ALTERNATIVE TECHNOLOGIES TO MODIFY AND MEASURE RED WINE ASTRINGENCY AND QUALITY

Wenyu Kang

A thesis submitted for the degree of Doctor of Philosophy

Schools of Agriculture, Food and Wine

Faculty of Science

The University of Adelaide

May 2020

THE UNIVERSITY of ADELAIDE

TABLE OF CONTENTS

THESIS SUMMARY

Astringency is an important mouthfeel factor driving wine quality, complexity and consumer preferences. Wine astringency is mainly perceived due to the interactions between polyphenols in wine and salivary proteins during consumption. The wine industry has invested heavily in the analysis of wine phenolic composition and its effects on flavour/mouthfeel. However, our understanding regarding the relationships between specific phenolic fractions/compounds and their respective astringent mouthfeel and sub-qualities (e.g. grippy, puckering), as well as novel and improved techniques for measuring astringency perception and modification of wine astringency levels, are still limited. This thesis comprises a number of studies to investigate these research gaps. The findings of these studies are contained within the thesis chapters two through to and inclusive of chapter five. These are presented here as two published, peer-reviewed papers, one submitted manuscript and one un-submitted work written in a short research communication format following the introductory chapter one and are outlined in the following summary.

Firstly, in an attempt to improve methods to examine human astringency perception and elucidate the different yet more subtle astringent sub-qualities caused by different chemical parameters (basic wine composition and phenolic profiles), a modified progressive profiling was explored. Dynamic astringency profiles of 13 Australian commercial red wines and 2 rosés made from 11 grape varieties were generated using a trained, modified progressive profiling sensory panel. Overall astringency intensity and 6 sub-qualities: pucker, mouth coat, dry, grippy, adhesive and graininess defined by the panel were rated at six time periods (lasting 10 seconds each), with 20 second gaps between each period. Wine composition and phenolic profiles were also determined to establish correlations with mouthfeel attributes. This alternative sensory methodology enabled dynamic and quantitative

intensity measurement of astringent attributes, providing enhanced understanding of the chemical basis of subtle wine astringent sub-quality differences.

Secondly, due to consumer demand for non-animal-derived processing aids, the efficacy of potato proteins to manipulate astringent compounds in red wine and the steps required for its optimisation of fining were investigated. This represented the first study to examine the potato protein dose-response kinetics of tannin and phenolic compound removal for two unfined Cabernet Sauvignon wines. Testing the influence of wine matrix and fining parameters (including pH, ethanol concentration, sugar concentration, temperature, and agitation) were according to a fractional 2^{5-1} factorial design. Insights into potato proteins' optimal use revealed that fining efficiency could be increased by treating wines at higher than usual cellar temperatures (20˚C), and at both a lower pH and/or alcohol concentration.

Thirdly, an investigation of a new grape-must polyphenol extraction technique: Accentuated-Cut-Edges (ACE) revealed its capacity for modifying wine astringency. This study reported the effect of the ACE technique on non-volatile chemical composition of Shiraz wine (basic wine chemistry, colour, phenolic components and polysaccharides) and sensory profiles (using rate-all-that-apply and modified progressive profiling) for the first time. Furthermore, any potential improvement provided by ACE for the pre-fermentation water addition to must to reduce alcohol was investigated. The ACE technique increased the intensities of adhesiveness and graininess, which partly overcame the impact of water addition on the astringent sensation.

Fourthly, as the experimental Shiraz wines for the ACE study were produced in small-scale fermentation batches (25kg), an investigation at the industrial scale was warranted. Therefore, two pilot commercial wines (ACE with 5-day skin contact and NOACE with 8 days on skins) were produced in 2018 by the Coriole winery at industry scale (averaged 2.45

tonnes for each treatment) and were chemically analysed and underwent sensory profiling in 2019 alongside the ACE research wines in Chapter four. It was a preliminary experiment investigating the feasibility of ACE grape must extraction technique on Shiraz wines at an industry scale. This study indicated that ACE could potentially be used by the wine industry to combat one of the challenges of climate change, vintage compression, caused by climate change, by pressing wine ferments earlier, freeing up tank space for other wines.

In conclusion, the research contained in this thesis provides advanced insights and alternative tools for researchers and the wine industry. Uncovering what components impact wine astringency, knowing how to better evaluate perceived wine astringency along with its sub-qualities and modify this important wine sensory attribute with a more informed approach, will enhance the capability of wine producers to better cope with some of the ramifications of climate change such as higher alcohol levels and vintage compression, target product style and quality plus meet consumer expectations.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Wenyu Kang	29 May 2020
	Date

PUBLICATIONS

This thesis contains a collection of manuscripts published in Food Research International, Molecules, and Foods during candidature. The impact factor of Food Research International according to Thomson Reuters Journal Citation Reports (JCR) was 4.972 in 2019 and the 5-year impact factor was 5.084. The impact factor of Molecules (2019) was 3.267 and its 5-year impact factor was 3.589. The impact factor of Foods was 4.092 in 2019.

