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REVIEW ARTICLE

Exploring the role of grape cell wall and yeast polysaccharides in the extraction and stabilisation of anthocyanins and tannins in red wines

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ABSTRACT

This paper explores the relationships between polysaccharides/oligosaccharides and anthocyanins/tannins from berry to wine. It is in the form of a review in which the literature has been considered in the light of the following model: in order to remain stable in musts or wines, anthocyanins or tannins need to be surrounded by oligosaccharides or polysaccharides. Moreover, the extraction and stabilisation of anthocyanins and tannins seem to be driven by polysaccharides at all winemaking stages from berry to wine. Polysaccharides can contribute either negatively or positively to the extraction and stabilisation of polyphenols in wines.

KEYWORDS: wines, binding, complexes, polyphenols, anthocyanins, tannins, polysaccharides, oligosaccharides

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INTRODUCTION

Unlike many other food products, wine is consumed primarily for its sensory properties, highlighting the importance of visual, olfactory and gustatory aspects in quality appreciation. Colour and mouthfeel properties are two main criteria for assessing red wine quality. Native anthocyanins, or evolved anthocyanins (e.g., pyranoanthocyanins and oligomeric and polymeric pigments formed as a result of reactions between anthocyanins and tannins (Cheynier *et al*., 2006)) are responsible for red wine colour. Ideal mouthfeel properties are considered to be soft and unaggressive astringency, balanced with sweetness and acidity. Astringency is a sensory stimulus that is experienced as a dryness or roughness in the mouth often characterised as graininess (De Freitas and Mateus, 2002). It is mostly ascribed to the polymerised forms of flavan-3-ol monomers (catechin, epicatechin and epigallocatechin), known as proanthocyanidins or condensed tannins. Anthocyanins, phenolic acids, and flavonols, also contribute to astringency, and they sometimes amplify this perception, as has been observed for phenolic acids (Ferrer-Gallego *et al.*, 2015b; Ferrer-Gallego *et al.*, 2014; Ferrer-Gallego *et al*., 2016). The astringency mechanism involves complexation between tannins and salivary prolin-rich proteins (PRPs), which precipitate on the mucosal pellicle and thereby alter its lubrication (Ployon *et al*., 2018; Soares *et al.*, 2020). Complexes between mucins (other salivary proteins) and anthocyanins can also be involved (Torres-Rochera *et al.*, 2023). PRPs can also prevent tannins from interacting with the mucosal pellicle, epithelium cells and chemoreceptors (Canon *et al.*, 2021). In any case, binding with PRPs - with or without precipitation - has been found to contribute to most astringent compounds (Soares *et al.*, 2020). Tannin composition influences the perception of astringency. Differences have been observed between skin and seed tannins: when tasted as purified fractions, skin tannins containing (epi)gallocatechin (tri-hydroxylated monomeric units) were preferred to seed tannins containing only (epi)catechin (di-hydroxylated monomeric units) (Ferrer-Gallego *et al.*, 2015a). Moreover, a higher galloylation rate of seed tannins increases the perception of their astringency (Vidal *et al.*, 2003a; Soares *et al.*, 2020). However, the composition of tannin is highly modified during aging. The acidic conditions in wine lead to tannin cleavage and therefore a drop in their mean degree of polymerisation (Vidal *et al.*, 2002). Simultaneously, tannins are involved in reactions with different compounds (e.g., other tannins, anthocyanins, flavonols and phenolic acids), resulting in molecules with higher molecular weight and giving the wine better sensorial properties, as observed for flavanol units (Ferrer-Gallego *et al.*, 2015a). After transformation due to chemical reactions, seed tannins lose their astringency, as do skin tannins; as a consequence, aged wines are less astringent (McRae and Kennedy, 2011). We cannot rule out the hypothesis that the contribution of seed tannins to mouthfeel is equivalent to that of skin tannins; extraction of seed and skin tannins are both considered desirable in this review.

Several methods have been developed to address the issue of the substantial losses of anthocyanins and tannins occurring during red wine making (Sacchi *et al.*, 2005). For example, only 25 % of berry tannins have been recovered in wine (Bindon *et al.*, 2010). Such losses can be explained by oxidation and binding to the cell walls. Oxidised polyphenols are highly reactive; they polymerise and can be lost in precipitates when the molecular weight of the molecules is too high. Tannin quantity can be underestimated due to artifacts forming, such as phloroglucinolysis on evolved tannins, when depolymerisation methods are used for quantification. Newly formed bonds between polyphenols are resistant to acidic catalysed cleavage (Poncet-Legrand *et al.*, 2010), thus leading to the underestimation of tannin quantity (Vernhet *et al.*, 2020). However, methods based on size exclusion chromatography (SEC) result in general but more accurate quantifications. On the other hand, wine polysaccharides can play a positive role, as the complexes they form with polyphenols contribute to a softer, less aggressive astringency; for example, pectins have been shown to affect the binding of PRPs salivary proteins with polyphenols (Gonzalez-Munoz *et al.*, 2021). Therefore, more attention should be paid to polysaccharides and their interactions with polyphenols.

Based on a literature review, the aim of this paper is to propose a simple explanation of the relationships between polyphenols and polysaccharides, from mature red grape berries to wine tasting, in the context of polyphenol extraction and stabilisation during or after wine making.

For simplification, the term ‘polyphenols’ will hereafter be used to refer to anthocyanins and tannins, on which there is extensive literature. More precisely, ‘tannins’ will refer to proanthocyanidins and their adducts with, for example, other proanthocyanidins, anthocyanins, flavonols and phenolic acids.

AN EXPLORATION OF ANTHOCYANINS, TANNINS AND POLYSACCHARIDES

Before introducing our model, in this section we draw on relevant literature to describe the location of anthocyanins and tannins, and how they bind to cell walls.

1. Location of anthocyanins, tannins and polysaccharides in the berries

Berries can be divided in three parts: skins, pulps and seeds. At harvest, most anthocyanins and tannins are located in either the skins (anthocyanins and tannins) or the seeds (tannins). At the cell scale, tannin concentrations in the pulp and skin cell walls are equivalent. However, pulp cell walls represent roughly 10 % of skin cell walls (Ortega-Regules *et al.*, 2008); therefore, the quantity of wine tannins that come from pulp tannins is negligible, and this paper will focus on the extraction of polyphenols from skins and seeds.

