme range manager for Laffort, **Bastien Nazaris**, from Laffort Company France, **Virginie Moine**, from Biolaffort, and **John Moore**, from the Institute of Wine Biotechnology at the University of Stellenbosch, South Africa, explain.

Wine quality is a result of vintage, varietal, grape health, vineyard and winemaking practices. Climatic anomalies can affect the grape development and resultant wine quality. Whilst white wines may be pressed straight away, red grapes are normally crushed to allow for maceration during alcoholic fermentation. The objective of this maceration period is to extract beneficial compounds from the different fractions of the berry to increase the resultant wine quality, including phenolic compounds, organic acids and sugars. Enzyme preparations may be utilised to enhance the extraction of beneficial compounds from the grape skin due to highly specific enzymatic activities. Understanding of the grape cell wall enables better understanding of the maceration process but greater control over downstream processing in terms of colouring matter stabilisation, tartrate stabilisation and clarification. This article is a summary of recent findings on the impact of commercial enzyme preparations with new information relevant to grape cell wall structure.

Grape berry

The grape berry is comprised of three main sections: skin, pulp and seeds (Figure 1). A wax layer of soluble lipids covers the grape berry. Aside from providing some protection from pathogens and regulating water loss, it has been suggested that the wax layer prevents the cell degrading enzymes found here from degrading the rest of the berry (Gao *et al*. 2019). It is only after the grapes are crushed that they may commence degrading the berry.

The skin cells form a condensed layer rich in phenolic compounds and aromatic precursors. It also contains a large percentage of neutral polysaccharides as well as acidic pectic components.

The main storage of sugars is the pulp fraction, rich in sugars and organic acids like tartaric acid. This fraction expands significantly during ripening and contains pectins that are more easily hydrolysable.

The seeds are rich in phenolic compounds including tannins, often undesirable in the high concentrations found here as they may confer harsh, bitter notes.

Pectins are a group of polysaccharides derived from plant cell walls that have many different forms and properties. Pectins are polyelectrolytes with gelling behaviour influenced significantly by the presence of Calcium ions (Donald, A.M., 2001). Typically, pectins have a backbone that consists primarily of a linear chain of alpha-(1-4)-D-galacturonic acid (GalA) units with rhamnose residues present. They act as a setting agent in fruit jams, but can hinder colouring matter stability and filtration. As the grapes ripen, a large proportion of pectins go from being insoluble to soluble due to specific enzymes in the grapes which are turned on post-veraison. Also, pectins may have a methyl group or an ester group attached making them methyl-esterified. The take-home from this is that many different enzymatic activities are required to break down the cell wall and access specific sugars and phenolics found in the different layers of skin.

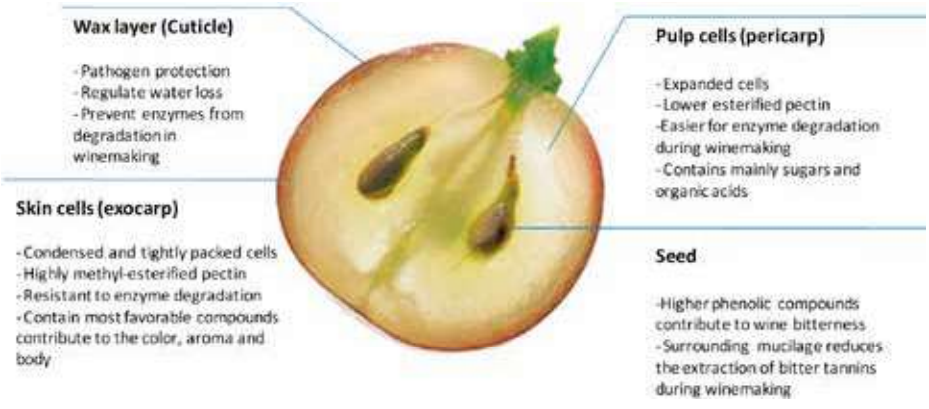


Figure 1. The biological anatomy and biochemical composition of a typical wine grape berry with reference to extractable components taken from Gao and Zietsman *et al*. (2019).