The text and figures in Chapter 2 to 4 appear in different formats due to each journal's specific requirements. A statement of authorship, signed by all of the authors and listing individual contributions to the work, is included at the beginning of each chapter.

The thesis is based on the following publications (* indicates peer reviewed publications.)

Chapter 2. Kang, W., Niimi, J., Muhlack, R. A., Smith, P. A., & Bastian, S. E. P. (2019). Dynamic characterization of wine astringency profiles using modified progressive profiling. Food Research International, 120, 244-254.*

Chapter 3. Kang, W., Muhlack, R. A., Bindon, K. A., Smith, P. A., Niimi, J., & Bastian, S. E. P. (2019). Potato protein fining of phenolic compounds in red wine: A study of the kinetics and the impact of wine matrix components and physical factors. Molecules, 24 (24), 4578.*

Chapter 4. Kang, W., Bindon, K. A., Wang, X., Muhlack, R. A., Smith, P. A., Niimi, J.,& Bastian, S. E. P. (2020). Chemical and Sensory Impacts of Accentuated Cut Edges (ACE) Grape Must Polyphenol Extraction Technique on Shiraz Wines. Foods, 9 (8), 1027. *

During the candidature, PhD student Mr Kang wrote a first author research paper published in 2018. This paper examined the ability of four vegetable proteins (derived from rice, soy, pea,

and potato) as alternative fining agents to reduce perceived wine astringency relative to traditional fining agents (gelatin and polyvinylpolypyrrolidone [PVPP]). This paper, written using data from experiments of Mr Kang's Master of Viticulture and Oenology research project, is an additional peer-reviewed paper presented in the thesis as an appendix (American Journal of Enology and Viticulture. 69:1, 2018).

AWARDS

Mr Kang gained a full scholarship from the University of Adelaide (The Adelaide Graduate Research Scholarship) for the PhD candidature.

In addition, an Australian Grape and Wine Authority (AGWA, trading as Wine Australia) Supplementary Scholarship was awarded to support the research (AGW Ph1605). A Travel Bursary (WAT1710) from Wine Australia was awarded for the financial support of conferences and laboratory visits. Wine Australia invests in and manages research, development and extension on behalf of Australia's grape growers and winemakers and the Australian Government.

CONFERENCES

The eighth European Conference on Sensory and Consumer Research (EuroSense), August to September 2018, Verona, Italy.

Presented a poster entitled “Dynamic characterization of wine astringency profiles using modified progressive profiling”, and additionally visited wine research laboratories at the University of Verona and the University of Bordeaux.

12th Annual Post-graduate Symposium, The University of Adelaide School of Agriculture, Food and Wine, September 2018, Adelaide, Australia.

Presented a talk titled “Reduction of red wine astringency perception using vegetable protein fining agents”

The CRUSH 2018 -- the grape and wine science symposium, September 2018, Adelaide, Australia.

Presented a talk titled “Reduction of red wine astringency perception using vegetable protein fining agents”

17th Australian Wine Industry Technical Conference, July 2019, Adelaide, Australia.

Presented a poster titled “Dynamic characterization of wine astringency profiles using modified progressive profiling”

PANEL OF SUPERVISORS

Principal Supervisor:

Associate Professor Susan Bastian

School of Agriculture, Food and Wine

The University of Adelaide

Co-Supervisors:

Dr Richard Muhlack

School of Agriculture, Food and Wine

The University of Adelaide

External Co-Supervisors:

Dr Paul Smith

Wine Australia

Dr Keren Bindon

The Australian Wine Research Institute (AWRI)

Independent adviser:

Dr Jun Niimi

Institut für Molekulare Biowissenschaften

Goethe University Frankfurt

ACKNOWLEDGEMENTS

“If I have seen further, it is by standing on ye shoulders of giants.”

- Letter from Sir Isaac Newton to Robert Hooke, 1675

To the giants…

Susan E.P. Bastian, Richard A. Muhlack, Paul A. Smith, Keren A. Bindon, Jun Niimi

Thank you!

This project would not have been possible without the financial support of the University of Adelaide and Wine Australia, thank you.

I would like to take this opportunity to acknowledge my parents, Junyong Kang and Caixia Deng, for their unconditional love and support. A special thanks goes to my fiancée, Peiyu Liu, you are always the motivation that drives me to go further.

CHAPTER 1

Literature Review

This literature review was predominantly prepared within the first 6 months of candidature and updated in March 2020. It mainly covers the literature up to April 2017 but has been updated to include some more recent references. The relevant literature beyond this review has been included in the introduction sections of the publications covered in Chapters 2 to 5.