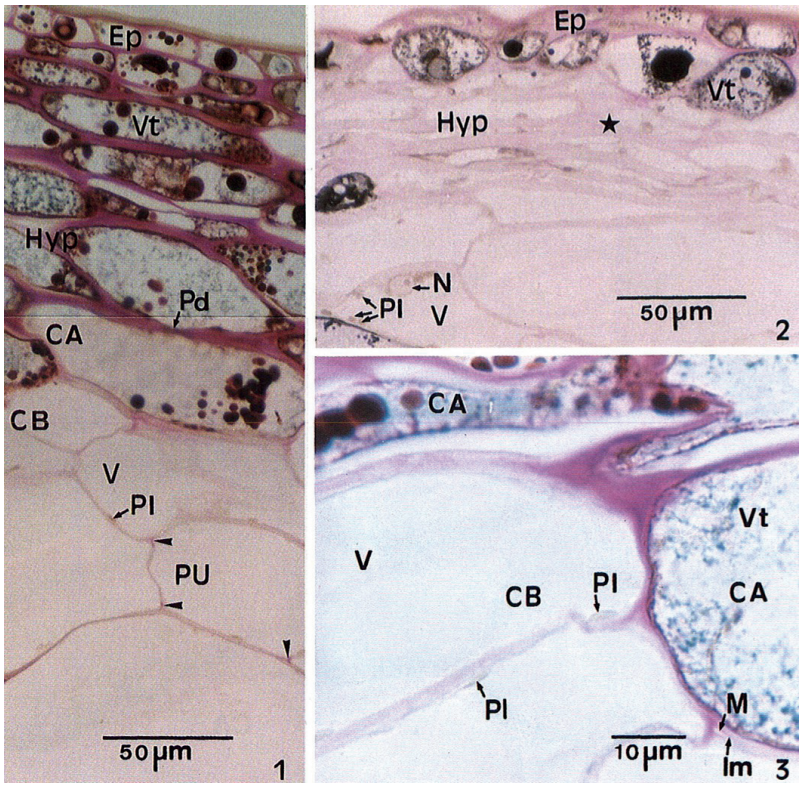


FIGURE 1. Polysaccharides and anthocyanins in the cell walls (Fougere-Rifot *et al.*, 1996).

CD = cell type A, CB = cell type B, Ep = external epiderm, Hyp = hypoderm, Lm = medium lamella, M = Meatus, Pd = plasmodesma, PI = plaste, PU = pulp, V = Vacuole, VT = tannic vacuole, and N = nucleus.

1.1. Location in the skins

In a study by Fougere-Rifot *et al.* (1996), polysaccharides were stained red by PAS (Periodic Acid Schiff) to reveal the skin cell walls (Figure 1). Anthocyanins, naturally red in colour, were also visible in spheric inclusions. Epidermic cells were homogeneous and had thick walls containing anthocyanins. No tannins are visible in this image, but other works have shown tannins to be mainly located in the vacuole (Amrani-Joutei *et al.*, 1994), as were anthocyanins, but in separate inclusions. Such homogeneity has not been observed for the hypoderm: some hypodermic cells were the epidermic-cell type, while others had thick cell walls, similar to epidermic cells but no anthocyanins, similar to pulp cells. The hypoderm is a transition zone, but due to its thick cell walls, it is collected along with the skin and not the pulp when the berries are peeled. The epiderm is protected by a layer of wax, known as the pruine; therefore, it is likely that polyphenols and polysaccharides located in the epidermic cells have to cross the hypodermic zone prior to dissolving in the must (Gao *et al.*, 2019).

1.2. Location in the seeds

Seeds do not contain anthocyanins, but they nonetheless represent a large part of total tannins in the berry depending on the variety: around 60–70 % in Cabernet-Sauvignon (Hanlin *et al.*, 2010) and Pinot noir (Mane *et al.*, 2007; Terrier *et al.*, 2009), and only 30 % in Maccabeo (Terrier *et al.*, 2009). Seed tannins are composed of the three monomeric units catechin, epicatechin and epicatechin-3-gallate (Prieur *et al.*, 1994) and not epigallocatechin units, which are only found in berry skin (Souquet *et al.*, 1996; Llaudy *et al.*, 2008). Seeds are made up of several layers (from outer inner): the cuticle, epidermis and tegument, then the endosperm and embryo. Most seed tannins are located in the tegument (Cadot *et al.*, 2006), which is in the external part of the seed and is therefore favourable for tannin extraction. However, other factors can affect tannin extractibility, namely lignification (mostly occuring at maturation), epidermis thickness which considerably varies from one side of the seed to the other, and polyphenol oxidation (Cadot *et al.*, 2006).

2. Binding of polyphenols to cell walls: positive or negative effect on their extraction?

In the literature, polysaccharides in cell walls are often described as being polyphenol traps (Hanlin *et al.*, 2010); in other words, binding is a mechanism that impedes polyphenol extraction. However, there are some inconsistencies linked to this theory, which are briefly reviewed below.

2.1. First inconsistency: tannin extraction between veraison and maturation

The ripening process greatly modifies skin composition in terms of both polyphenols and polysaccharides. Here we focus on the period between veraison and maturation. While the binding capacity of skin and pulp cell walls increases with maturation (Hazak *et al.*, 2005), tannin concentrations either

remain stable or they decrease in seeds and skins between veraison and maturation (Downey *et al.*, 2003). Therefore, if tannin losses are explained as being a consequence of binding, then maximum tannin extraction would be expected to be achieved at veraison, when binding is at a minimum and tannin concentrations their maximum. This is true for extraction methods involving stirring and acetone, for example. However, in practice, this hypothesis does not hold: in winemaking, the ripening period is the best moment for harvest due to high tannin concentrations in the berry, which are sought for producing high quality wines. Moreover, this hypothesis is also not consistent with the results of work by Abi-Habib and co-workers (Abi-Habib *et al.*, 2021; Abi-Habib *et al.*, 2023), in which two varieties, Carignan and Grenache, were harvested during two stages of ripening for three years. In the first two years, the berries were divided into two groups depending on their volumes (high (V+) and low (V-) respectively) before microvinification. The wine tannins were quantified by SEC (the results are reported in Supplementary Table S1). In nine out of ten comparisons on the two ripening dates, higher quantities of tannins were found in wines when the berries were riper. The exception was the low volume berries of Grenache in 2017; however, their ethanol content of 15.3 %vol. suggests a case of over ripening, explaining the loss of tannins.