Interaction of commercial maceration enzymes with grape cell wall components – recent findings

Once harvested, maceration of red grapes is influenced by many factors including yeast strain, temperature, SO_2 level, vinification conditions including physical and mechanical interventions (crushing, plunging, pump-overs, header boards). Alternatively, fungal derived commercial enzyme preparations may be used to facilitate maceration process. The research summarised here was carried out by a PhD student Yu Gao in 2016 (South

Africa), supervised by John P. Moore in collaboration with BioLaffort.

Finding the perfect balance of cell extraction can be challenging given the localisation of very different compounds in very different sections of the berry, many of which can bring astringency, bitterness and excessive vegetal notes. Variations in ripeness can further pronounce these differences. There is no doubt that best practice maceration techniques can improve the organoleptic quality of the wine by way of extracting key phenolic compounds (anthocyanins and tannins), polysaccharides and aroma compounds which can shape the sensory profile of the resulting wine.

The structure of the grape cell wall is highly complex and has been a limiting factor in thoroughly understanding the mechanisms and impact of commercial enzyme preparations. Commercial enzyme preparations possess a multitude of principal activities targeted at pectin degradation (pectin-lyases, polygalacturonases, pectine-methylesterases) and a vast array of secondary activities. These secondary activities can facilitate the breakdown of the grape berry allowing access for the primary de-pectinisation activities to function. Previous work from Ducasse (2009) has demonstrated that commercial enzyme preparations with specific activities can liberate specific polysaccharides (RGI, RGII, AGP) which have both a significant sensory impact and effect on stabilisation (Vidal *et al.* 2003).

Investigating the impact of commercial enzymes on grape berry cell wall variation (Cabernet Sauvignon)

First developed by Moore *et al.* (2014) a novel method was developed to enable analysis on the grape marc post fermentation as opposed to compounds found in the wine itself. Comprehensive Microarray polymer profiling (CoMPP) involves extracting the components in the grape skin after fermentation and then dissolving this in different medium. These different mediums (acidic, alkaline) enable solubilization of different compounds, giving fractions either rich in pectin or hemicellulose. This is then printed onto a nitrocellulose membrane and probed with monoclonal antibodies (mAbs) as well as carbohydrate-binding modules (CBMs) (Moore *et al.* 2014); Gao *et al.* 2015) then conducted work on Cabernet Sauvignon grapes from South Africa to assess changes in polysaccharide composition/turnover throughout the winemaking process.

The above study provided framework to specifically target key commercial enzyme preparations and their interactions with the grape cell matrix. This second study was carried out so as to represent even distribution in the vineyard using three commercial enzyme preparations from Laffort (Table 1, page 62) factoring in a variety of grape maturities (Figure 2, page 62). The Cabernet Sauvignon grapes were sourced in the 2014 vintage from the experimental vineyard (Stellenbosh Univerisity) and processed to ensure a homogenous sample before being divided into three 5kg replicates. Chemical analysis at the end of fermentation was conducted to ensure consistency among all replicates. The ferments were inoculated with the same

Table 1. Information on the commercial enzymes used in this trial (www.laffort.com/en).

Commercial enzyme preparation	Properties	Application
Lafase® XL Extraction	Liquid enzymatic preparation for red wine maceration and clarification	Extraction and clarification
Lafase® HE Grand Cru	Pectolytic enzyme preparation, purified in CE for the production red wines that are rich in colouring matter and structured tannins, destined for ageing.	Maceration
Lafase® Fruit	Purified pectolytic enzyme preparation for the production of fruity, colourful and round red wines.	Maceration