1.1 Astringency

1.1.1 Perception of astringency

Astringency is a tactile (touch) sensation that occurs on the mouth surfaces (Breslin et al. 1993; Lyman and Green 1990) and refers to the drying, roughing and puckering perceptions felt in the oral cavity (Gawel 1998; Lee and Lawless 1991; Vidal et al. 2015). This sensation is perceived in numerous food and beverage products such as unripe banana, tea and wine, which is generally caused by the plant based polyphenols (Hanlin et al. 2010; Hayashi et al. 2013). The astringency sensation influences the consumers' preference and choice of food and beverage products, therefore it is vital to comprehend the perception of astringency.

The generally accepted mechanism of astringency perception is primarily due to the lack of lubrication of the oral epithelium (Bennick 2002; Kallithraka et al. 2001). This is caused by the interactions of astringent stimuli (e.g. polyphenols etc.) with salivary proteins, such as proline-rich proteins (PRP), mucins and histatins as well as the subsequent precipitation of these complexes (Jöbstl et al. 2004; Mehansho et al. 1987; Poncet-Legrand et al. 2007; Scollary et al. 2012). There are three interaction stages proposed for this mechanism: initially, weak and reversible hydrophobic interactions occur between PRP and astringent stimuli; followed by hydrogen bonding; and finally the formation of cross-links between multiple protein-astringent stimuli complexes (Figure 1). Although it has been reported that ionic and covalent bonds may impact on the interaction process, they are not considered as a main driver (de Freitas and Mateus 2012).

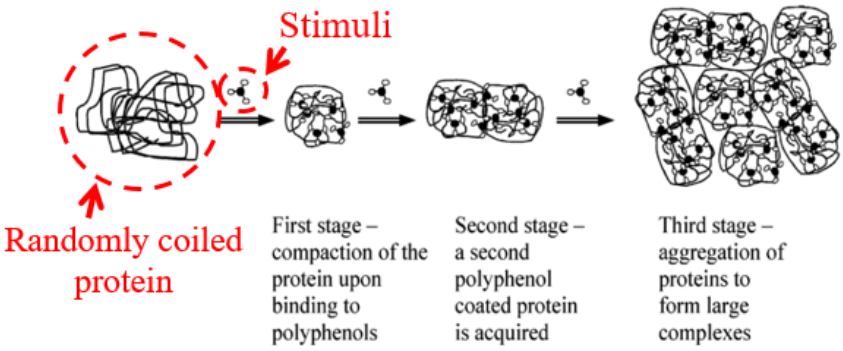


Figure 1 Binding model between original random coiled PRP and astringent stimuli (polyphenols) adapted from Jöbstl et al. (2004).

However, recently, other mechanisms of astringency perception have been reported. For instance, Gibbins and Carpenter (2013) proposed that the astringency sensation is possibly caused by 'free' stimuli or soluble protein-stimuli complexes depleting the protective and lubricating properties of salivary film and eventually binding to the saliva pellicle or even to the exposed receptors, for example, hTAS2Rs, the human bitter receptors (Narukawa et al. 2011) or trigeminal G protein–coupled receptor (Schöbel et al. 2014). Other astringency perception mechanisms postulated are through the binding of wine phenols and cell membranes in the oral cavity, because a strong interaction was found between monomeric flavan-3-ols and lipid liposomes (Furlan et al. 2014; Kaneda et al. 2002; Payne et al. 2009). However, the mechanism for astringency perception is not yet fully understood, and therefore requires further research.

Astringency sensations are not only confined to simple intensity. That is to say, even at the same astringency level, the astringent sub-qualities (texture or mouthfeel) can be different (Vidal et al. 2016). Thus, astringency level alone is insufficient to fully estimate the complicated wine astringency sensation. Although a number of astringent sub-qualities have been characterised and suggested to describe wine mouthfeel, such as drying, velvety,

puckering, dusty, adhesive and chamois etc. (Ferrer-Gallego et al. 2014; Gawel et al. 2001; Gawel et al. 2000; Pickering and Demiglio 2008; S Vidal et al. 2004), the mechanism of astringent sub-quality perception is likely to be intricate and is not understood.

1.1.2 Astringent stimuli and factors influencing astringency perception in wine

Astringency perception is generated by several stimuli: various phenolic components (Kallithraka et al. 1997), multivalent salts like alum (Peleg et al. 1998), organic acids (Rubico and McDaniel 1992; S. Vidal et al. 2004), and charged polysaccharides such as chitosan (Rodriguez et al. 2003). In wine, it is widely accepted that a strong and positive relationship exists between astringency intensity and concentration of phenolic components. Grape-derived tannins (condensed tannins or proanthocyanidins), polymeric pigments, flavonols and possibly flavan-3-ols cause astringent sensations and these structures are covered in detail in section 1.3. A small percentage of oak-derived tannins (hydrolysable tannins) can be released during treatment of wine with oak barrels or chips (Cliff et al. 2012; Sarneckis et al. 2006). However, these oak tannins are unlikely to contribute to astringency alone due to the presence of such small amounts (Pocock et al. 1994), but also because they are hydrolysed readily at wine pH (Puech et al. 1999).