2.1. Second inconsistency: positive correlations between skin cell wall polysaccharides and polyphenols in wines

CoMPPs or comprehensive microarray polymer profiling is a new technique involving the use of antibodies and targeting specific parts of cell wall polysaccharides; for example, LM19 targets non-methylated homogalacturonans, while LM20 targets highly-methylated homogalacturonans. The composition of skins and pulp cell walls was investigated using CoMPPs (Abi-Habib *et al.*, 2021; Boulet *et al.*, 2023). The results are summarised in Table 1. In the skins, all the correlations between CoMPPS and polyphenols were positive. This was the case for homogalacturonans, which showed high binding affinity with tannins (Le Bourvellec *et al.*, 2005). Positive correlations were even found in the pulps; e.g., with arabinans. In other words, the high concentrations of anthocyanins and tannins in the wines were associated with high polysaccharide concentrations in the skin cell wall. These results conflict with the hypothesis that cell wall polysaccharides impede anthocyanin and tannin extractions.

PROPOSED MODEL

The proposed model will be described first, with an explanation of the behaviour of polyphenols and polysaccharides from grape berries to wine. The terms adducts, aggregates and complexes refer to covalent bonds between polyphenols, weak associations between polyphenols (same family) and weak associations between polyphenols and polysaccharides (different families) respectively. Polyphenols, especially anthocyanins and tannins, are very reactive molecules, prone to forming adducts with or without oxidative context (Cheynier *et al.*, 2006). Precipitation, which causes losses, is triggered by high molecular weights and other parameters (e.g., absence of glycosylation), as well as the chemical environment; for example, polysaccharides are protective colloids in wines and they therefore limit precipitations (Lubbers *et al.*, 1993). Polyphenol-polysaccharide complexes have long been observed (Glories, 1978). Red wines contain three main families of polysaccharides (Vidal *et al.*, 2003b): i) cell wall polysaccharides, such as polysaccharides rich in arabinose and galactose (PRAGs), ii) type II (RG-II) rhamnogalacturonan in monomer or dimer form, and iii) polysaccharides released by yeast during winemaking, such as mannoproteins (MPs). PRAGs mainly comprise

TABLE 1. CoMPPs associated with the high (+) or low (-) concentrations of extracted anthocyanins and/or tannins from pulps (left) and skins (right). Data from Boulet *et al.*, 2023.

Pulps		Skins	
Polysaccharide structures	Monoclonal antibodies	Polysaccharide structures	Monoclonal antibodies
+ arabinan	CDTA[a] LM6, LM13	+ arabinan	CDTA LM6
	NaOH[b] LM13		
+ extensin CDTA	CDTA LM1, JIM20		
- extensin NaOH	NaOH JIM11	+ extensin NaOH	NaOH JIM11, JIM20
- homogalacturonan	CDTA LM18, LM19	+ homogalacturonan	CDTA LM18, LM19
+ xylan, arabinoxylan	NaOH LM11		
- glucan, xyloglucan	NaOH BS400-2		
	CDTA LM25		
- backbone RG-I	CDTA INRA-RU1	+ backbone RG-I	CDTA INRA-RU2
	CDTA INRA-RU2		
- galactan	CDTA LM25		
		+ AGP	CDTA JIM13

[a] CDTA used to extract pectin rich fractions from grape pulp or skin cell walls.

[b] NaOH used to extract hemicellulose rich fractions from grape pulp or skin cell walls.

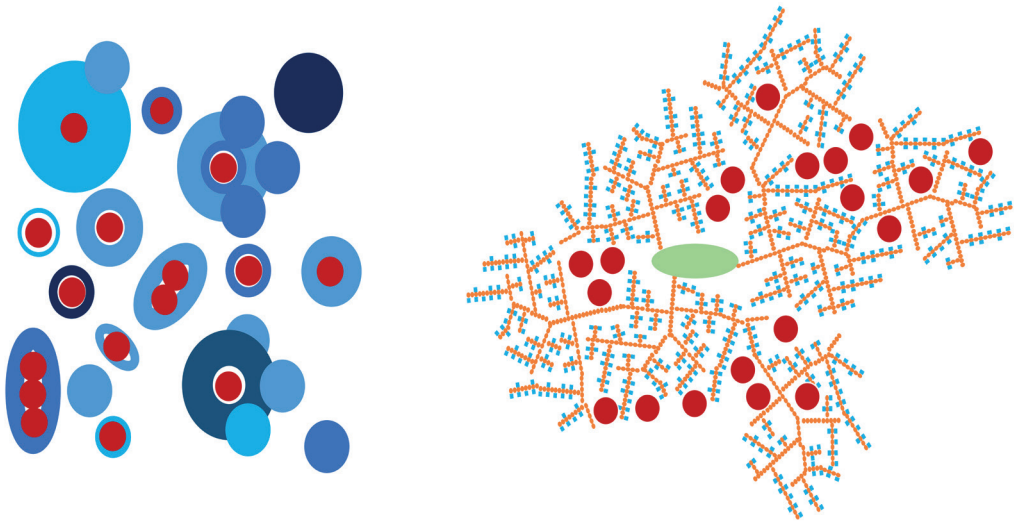


FIGURE 2. Polysaccharides (blue and orange) surrounding polyphenols (red) via encapsulation (left) and nesting (right).