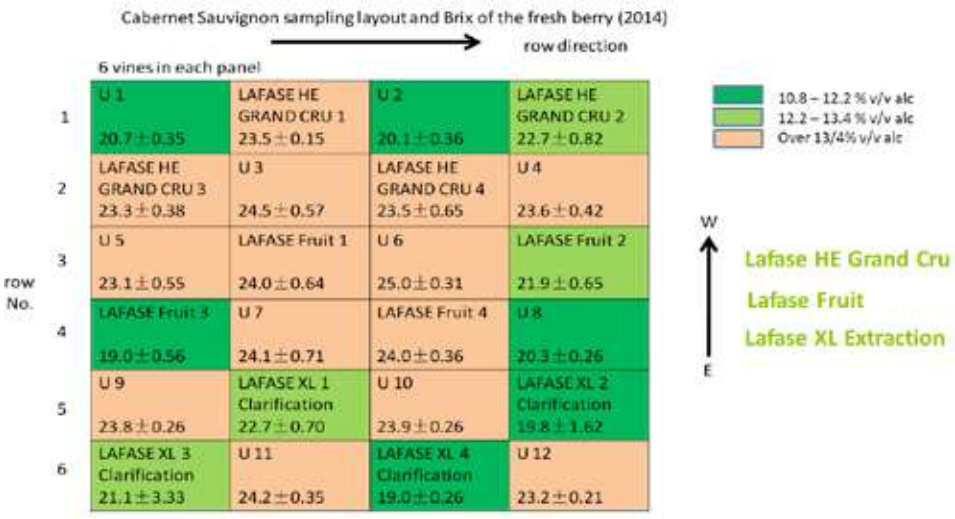


Figure 2. Harvest plan and ripening level variation of Cabernet Sauvignon. Each block represents a panel, which consists of six vines. U refers to untreated fermentations. The level of ripening was categorized into three stages depending on °B value (mean values from three biological repeats). Sourced from Gao *et al*. (2016).

active dry yeast, temperature controlled to 25°C and plunged each day. At the end of alcoholic fermentation the must was pressed in a cage press. Malolactic fermentation was not conducted.

Discussion

There is a clear difference between treated and untreated samples as represented by the two distinct groups in Figure 3. The untreated samples cover a broad area demonstrating a high variability between samples, likely due to the influence of maturity. In contrast, the samples treated with enzymes were more consistently grouped, and minimized variation between maturity levels. Depectinisation is significantly facilitated with the addition of enzymes and there is clustering of the specific enzyme preparations, meaning that each enzyme preparation is able to influence the final wine outcome.

Extraction with NaOH (Figure 4) again distinctly separates enzymes treated from untreated samples. This type of analysis is able to target the different

polysaccharides in the hemicellulose rich cell wall fraction in the grape marc post fermentation. The samples in the bottom right hand corner are predominantly untreated samples which are rich in homogalacturonans (HG), rhamnogalacturonanes I (RGI) and mannanes with a poor degree of esterification. This indicates a poor level of cell wall extraction. The samples which have had enzyme treatment can mostly be found in the top left-hand quadrant of the graph. These samples were found to be higher in xyloglucans with a higher level of esterification, generally indicating a higher level of cell wall degradation. As a consequence, samples treated with XL extraction (high levels of extraction) vs Lafase fruit (lower levels of extraction) can be found on either side of this cluster. Differences between preparations can be visualized from Figure 5 (Page 64) which depicts comprehensive microarray polymer profiling (CoMPP) of the pectin-rich fraction of pomace after alcoholic fermentation. This data also suggests that some enzymes might be better at working on the esterified

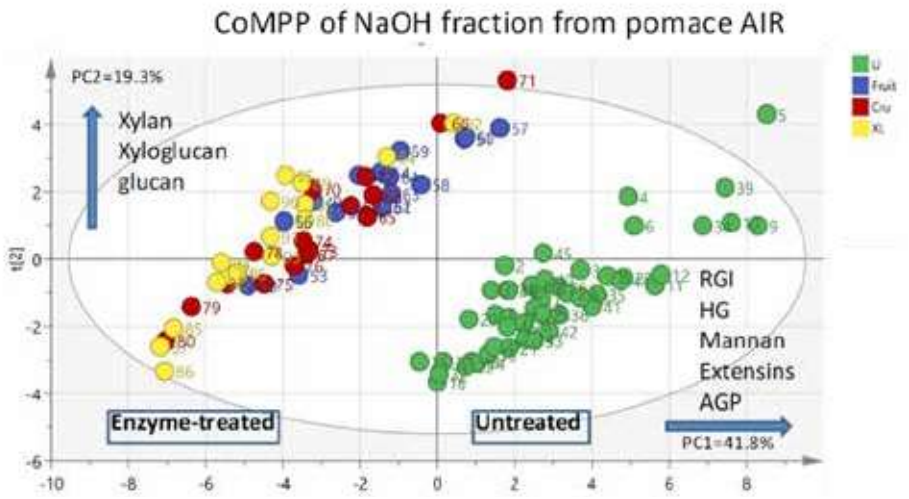


Figure 4. PCA score plot of the NaOH (hemicellulose-rich) extract from AIR sourced from fermented berry pomace. Untreated fermentation; Fruit, Lafase fruit; Cru, Lafase HE Grand Cru; XL, Lafase XL extraction. The colour is according to the treatment.