During wine consumption, the intensity of the astringent sensation may be influenced by a number of factors. The intensity can increase due to lower levels of pH, alcohol (Demiglio and Pickering 2008; Fontoin et al. 2008; Payne et al. 2009), viscosity and sweetness (Courregelongue et al. 1999; Guinard et al. 1986; Lyman and Green 1990; Payne et al. 2009; Peleg and Noble 1999a; Smith et al. 1996); or due to higher temperatures, acidity, number of repeated exposures and longer resonance time on the palate (Lee and Lawless 1991; Lyman and Green 1990; Payne et al. 2009; Valentová et al. 2002). Although certain polysaccharides are astringent stimuli (Rodriguez et al. 2003), some have been shown to clearly reduce the intensity of perceived astringency (Chong et al. 2019; Quijada-Morín et al.

2014). These polysaccharides are released from the grape itself or are yeast-derived. For instance, polysaccharides rich in arabinose and galactose (PRAG, including arabinogalactan proteins (AGP) and arabinans) and rhamnogalacturonans II (RG-II) stem from grape berry cell walls; whilst mannoproteins (MP) are derived from yeast (Quijada-Morín et al. 2014). In addition, physiological factors affect the intensity of astringency perception as well. The intensity can increase due to increased whole saliva protein concentration (Naczk et al. 1996), but can reduce owing to higher salivary flow rate (Fischer et al. 1994), saliva volume (Nayak and Carpenter 2008) and haze developing capacity (Condelli et al. 2006). Some classes of salivary proteins e.g. PRPs are precipitated by tannins and alum, but not by acid. Meanwhile acid and alum precipitate mucins, but tannins do not (Lee et al. 2012). An individual tasters' sensitivity to 6-n-propylthiouracil (PROP status) (Melis et al. 2017; Pickering et al. 2004) has similarly been demonstrated to influence the intensity perception of the astringent sensation.

In terms of perceived astringency sub-qualities, the chemical structure of the phenolic components is believed to be one of the most important factors (see 1.3). pH, ethanol levels (DeMiglio et al. 2002) and physiological factors (Pickering and Robert 2006) also demonstrate impacts on sub-quality perception. In addition, it has been proposed that polysaccharides (such as AGP, RG and MP) provide a 'fullness' sensation to wine (Li et al. 2017; S. Vidal et al. 2004), and hydroxycinnamic acids (e.g. (E)-caftaric acid) provide a 'puckering' sensation (Hufnagel and Hofmann 2008a).

Overall, wine is a complex matrix and the mechanism of astringency sensation is not yet fully understood (in terms of either intensity or sub-quality). Therefore, there is room for further, albeit challenging, research to determine the stimuli and influencing factors responsible for wine astringency perception.

1.1.3 Astringent phenolic compounds in wine

A range of classes of flavonoid compounds are found in grape and wine. Four classes have been proved to have an effect on astringency sensation including flavan-3-ols, condensed tannins (these are flavan-3-ols but polymeric), polymeric pigments, and flavonols (Figure 2).

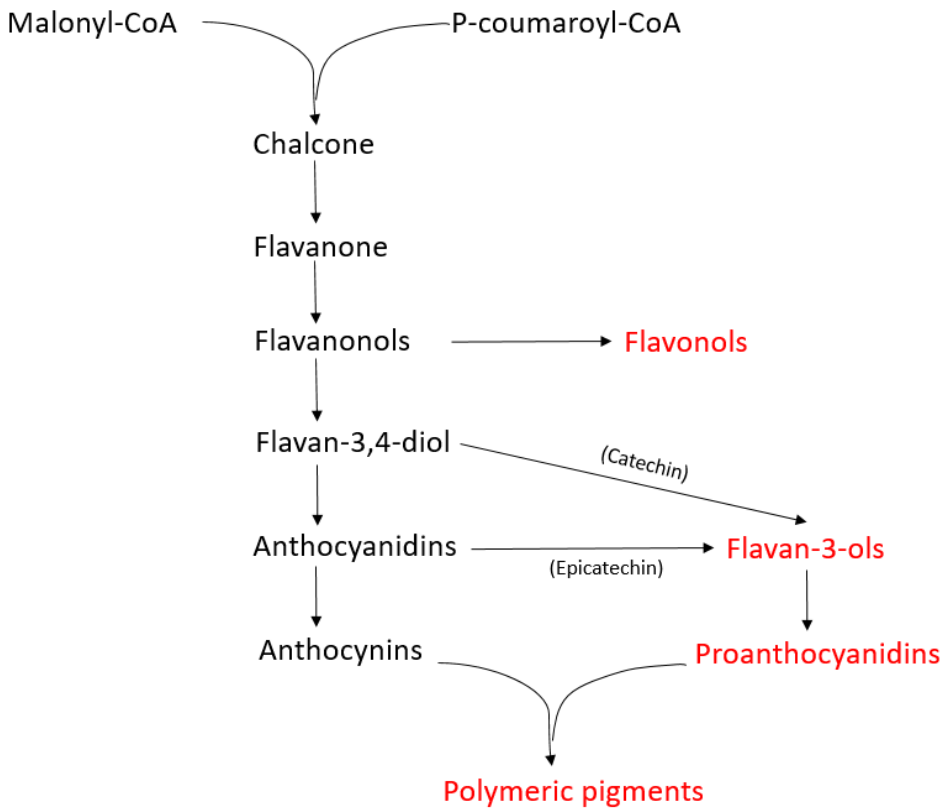


Figure 2 The biochemical pathway to the major flavonoids found in grape and wine.