arabinogalactan-proteins (AGPs), but they also comprise arabino-galactans (AGs) and arabinans. Concentrations have been observed in the ranges of 18–616 mg/L for PRAGs, 67–291 mg/L for RG-II, and 76–216 mg/L for MPs (Boulet *et al.*, 2016). The stabilisation properties of these three families (i.e., their ability to prevent enlarged aggregation) have been studied using tannin fractions prone to forming aggregates and precipitating in model wine: the MPs clearly stabilised the polyphenols (Riou *et al.*, 2002), preventing enlarged aggregation and precipitation, and low and medium molecular weight MPs (51 and 62 kD) were more efficient for the stabilisation of tannins than higher molecular weight MPs (337 kD) (Poncet-Legrand *et al.*, 2007). For AGPs and RG-II, conclusions cannot be drawn so easily. The observed molecular weights of the AGPs were between 48 and 192 kDa (Vidal *et al.*, 2003b), which is within the 30 to 400 range - the molecular weight range of MPs that form complexes with polyphenols (Poncet-Legrand *et al.*, 2007). AGPs and MPs both comprise neutral chains of polysaccharides linked to a protein which represents 5–10 % of the AGP or MP weight. Therefore, like MPs, AGPs should stabilise polyphenols. Fractions of AGPs have been purified according to their uronic acid content, from neutral AGP0 to acidic AGP4 (Pellerin *et al.*, 1995; Vidal *et al.*, 2003b). AGP4 has been found to inhibit the formation of large polyphenol aggregates, while AGP0 has not (Riou *et al.*, 2002). These results align with the literature: acidic polysaccharides have been shown to be more effective than neutral polysaccharides at stabilising polyphenols (Le Bourvellec *et al.*, 2004). Therefore, the efficiency of AGPs should depend on the proportions of AGP0 and AGP4 respectively, which seem to dramatically vary. AGP4 and AGP3 are similar in terms of their composition: they both contain galacturonic acid, and a higher concentration of rhamnose and a lower concentration of proteins than AGP0, AGP1 and AGP2 (Vidal *et al.*, 2003b). AGP3 plus AGP4 have been found to represent 19 % (Pellerin *et al.*, 1995) and 78 % (Vidal *et al.*, 2003b) of total AGPs, thus showing high variability. Moreover, AGPs can provide colloidal stabilisation at very low concentrations. The aggregation of polyphenols has been shown to be dramatically limited by the addition of 50 mg/L of AGP4 (Riou *et al.*, 2002) and even 12 mg/L of an AGP extract from Acacia senegal gum (Nigen *et al.*, 2019). RG-II is present in the form of a monomer or a dimer. The RG-II monomer has been found to slightly limit tannin aggregation and the RG-II dimer to increase it. The explanation for this lies in the hydrophobic region of RG-II, which is able to bind polyphenols. Having two hydrophobic regions, the RG-II dimer was able to link the polyphenol aggregates, while RG-II monomer was not. The efficiency of the RG-II monomer increased as it increased in concentration, but these were too low to have a real effect (Riou *et al.*, 2002).

To conclude, monomeric RG-II, MPs and acidic AGPs have been found to contribute to polyphenol stabilisation. Some explanations for this have been proposed. Wine polyphenols are encapsulated by a layer of polysaccharides, which may provide them steric protection (Luck *et al.*, 1994; Saucier, 1997; Le Bourvellec *et al.*, 2004; Mateus *et al.*, 2004; Poncet-Legrand *et al.*, 2007; Brandao *et al.*, 2017). As regards large branched polysaccharides (e.g., from MP or AGP families), polyphenols may be nested between the branches (Carn *et al.*, 2012) (Figure 2). A synergic effect likely occurs between RG-II, AGPs and MPs, which each surround a part of the polyphenol pool by encapsulating or nesting, depending on the polysaccharide family, size and conformation.

Observed under non-wine conditions, the decrease in density of polyphenol-polysaccharide complexes when compared to polyphenol aggregates enhanced stabilisation (Carn *et al.*, 2012). Polyphenol-polysaccharide complexes have been observed in wine in an AF4 analysis (Marassi *et al.*, 2021). Proteins have been evidenced in polyphenol aggregates (Maury *et al.*, 2016; Marassi *et al.*, 2021), which may or may not belong to AGPs or MPs (not discussed here, since proteins not belonging to AGPs or MPs are not within the scope of this article, and they do not alter the general picture).

To summarise, the previously described model (e.g., polyphenol aggregates surrounded by polysaccharides), is largely supported by the literature. However, it does not address the involvement of oligosaccharides in encapsulating tannins, nor the presence or not of free tannins, i.e. not surrounded by polysaccharides, in wines. Unpublished studies by our team show that there are interactions between oligosaccharides and polyphenols, which supports the theory that oligosaccharides contribute to encapsulating tannins, as some polysaccharides do. Furthermore, if significant quantities of free tannins were present in the wines, then the oligosaccharide contribution to astringency would be negative (as was the polysaccharide contribution) and not positive as has been observed (Boulet *et al.*, 2016). It should be noted that an alternative model was considered that relied on tannin-oligosaccharide complexes that had higher astringency than free tannins - themselves more astringent than tannin-polysaccharide complexes; however, this model was dismissed as explanations could not be provided. In the end, the most probable model from our point of view is as follows: *to remain stable in musts or wines, anthocyanins or tannins need to be entirely surrounded by polysaccharides or oligosaccharides.* The time scale for stability is measured in years. The wine-making process, from berry crushing to wine tasting, will hereafter be reviewed in the light of the proposed model.

1. Crushing of berries

Maturation involves the degradation of the middle lamella located between the walls of two adjacent cells, which increases the porosity of the cell walls and is associated with a wavy appearance (Bindon *et al.*, 2014), enabling better access to the binding sites within the cell walls (Hanlin *et al.*, 2010). As soon as the cell walls, and thus the vacuole membrane, are disrupted, anthocyanins and tannins are released, of which one part bind to the cell walls and the other will dissolve in the must.

1.1. Polyphenols bound within the cell walls

Cell walls have a very high capacity for binding polyphenols (Renard *et al.*, 2001; Hanlin *et al.*, 2010), those of skins more so than those of pulp (Abi-Habib *et al.*, 2023). It has been hypothesised that hydrophobic cavities in the cell walls are able to store polyphenols (Le Bourvellec *et al.*, 2004). The stacking of polyphenols within these cavities very likely occurs, thus explaining such high binding capacity (Renard *et al.*, 2001; Le Bourvellec and Renard, 2005; Hanlin *et al.*, 2010). Of the cell wall polysaccharides, pectins have the highest affinity for polyphenols (Le Bourvellec *et al.*, 2005; Ruiz-Garcia *et al.*, 2014). Pectins can be categorised as linear homogalacturonan (HG) or hairy rhamnogalacturonans, namely rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II).