HG (Lafase fruit) whilst others are more efficient on the de esterified HGs (XL extraction).

A new cell wall structure and better understanding of Laffort enzyme preparations

The results from Gao *et al.* (2016) were able to support the proposal of a new cell wall structure, depicted in a simplified format (Figure 6, page 64). These studies were focused on musts and reflect a degree of esterification of polysaccharides, a function of localization in the berry as well as grape maturity. As the grape berry matures, it is likely that the cell wall and pulp start to depolymerise after veraison, increasing cell size and decreasing cell wall size (thinning) as a consequence.

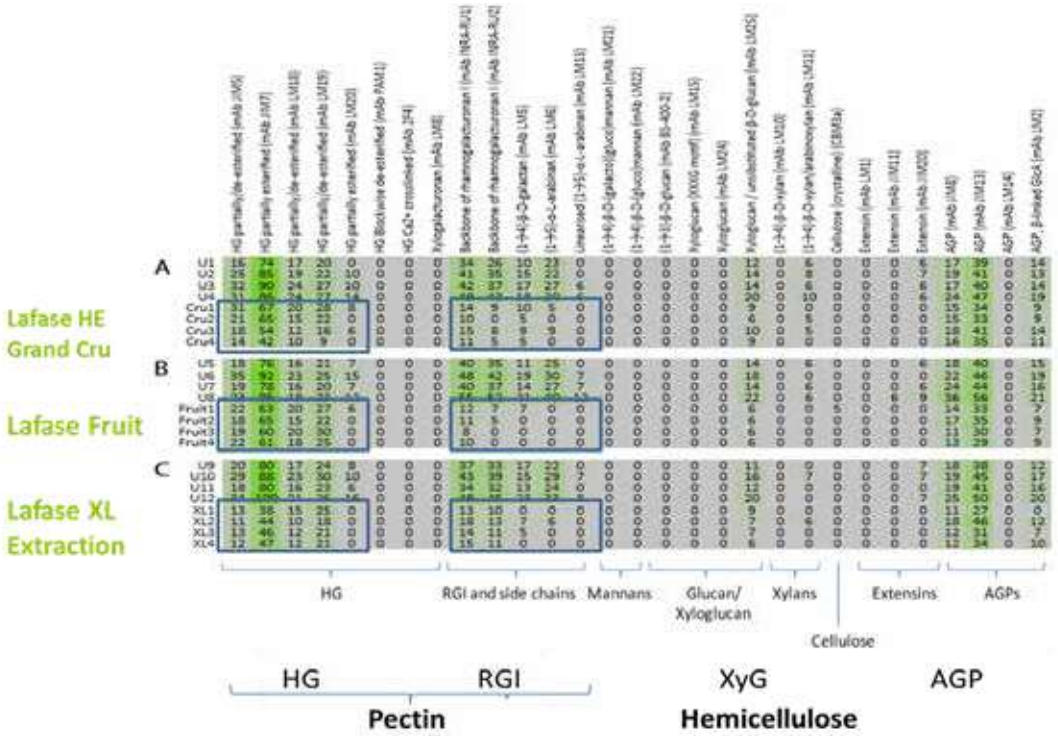
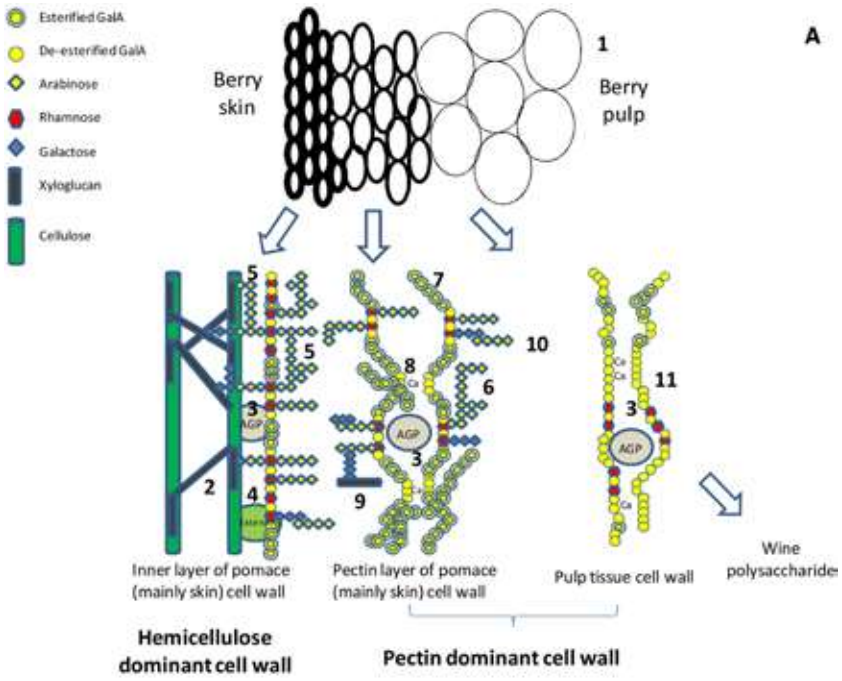