Firstly, within the class of flavan-3-ols, four monomeric units (catechin, epicatechin, epigallocatechin and epicatechin-gallate) are found in *V. vinifera* grapes and their wines (Adams 2006; Czochanska et al. 1980; Souquet et al. 2000; Su and Singleton 1969) (Figure 3). In grape berries, there are differences between flavan-3-ol content between skins and seeds. Skins contain all four flavan-3-ol types, whereas seeds lack epigallocatechin.

Furthermore, seeds comprise the majority of a berry's total flavan-3-ol content, e.g. a maximum of 96% of Cabernet Sauvignon flavan-3-ols are reportedly derived from seeds (Casassa and Harbertson 2014; Cheynier 2005; Escribano-Bailón et al. 1995; Guerrero et al. 2009). In grape stems, all four types of flavan-3-ols have been identified, but they contain a higher quantity of catechin than other berry parts (Souquet et al. 2000).

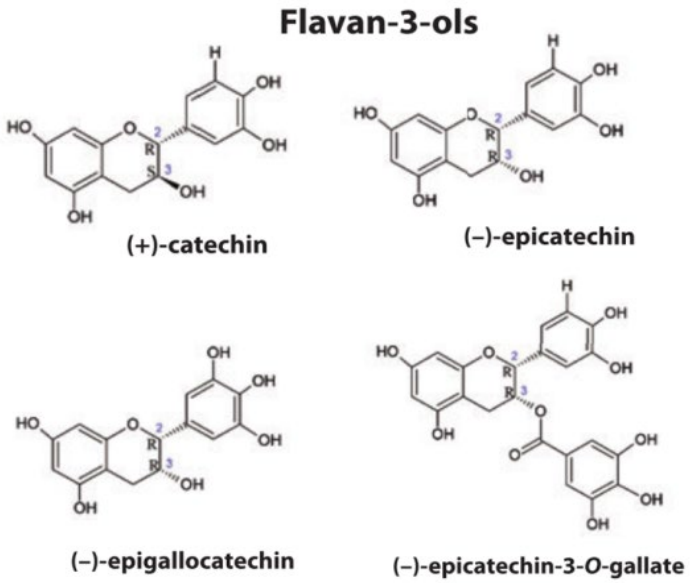


Figure 3 Structure of four monomeric units of flavan-3-ols: catechin, epicatechin, epigallocatechin and epicatechin-gallate (Casassa and Harbertson 2014).

Flavan-3-ols in grape are released into juice or must during the maceration step of wine making. Generally, the extraction rates of these compounds are faster from skins than seeds, but 90% of the total seed flavan-3-ols can be released in 2~3 weeks' maceration (González-Manzano et al. 2004; González-Manzano et al. 2006; Hernández-Jiménez et al. 2011; Koyama et al. 2007).

Flavan-3-ol compounds not only elicit the perception of astringency, but can also give rise to bitterness (Thorngate and Noble 1995). The duration and intensity of bitterness varies

depending on the compound type; for example, epicatechin is longer lasting and more intense than catechin at equal concentrations (Kallithraka et al. 1997; Macheix and Fleuriet 1990; Peleg et al. 1999; Thorngate and Noble 1995).

However, Scollary and co-workers (2012) reported that flavan-3-ols may be able to form colloidal particles at high concentrations (1200 mg/L), which can interact with and precipitate salivary PRPs readily. Nevertheless, 1200 mg/L is much higher than the threshold of flavan-3-ol bitterness perception (270 to 290 mg/L) (Hufnagel and Hofmann 2008b). Thus, compared with the well-established contribution to bitterness, the role of flavan-3-ols on astringency remains to be explored in wine matrix conditions (Casassa and Harbertson 2014).

Secondly, condensed tannins correspondingly contribute to the astringency sensation, which are oligomeric and polymeric proanthocyanidins (PAs) (2-5 and > 5 polymer subunits, respectively). These are the most abundant class of soluble, polyphenolic compounds in grape berries. PAs are located in the skin hypodermal layers, pulp and the soft parenchyma of the seed between the cuticle and the hard seed coat (Adams 2006; Bindon et al. 2017). The differences between skin, seed and stem tannins are shown in Table 1 (Cheynier 2005; Downey et al. 2003; Escribano-Bailón et al. 1995; Herderich and Smith 2005; Souquet et al. 2000).