1.1.1. Skin cell wall homogalacturonan

HGs are linear chains of galacturonic acids and the methylation rate of their acidic functions affects binding capacity. Highly methylated HGs have been found to have a high affinity for tannins, especially those with high mean degrees of polymerisation (mDP) (Watrelot *et al.*, 2013), as well as a high affinity for *para*-coumaroylated anthocyanins (Goncalves *et al.*, 2012). On the other hand, low methylated HGs have been found to have a higher affinity for non acylated anthocyanins in studies on cyanidin-3-o-glucoside and blueberry anthocyanins (Fernandes *et al.*, 2020a; Koh *et al.*, 2020). The capacity of HGs for binding anthocyanin increases during ripening (Campbell *et al.*, 2021) due to a decrease in methylation rate. The methylation rate in green berries is around 70 %, dropping to around 20 % on average in ripe berries (Barnavon *et al.*, 2001). Because this methylation rate is low at maturation, anthocyanins are expected to bind more easily than tannins. We thus propose the following scenario, which is illustrated in Figure 3. A first layer of polyphenols is formed when they bind to the HGs: non-acylated anthocyanins bind to slightly methylated HGs, tannins to highly methylated HGs, and *para*-coumaroylated anthocyanins bind between non-acylated anthocyanins and tannins, since they are more hydrophobic than non-acylated anthocyanins but not as hydrophobic as tannins. Thus a first layer is formed, as already suggested by Campbell *et al.* (2021), which contains more anthocyanins than tannins. Then, a second layer is formed by stacking (Renard *et al.*, 2001), and so on, until the cavity has been filled. This scenario provides an explanation for why tannin recovery in wines increases with anthocyanin concentration (Singleton & Trousdale, 1992; Kilmister *et al.*, 2014;

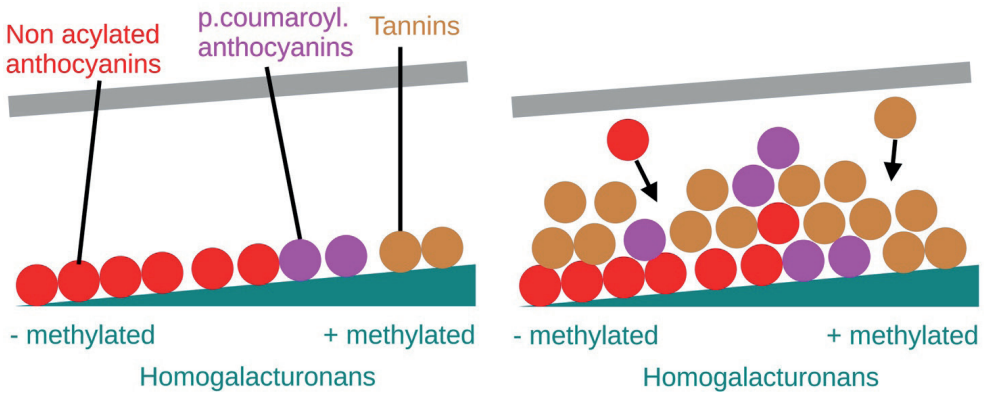


FIGURE 3. Binding of anthocyanins and tannins onto homogalacturonans within the cavities of skin cell walls. Left: filling of the first layer. Right: filling by stacking. The number of layers is illustrative.

Campbell *et al.*, 2021). The contribution of anthocyanins to binding capacity will be discussed in detail hereafter.

1.1.2. Skin cell wall rhamnogalacturonan-I

RG-Is are linear chains of rhamnose and galacturonic acid, which support lateral branches of arabinans, galactans and arabinogalactans (Watrelot *et al.*, 2014). Limited interactions have been observed between tannins of mDP 9 and rhamnogalacturonans when compared to HGs. However, arabinans, which are side chains of RG-Is, are of interest in the binding mechanism. Due to their higher binding capacity than that of RG-Is (Watrelot *et al.*, 2014), arabinans are another significant contributors to binding after HGs (Fernandes *et al.*, 2020b), non-branched arabinans showing high efficiency. Another role of arabinans within the cell walls may be to faciliate the access of polyphenols, since they increase cell wall porosity (Verhertbruggen *et al.*, 2009).

1.2. Polyphenols released in the must

After the berries have been crushed, the conditions in the must are not favorable for anthocyanins and tannins. Oxidative enzymes associated with dissolved oxygen lead to the formation of adducts of higher molecular weight that are more unstable. This situation is worsened by the presence of proteins, and by laccase in the case of harvest with *Botrytis cinerea* (Ribereau-Gayon *et al.*, 2000). Polysaccharides contribute to either the precipitation or the stabilisation of polyphenols. An example can be given with HGs and arabinans based on the results presented in Table 1 and our model. Soluble HGs result from the degradation of the pulp and around 100 mg/L is present in fresh must (Vidal *et al.*, 2000). They have a high capacity for binding polyphenols (Watrelot *et al.*, 2013), but not for surrounding them due to their linear form and their lower flexibility. Therefore, homogalacturonan-polyphenol complexes precipitate: this is supported by no HGs being found in wines (Pellerin and Cabanis, 1998). In contrast, soluble arabinans, however, surround polyphenols and therefore lead to stable complexes due to their high flexibility (Verhertbruggen *et al.*, 2009). However, the concentration of arabinans is limited in musts, with values of 26 mg/L having been found in a fresh Ugni blanc must (Vidal *et al.*, 2001). Other stabilising polysaccharides present in musts are RG-II (30–50 mg/L) and AGPs (113 mg/L) (Vidal *et al.*, 2001), but their concentrations are also low. Thus a minor part of polyphenols released into the must before alcoholic fermentation starts can form stable complexes with polysaccharides, but on the whole, they end up being lost and never recovered in wine.

2. Alcoholic fermentation

Several explanations have been proposed for the release of polyphenols from skins or seeds. During alcoholic fermentation, yeasts produce ethanol and, in the case of red wines, the temperature reaches 30 °C. Tannins are more soluble in ethanol than in water (Sacchi *et al.*, 2005). Ethanol and temperature have been shown to be favorable for the extraction of tannins, because they limit their interactions with the polysaccharides of the cell walls (Le Bourvellec *et al.*, 2004). In addition, the concentration gradient produced by the higher polyphenol concentrations in skins than in must is favourable for their extraction.