Figure 5. Comprehensive microarray polymer profiling (CoMPP) analysis of the pectin-rich fraction(A) U1–U4 and Cru1–Cru4; (B) U5–U6 and Fruit1–Fruit4; (C) U9–U12 and XL1–XL4. HG, Homogalacturonan; RG, Rhamnogalacturonan; AGPs, Arabinogalactan protein. The heatmap shows the relative abundance of plant cell wall glycan associated epitopes present in alcohol insoluble residue (AIR) sourced from fermented berry pomace. The highest signal was set as 100 and others were adjusted accordingly; the colour intensity is correlated to the mean spot signal. A cutoff (<5) was applied to all heatmaps. Sourced from Gao *et al.* (2016).



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No.	Description	Reference
1	Tissue differences in grape berry,	Nunan et al, 1998
2	Tethering model of hemicellulose -dominant fraction	This study
3	Parallel form of cellulose and associated xyloglucan	Guerriero et al, 2010, Brett 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, Zietman 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	RGI-dombinant pectin layer coating hemicellulose fraction	Gao et al, 2015 and this study
7	Branched arabinan dominant side chain of RGI	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, RGI and AGP in pulp cell walls and wine polysaccharides	Gao et al, 2015

Figure 6. Proposed model of the grape berry cell wall (taken from Gao et al. 2016). Number legend: 1) Tissue differences in grape berry; 2) Tethering model of hemicellulose-dominant fraction; 3) Parallel form of cellulose and associated xyloglucan; 4) AGPs are associated with all fraction of cell wall; 5) Extensins are mainly associated with pomace cell walls; 6) RGI-dominant pectin layer coating hemicellulose fraction; 7) Branched arabinan dominant side chain of RGI; 8) High esterification levels of HG in pectin-rich fraction; 9) Ca2+ cross-linking in pectin-rich fraction, masked by esterified HG; 10) Low amounts of xyloglucan are associated with pectin; 11) Unusual linkage "Ara-Gal-Rha" in pectin rich fraction; 12) De-esterified HG, RGI and AGP in pulp cell walls and wine polysaccharides.

During vinification, the grape berries are crushed and the cell walls of the pulp are easily degraded/solubilized in the wine. As proposed by previous studies, it is likely that this generates a must rich in polymers of homogalacturonanes (HG) and rhamnogalacturonan I (RGI), de-esterified, with the presence of arabinogalactan-proteins (AGP) and in a smaller capacity xyloglucans (XyG). The process of de-esterification is likely to start in the pulp and progress outwards versus the wax cuticle.

The structure of the pectins in the pulp is relatively simple in comparison, requiring enzymatic degradation through the action of pectin-lyases, facilitated through the use of mechanical intervention (crushing or thermovinification) pre-fermentation. On the other hand, the structure of the pectins in the cell wall is far more complex. In order for enzymes to access the main pectin chain, secondary activities are required to lyse the side pectin chains. These secondary activities are also of interest as they affect the liberation of anthocyainins, tannins and important aroma precursors.

Conclusions

These studies have enabled a better understanding of the grape cell and how commercial enzyme preparations can interact with the individual components. It highlights the complexity of the enzyme preparation required for depectinisation, maceration for particular wine styles. The use of enzyme preparations conclusively reduced variation between different levels of maturity (up to 3% v/v alcohol difference). The data also demonstrated that all of the enzymes were able to open up the hemicellulose component of the cell wall, exposing components from inner layers. Those of particular interest were polisaccharides with known sensory impact such as RG II.

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