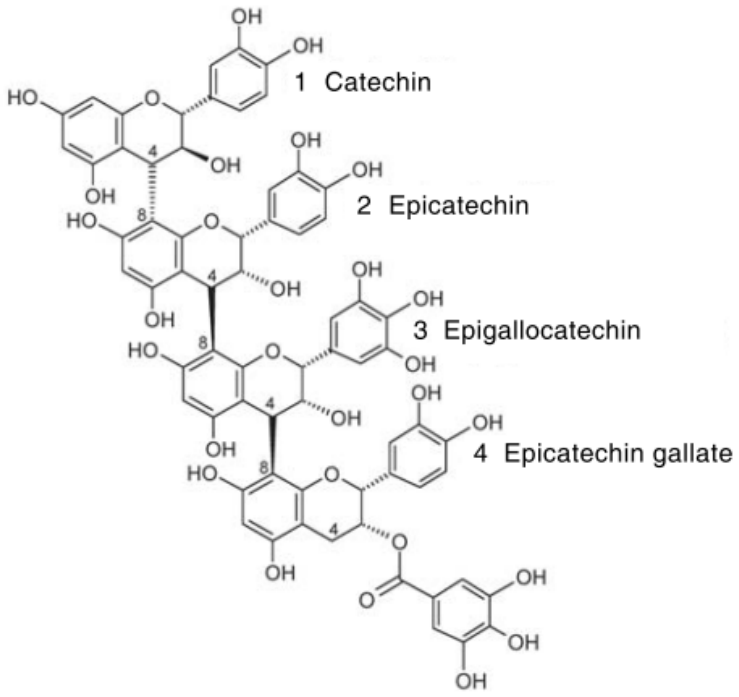


Figure 4 Structure of a hypothetical proanthocyanidin (condensed tannin) comprising of four flavan-3-ol monomers: catechin, epicatechin, epigallocatechin and epicatechin-gallate (Adams 2006).

Table 1 Differences between skin, seed and stem condensed tannins.

	Average range of polymerisation	**Subunits**
Skin	20 to 40+	All types, but less galloylated subunits
Seed	5 to 20	Lack of epigallocatechin
Stem	Up to 27	All types, mainly consisting of epicatechin

These tannins are considered to be primarily responsible for the sensation of astringency in wine (Gawel 1998; McRae et al. 2013; Sun et al. 2013). From their observations of phenolics in a series of aged wines, McRae et al. (2012) concluded that

neither astringency intensity nor mouthfeel are simply due to the tannin concentration in wine. In fact, the degree of polymerisation also affects astringency, where higher polymerisation leads to higher astringency intensity (Noble 1994). Additionally, the percentage of galloylation can also influence the astringency intensity (S. Vidal et al. 2004). It is thought that the galloyl aromatic ring enhances the hydrophobic interactions with the PRPs' proline ring, thus leading to further precipitation of salivary proteins (Charlton et al. 2002; Zhu et al. 1997).

Thirdly, polymeric pigments, this category are a combination of anthocyanin and PAs (Figure 5). In wine, the formation of polymeric pigments increases as a function of wine aging (Casassa et al. 2013; González-Neves et al. 2012; Harbertson et al. 2009; Sipiora and Granda 1998).

Polymeric pigments contribute less to astringency sensations than PAs, even at similar molecular sizes (Somers 1971; S Vidal et al. 2004). It is thought that the higher degree of hydrophilicity of polymeric pigments results in a reduced ability to interact with salivary PRPs, thereby limiting the perception of astringency (McRae and Kennedy 2011).

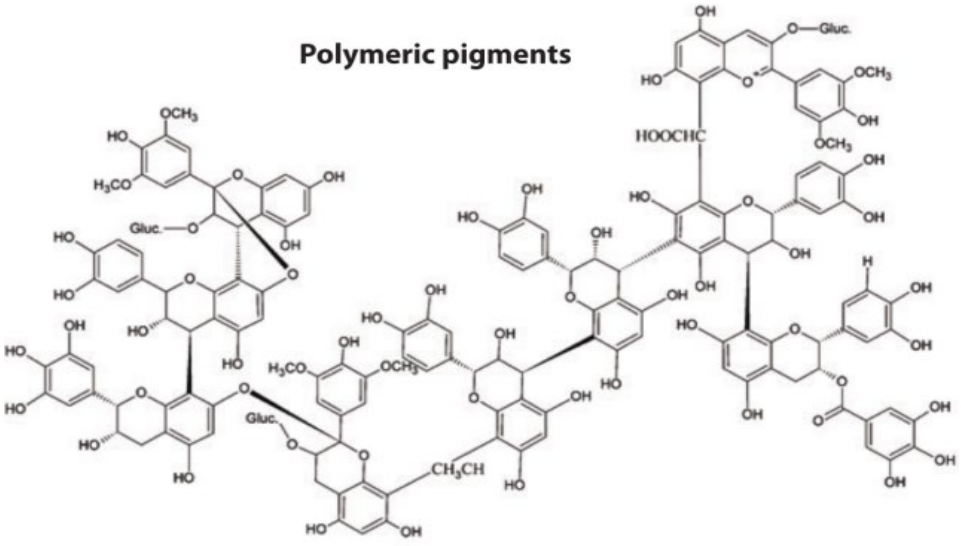


Figure 5 Structure of hypothetical polymeric pigments (Casassa and Harbertson 2014).