The release of polysaccharides depends on their origin and will affect the final wine composition, in terms of MPs, RG-II and PRAGS, which include AGPs, AGs, arabinans and galactans (Vidal *et al.*, 2003b). Skin cell walls contain a few MPs, but their contribution is negligible. Wine MPs are derived from yeasts, and thus they are released via alcoholic fermentation. Skin cell walls also contain two other families of polysaccharides, namely RG-II and AGPs. The RG-II monomer and dimer are specific and well-defined structures embedded within the pectins; however, it has not yet been determined whether RG-II is located in linear HGs or in hairy RG-Is. RG-II is a marker of the degradation of cell walls, because it is spared by pectolytic enzymes. RG-II concentration in berries is around 250 mg/kg (Vidal *et al.*, 2001); thus the production of 0.6 L of wine per kg of berries would lead to a potential RG-II concentration of 400 mg/L (250/0.6) in wines. As 3/4 of berry pectins are found in skins and a 1/4 in pulps (Vidal *et al.*, 2001), skins and pulps could contribute 300 mg/L and 100 mg/L respectively. However, these values are still far from the observed concentrations in wines: 30–50 mg/L in white wines and 100–150 mg/L in red wines (Pellerin and Cabanis, 1998). RG-II is present in fresh musts and is extracted during alcoholic fermentation. Its concentration was doubled during the alcoholic fermentation of a red variety (Doco *et al.*, 1996). The location of AGPs has been studied in the cell walls of apples using the JIM13 epitope (Leszczuk *et al.*, 2020): the AGPs showed a uniform distribution in the cell walls of the skin of unripe apples, but during maturation, they accumulated and moved towards the edge of the cell walls, close to the plasma membrane. These locations and the unusual mobility for cell wall polysaccharides could explain why high quantities of AGPs are extracted and recovered in wines.

To summarise, RG-II is present in must and is steadily extracted during alcoholic fermentation (Doco *et al.*, 1996). The extraction of AGPs begins as soon as the berries are crushed, and ends when the skins are separated from the fermented juice. Some MPs are released at the early stage of fermentation, and others during autolysis which begins at the end of the alcoholic fermentation (Vidal *et al.*, 2003b). On the whole, polysaccharides are either already present in the must (RG-II) or are released during alcoholic fermentation (RG-II, AGPs, MPs), or even during post fermentation maceration (AGPs, MPs). Therefore, complexes can be formed between polyphenols on the one hand, PRAGs (e.g., AGPs and arabinans) and MPs and RG-II on the other. Polysaccharides in wines have been observed to be stable over 10 years without any significant losses (Doco *et al.*, 1999). Meanwhile, polyphenols may continue to evolve, but they could still benefit from the stabilisation provided by polysaccharides.

3. The contribution of oligosaccharides

Monomeric RG-II is considered a polysaccharide, but its molecular weight of around 4,800 Da is close to the threshold between polysaccharides and oligosaccharides.

Structures corresponding to HGs, arabinogalactan, arabinan, xylan side chains of RG-Is and mannans have been found in Merlot and Carignan wines (Ducasse *et al.*, 2010). Because they share almost the same structures, although differing in size, oligosaccharides should logically have the same binding properties as polysaccharides. Unpublished results obtained by our team support this hypothesis. Oligosaccharide concentrations are quite high in wines: in an analysis of 21 red wines, mean values of 484 mg/L of polysaccharides and 359 mg/L of oligosaccharides have been found (Boulet *et al.*, 2016); i.e., oligosaccharides represented 42 % of the (oligosaccharide + polysaccharide) concentration. It has been reported that 35 to 60 % of polyphenols are associated with polysaccharides (Fernandez *et al.*, 2017). These values indicate that oligosaccharides could support polysaccharides to achieve complexes that completely surround anthocyanins and tannins, in accordance with our model.

Polysaccharides and oligosaccharides have direct consequences on mouthfeel. Polysaccharides slow down reactions between polyphenols and salivary proteins, or between polyphenols and chemoreceptors of the epithelium cells, thus affecting astringency (Canon *et al.*, 2021). Passive (steric repulsion) or active (competition) mechanisms have been proposed. MPs have been found to limit the increase in tannin-BSA aggregates by steric repulsion (Assuncao Bicca *et al.*, 2023), and AGPs to compete with tannins when binding with salivary proteins (Kuhlman *et al.*, 2023), even if proteins have higher affinity for tannins than for polysaccharides (Assuncao Bicca *et al.*, 2023). Oligosaccharides also slow down the formation of tannin-salivary protein complexes, but to a lesser extent than polysaccharides due to their smaller size; as a consequence, the perception of astringency will be lower with polysaccharides than with oligosaccharides, as has been observed (Quijada-Morin *et al.*, 2014; Boulet *et al.*, 2016).

DISCUSSION

In order to provide support for our model, the following five points are discussed in this section drawing on the literature: 1) oenological practices, 2) seed tannins, 3) anthocyanins, 4) enzymes, 5) cell wall material.

1. Oenological practices

Certain oenological practices are applied in almost all wineries, which are worth considering here.

1.1. Vinification using flash-detente

Images of skins obtained from flash-detente (FD) processing have revealed a network of furrows that are 0.3 µM deep (Boulet and Escudier, 1998); thus, extraction becomes possible from the outer face of the skin in addition to the inner face. FD was originally developed to extract more polyphenols (Morel-Salmi *et al.*, 2006), but more polysaccharides, namely RG-II and AGPs, have been extracted too (Doco *et al.*, 2007). A large variability in polyphenol concentrations has been obtained from the same grapes, depending on maceration duration (Ntuli *et al.*, 2023), and correlations have been observed between total polysaccharides and tannins. According to our model, this is because supplementary polysaccharides extracted during maceration after FD processing are able to form complexes, thereby stabilising the supplementary polyphenols also extracted and thus resulting in higher concentrations of polyphenols in the wines. In one of the trials conducted by Ntuli *et al.* (2023) only tannins were added and not polysaccharides, but the concentration of tannins in the wine did not increase. A likely explanation for this is that because all soluble polysaccharides were already involved in complexes with polyphenols, the additional tannins could not be stabilised and so they precipitated, leading to the observed result.

1.2. Vinification using frozen grapes

Cold conditions lead to the deep destructuring of cell walls from the inside due to the growth of ice crystals within them. This destructuring, which occurs at the cell scale, is usually more violent and effective than flash-detente, if the crystals have time to grow. Vinification of frozen berries is not usual, but it can occur naturally or be carried out in experiments. For example, it has been applied to the red berries of Cabernet-Sauvignon and compared to two hybrids, Frontenac and Frontenac blanc, red and white varieties respectively. Polysaccharides and tannins were quantified in the must at different moments of the fermentation (Nicolle *et al.*, 2021). On the whole, the three varieties showed the same trend: tannin concentrations decreased during the first two days of the alcoholic fermentation, then increased dramatically from Day 3 to the end. The final tannin concentrations were within the range of 200–300 mg/L, which is quite low for the Cabernet-Sauvignon variety. Meanwhile, polysaccharide concentrations increased at the beginning of fermentation, then remained stable to the end. Our explanation for this is that the ice crystals caused deep destructuring of the skin cell walls, resulting in the release of skin HGs into the must, which were then precipitated, along with pulp HGs; their presence and then their precipitation with polyphenols could explain the drop in polyphenols observed before the beginning of fermentation. Then three days after the beginning of alcoholic fermentation, all extractible HGs will have precipitated, probably due to enzymatic activities (Osete-Alcaraz *et al.*, 2022). The remaining polysaccharides are mainly RG-II, PRAGs and MPs, and therefore the newly formed complexes between polyphenols and polysaccharides are stable, leading to the increase in both polyphenol and polysaccharide concentrations (Nicolle *et al.*, 2021).

1.3. Fining and arabic gum

According to our model, polysaccharides and oligosaccharides can be a limiting factor for polyphenol concentrations in wines. Polyphenols not involved in complexes with polysaccharides/oligosaccharides precipitate. This is what usually happens when wine is aged in tanks or barrels, since the polyphenols that are not involved in complexes have time to precipitate. It should be noted that polyphenols continue to react (e.g., as a result of cleaveage, oxidation or polymerisation); the layer of polysaccharides/oligosaccharides just slows down the

reaction. Logically, tannins that are bound to oligosaccharides will react and perhaps be lost more rapidly than will tannins bound to polysaccharides. The ratio tannin-polysaccharide/tannin-oligosaccharide increases with time, leading to a drop in astringency perception, as is observed during aging.

Polysaccharides are very stable in wines (Doco *et al.*, 1999). However, losses can occur during certain processes; e.g., inadequate filtration can result in the largest polysaccharides being retained (Vernhet and Moutounet, 2002), and even if they cross the membrane, the corresponding polyphenols will no longer be stabilised and will be lost later on as a result of precipitation. Another example is given by primeur wines, which are put on the market too early for the excess tannins to stabilise naturally. Stabilization can be obtained by either removing polyphenols by fining with, for example, gelatin, or by adding polysaccharides, such as arabic gum which contains AGPs.

2. Seed tannins

Seed tannins constitute the majority of berry tannins (i.e., 75 %; Downey *et al.*, 2003), and despite them having lower extractibility due to the histological constraints described previously, they would be expected to represent the majority of wine tannins. However, this is not the case: skin tannins are present in higher quantities in wines than seed tannins (Rousserie *et al.*, 2019). Contradictory results have been obtained: the addition of seeds increased wine tannin concentrations in one experiment, but their removal did not lead to a drop in wine tannin concentration in another experiment (Rousserie *et al.*, 2019). Therefore, the recovery of seed tannins in wines is far from simple. Four different experiments addressing this issue are reviewed here. 1) extracted tannins were quantified after each of three processes had been carried out: i) real vinification, ii) skin vinification (fresh skins), and iii) seed vinification (fresh seeds) (Rousserie *et al.*, 2020). At the end of the post-fermentation maceration, the final concentrations of tannins after seed-vinification, as determined by phloroglucinolysis, were about three times higher than those after skin-vinification. 2) Tannins were extracted from fresh seeds and skins in model solutions with increasing ethanol content, mimicking fermentation (Llaudy *et al.*, 2008). The concentrations of the extracted tannins were comparable to those of ripe berries, with concentrations of around 2 g/L of tannin from both seeds and skins. However, seeds showed a delay in the extraction which began at 4 % ethanol, whereas no delay was observed for skins. 3) With an experimental design similar to the previous one (Abi-Habib *et al.*, 2023), extractions occured within 2 days, which was fast and may be due to a side-effect of freezing the seeds and skins, as well as to the rapid increase in ethanol concentrations: 0 and 5 % vol. on the first and second days respectively. However, this increase corresponded to the increase in ethanol concentrations observed in real micro-vinifications. 4) Destemmed grapes were subject to three preprocessing stages: freezing using dry ice, low temperature prefermentative maceration (10 °C for 10 days) and the addition of commercial enzymes. Fermentations were then performed for 10 days and tannin concentrations compared to a control vinification process (Busse-Valverde *et al.*, 2011).

The first three experiments confirm that seed tannins are extractable in large quantities. However, they still have a low recovery rate in wines. In contrast to skin cell walls, seed cell walls are poor in pectins and other polysaccharides; they release tannins without providing soluble AGPs or RG-II to quickly stabilise them. Logically, soluble AGPs or RG-II from skins are already involved in complexes with skin polyphenols. Therefore, seed tannins will bind to pulp or skin cell walls, or be involved in chemical reactions (Abi-Habib *et al.*, 2023). Their higher galloylation rate will limit desorption from cell walls on the one hand (Vernhet *et al.*, 2020) and enhance the formation of adducts of higher molecular weight on the other, leading to losses. Nevertheless, it can be assumed that significant stabilisation and recovery of seed tannins begins with the presence of MPs, which is first released during alcoholic fermentation and which continues during post fermentative maceration. The fourth experiment resulted in the higher extraction of tannins - particularly of seed tannins - in the low temperature prefermentative maceration. Seed tannin extraction has been explained as being the result of the higher hydratation of seed tissues which occurs during maceration (Hernandez-Jimenez *et al.*, 2012). Our model can thus explain what happens to them after extraction. The 10-day maceration at low temperature allowed the enzymatic activities to clarify the must and probably to precipitate soluble HGs coming from the pulp, as has been previously observed under similar conditions (Osete-Alcaraz *et al.*, 2022). The seed and skin polyphenols released during alcoholic fermentation showed a lower precipitation rate due to the much lower quantities of pulp polysaccharides, which led to the observed higher concentrations (Busse-Valverde *et al.*, 2011). Seed tannins increased more than skin tannins when compared to the control, because of the higher quantities in the seeds, the absence of soluble HGs and the release of MPs.