Fourthly, a further group of flavonol compounds is relevant to the perception of astringency, which are considered yellow pigments (Price et al. 1995). These compounds are derived from grape skins (Cheynier and Rigaud 1986) and stems (Souquet et al. 2000), and their level of accumulation in berries depends upon light exposure (Downey et al. 2004; Price et al. 1995; Spayd et al. 2002). There are six types of flavonols that are found in *V. vinifera* grapes, which are differentiated by their functional groups: myricetin, quercetin, kaempferol, laricitrin, syringetin and isorhamnetin (Figure 6). In grape berries, only glycosidically bound flavonols with one of three units; either glucosides, galactosides or glucuronides, are found. By contrast, in wines up to 21 types of flavonols have been reported (Castillo-Muñoz et al. 2007; Flamini and Traldi 2010). This is because free flavonol aglycones can be released by the hydrolysis of glycosidic bonds by enzymes or under acidic conditions (Burns et al. 2001; Castillo-Muñoz et al. 2007; Jeffery et al. 2008b).

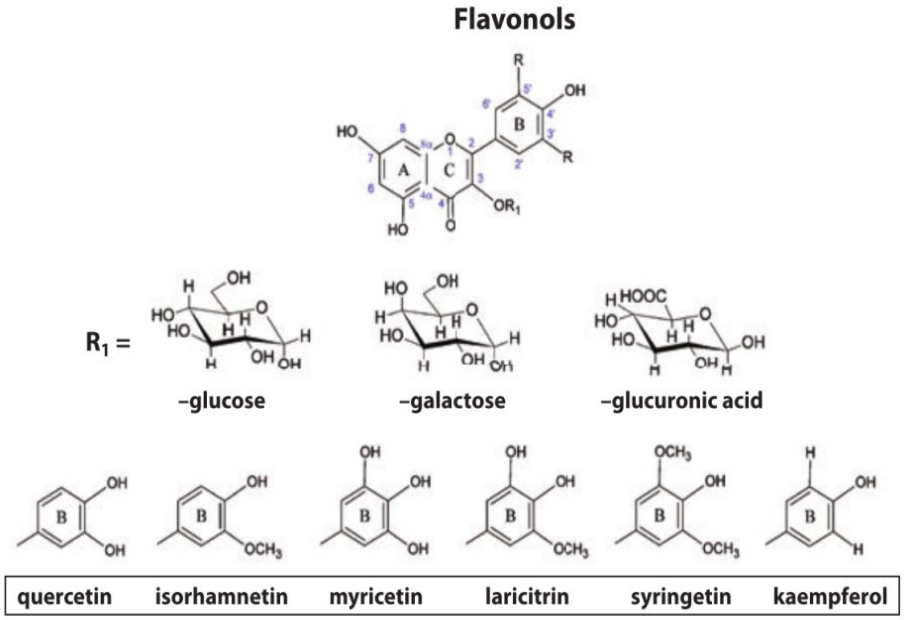


Figure 6 Structure of flavonols (Casassa and Harbertson 2014).

According to Hufnagel and Hofmann (2008a) and Sáenz-Navajas et al. (2010), the presence of quercetin and myricetin glycosides in wine is thought to impart bitterness. The authors also found that syringetin-3-glucoside and quercetin-3-glucoside give rise to velvety astringency (Hufnagel and Hofmann 2008b).

Overall, the content of these four classes of phenolic compounds can impact on the intensity and subtly discriminating sub-qualities of wine astringency, even though the smaller sized compounds are generally considered as mainly contributing to bitterness in and browning of wine (Margalit 2012; Zoecklein et al. 1995). The detection sensory thresholds of some of these compounds have been tested according to mean values of the three-alternative forced-choice test and the half-tongue test (Glabasnia and Hofmann 2006; Scharbert et al. 2004; Stark et al. 2005). However, the impact of interactions among these phenolic compounds on threshold values requires further research. As for the perception of astringent sub-qualities, little is known regarding the relationships between these and the content of phenolic compounds (specific fractions and/or overall concentration).

1.2 Methods for measuring astringency perception or the astringent components

1.2.1 Sensory evaluation methods

Sensory evaluation is a scientific discipline using the senses of human subjects as instruments to evaluate attributes of everything used and consumed (Meilgaard et al. 2006). Astringency is perceived by the sense of touch (Breslin et al. 1993), thus the use of sensory evaluation is appropriate to determine human's perception of this sensation. Sensory evaluation consists of different types of test methods (such as discrimination, descriptive, and affective), and a variety of sensory methods have been utilised in the study of the astringency sensation.