3. Anthocyanins

Although red and white grapes contain almost the same quantities of tannins, white wines have been found to contain much less tannins than red wines, even though both were made with pomace contact and the main difference between white and red varieties was the absence or presence of anthocyanins respectively (Singleton and Trousdale, 1992). In wines, tannin-anthocyanin adducts are quickly formed; for example, after the cleavage of tannins in acidic conditions (Cheynier *et al.*, 2006). These adducts are more stable than anthocyanins or tannins alone (Singleton and Trousdale, 1992), leading to an increase in the tannin concentrations (Kilmister *et al.*, 2014). Therefore, anthocyanins contribute to the stabilisation of tannins, and vice versa. However, their role during vinification remains unclear. In a study by Kilmister *et al.* (2014), fresh berries were selected for their high and low levels of anthocyanin and tannin concentrations respectively and were submitted to a real vinification process. Low anthocyanin/low tannin berries yielded the same concentration of tannins in wine as low anthocyanin/high tannin berries. High anthocyanin/low tannin berries

yielded the same concentration of tannins in the wine as high anthocyanin/high tannin berries. Thus clearly, tannin extraction was directly driven by anthocyanin concentration. The authors qualified their result as counter-intuitive, but in the light of our model we can propose an explanation. In the cases of a lack of anthocyanins (e.g., in pomace contact with white berries or with red varieties poor in anthocyanins), there will be gaps in the first layer of polyphenols in large regions of skin with lowly methylated HGs within the skin cell wall cavities. The binding capacity of tannins will thus be reduced, and once in the must they quickly precipitate. But if anthocyanin quantities are sufficient enough to fill out a first layer on the HGs, then the tannins will be stacked and preserved on this anthocyanin layer before alcoholic fermentation begins. It should be noted that the desorption mechanism involving anthocyanins and tannins mainly concerns the second and subsequent layers, and stacking involves very weak bonds. It is probable that most of the anthocyanins and tannins that form the first layer (i.e., bound to HGs) will remain trapped, even during skin maceration.

This closeness between anthocyanins and tannins could enhance the formation of more stable anthocyanin-tannin adducts in non-oxidative conditions (Campbell *et al.*, 2021). This would explain the correlations observed between not only total anthocyanins and tannins (Bindon *et al.*, 2017), but also between non acylated anthocyanins, *para*-coumaroylated anthocyanins and tannins (Abi-Habib *et al.*, 2021; Boulet *et al.*, 2023). The recovery rate of *para*-coumaroylated anthocyanins is very low compared to non-acylated anthocyanins, which is more dramatic in model solution than in wine (Abi-Habib *et al.*, 2021; Boulet *et al.*, 2023). This may be due to i) stronger bonds of *para*-coumaroylated anthocyanins with highly methylated HGs, limiting their release, or 2) the higher incorporation of *para*-coumaroylated anthocyanins within anthocyanin-tannin adducts by tannin cleavage (Campbell *et al.*, 2021) when compared to non-acylated anthocyanins.

4. Enzymes

Enzymes have the ability to degrade cell walls, more than any physical processing. However, in practice, their efficiency is not so clear and results are often contradictory. According to our model, the beneficial effect of enzymes is that they degrade cell walls enough to extract RG-II and AGPs. The degradation of pulps, leading to either early pulp precipitation (Osete-Alcaraz *et al.*, 2022) or to the release of oligosaccharides (Ducasse *et al.*, 2011) can also be beneficial. However, a detrimental effect is that long soluble chains of pectins are released from cell walls and then precipitate with polyphenols. Commercial enzymes are not pure, most of them constitute poly-galacturonase, pectin methyl-esterase, pectine lyase, and other residual activities. They have been found to increase polyphenol extraction by removing the pulp cell walls (Osete-Alcaraz *et al.*, 2022). However, in some cases of polyphenol extraction, enzyme effects can switch from benefical to detrimental. This would explain the mitigated effects of enzymes, depending on the experiment, despite their high degradation power.

5. Cell wall material

Cell wall material (CWM) comprises residue from skins after water extraction followed by ethanol precipitation. Here we consider the characteristics of CWM which could lead to the best recovery of anthocyanins and tannins in wine. According to our model, the price to pay would be the first layer of polyphenols bound to HGs. Polyphenol release concerns the second and subsequent layers. Therefore, the best conditions would involve a good balance between CWM, anthocyanins and tannins. In the case of skins that are too thick, too many anthocyanins and tannins would be lost in the first layer. In the case of skins that are too thin, not enough anthocyanins and tannins would be able to stack on the second and following layers, thus also leading to losses. Furthermore, as well as the quantities of CWM from the skins, the physiological characteristics of skins are also a very important factor. HGs need to be highly de-methylated and their cavities should remain intact before and during vinification. Ripening is a good example of the transformations of the skins. For example, drops of CWM have been observed during the ripening of Tempranillo cv. (Hernandez-Hierro *et al.*, 2014); meanwhile, the recovery of anthocyanins and tannins increased.

In fact, CWM is not of such great importance for winemakers, who are familiar with their varieties and environment and have the skills to precisely determine the maturation point and thus the best moment to harvest. However, they could play a role in the creation of new varieties: adequate characterisation of their composition in terms of polyphenols and polysaccharides could help to identify high or low oenological potential early on.

CONCLUSION

The aim of this paper is to describe the interactions between polyphenols and polysaccharides from grape to wine. Inspired by unexplained observations, the proposed model corresponds with most of the literature on the subject. Its novelty lies in the potential positive contribution of polysaccharides/oligosacharides to the recovery and stabilisation of anthocyanins and tannins in wines. The main idea is that all stable tannins and anthocyanins are surrounded by a layer of polysaccharides or oligosaccharides, namely RG-II, PRAGs and MPs, which are already known as protective colloids. While homogalacturonans do not have this stabilisation property, they can bind polyphenols. Such binding capacity enhances polyphenol recovery in wine when it occurs within the cavities of the skin cell walls. The contribution of anthocyanins to the recovery of tannins can be explained by their ability to bind to lowly methylated homogalacturonans; tannins can then stack on this anthocyanin layer. However, polyphenol binding on homogalacturonans in the must, whether in soluble form or in pulp tissues, will early precipitation.

We are aware that further research is required to reinforce and develop this initial study. Nevertheless, it seems clear that oligo and polysaccharides play a central role in the control

of anthocyanins and tannins; therefore, a characterisation of both oligosaccharides and polysaccharides would be required to increase our understanding of anthocyanin and tannin behaviour. Such an approach would be particularly valuable for carrying out quick but accurate evaluations of the oenological potential of new varieties.

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