Discrimination testing (e.g. triangle test or duo-trio test) is a simple and direct method, and it can quickly identify any perceived astringency differences between samples (Piggott et al. 1998). However, discrimination testing cannot assess how much of a difference in perception of astringency between samples exists. Thus, scales are used to objectively quantify astringency intensities, be it through categorical or line intensity scales (Peleg and Noble 1999b). A limitation of intensity rating is that it only captures an intensity that is time averaged despite astringency perception being dynamic, that is, it changes over time (Guinard et al. 1986; Lawless and Heymann 2010). Astringency sensations can gradually increase after repeated ingestion and the perception can last up to six minutes after expectoration or swallowing (Ishikawa and Noble 1995). To monitor the change in astringency intensity with time, Time Intensity (TI) testing has been used (Lee and Lawless 1991). The difficulty with a TI test however, is that TI is a continuous measurement over time and only allows intensity measurements of a maximum of two attributes at the same time (Castura et al. 2016; Duizer et al. 1996) and if the measurement of more than two attributes are required, each sample requires several rounds of evaluation.

Several methods exist to study astringent sub-qualities, such as classical Descriptive Analyses (DA), Temporal Dominance of Sensations (TDS) and Temporal Check-All-That-Apply (TCATA). DA is capable of acquiring detailed sensory descriptions of products to define the nature and magnitude of any sensory differences, in order to identify potential ingredient and process variables, and/or to ascertain which sensory attributes are significant to acceptance (Lawless and Heymann 2010). DAs are sophisticated methods but are very expensive and time consuming. A generic DA panel usually consists of 8 to 12 assessors, and the training of the assessors is the core component for this method. They use a range of reference standards determined by panel member consensus during training to define sensory attributes that differ within a product set. Reference standards are utilised, therefore, a panel can unambiguously

understand and agree on the meaning of the sensory attributes used to describe any differences between products tested. A quantitative scale is used for panelists to rate the intensity of each attribute, which ensures the data can be statistically analysed (Lawless and Heymann 2010). Multiple tastes of the samples are required, so this method is fatiguing particularly if assessors are evaluating astringent samples.

The perception of astringency sub-qualities are dynamic as well. TDS, a method that can measure the dominance of attributes as a function of time (Pineau et al. 2009), has been recently used in the study of astringent sub-qualities of wines (Vidal et al. 2016). In the TDS method, the assessors are provided a list of attributes (e.g. astringent sub-qualities), and are asked to select which attribute dominates their perception at each moment of the evaluation. Vidal et al. (2016) found that Tannat (*V. vinifera*) wines aged in oak barrels were associated with the dominance of the sub-qualities 'rough' and 'mouth coating' by TDS. In addition, the combined use of TDS and scaling (a 10-point line scale for rating maximum astringency intensity) in their work has indicated that wines with similar astringency intensity possessed different temporal astringent sub-qualities, and that there was a significant and negative correlation between the average highest astringency intensity and the dominance of 'velvety'.

Another method that has the capacity to capture the temporal sensory profile of products is called Temporal Check-All-That-Apply (TCATA). TCATA is a temporal extension of Check-All-That-Apply (CATA), which allows continuous checking and unchecking of relevant attributes from a list of attributes (Castura et al. 2016). TCATA and TDS are similar but they differ in that TDS involves choosing a single and dominant attribute at each moment of the evaluation, while TCATA allows selection of multiple attributes. TCATA has been applied for the study of wine astringency perception (Kemp et al. 2019), and the results indicated that TCATA is a reliable technique to discriminate red wines based on the mouthfeel and texture

profiles during consumption. Furthermore, it is important to note that the method of TDS or TCATA alone are qualitative only and capture the frequency of a chosen attribute/s. The intensity of the attributes within the samples as a function of time are not measured.

The methods mentioned above are usually conducted using experienced assessors/trained panels, which means they require assessor training sessions. In training, several materials have been utilised as astringent reference standards. In terms of intensity rating standards, different concentrations of tannin extract, tannic acid and alum solutions are commonly used (Antúnez et al. 2017; Chira and Teissedre 2015; Fleming et al. 2015). In terms of sub-quality, the reference standards used in previous articles are shown in table 2 (Antúnez et al. 2017; Pickering and Demiglio 2008; Pickering and Robert 2006; Vidal et al. 2016). Nevertheless, reference standards for astringent sub-qualities are not sufficient to cover all sub-qualities nor universal and they have largely relied upon the manual touching of cloth materials by hand rather than orally evaluated standards.

Table 2 Materials previously used as reference standards for astringent sub-qualities

Astringent sub-quality	**Materials**
Drying	Black tea
Velvety	Velvet
Silky	Silk
Satin	Satin
Suede	Suede
Chamois	Chamois
Puckering	Alum solution
Rough	Grape skin and seed extract solution/felt
Coarse	Fine sand
Mouth coating	Banana peel
Harsh	Green banana

Additionally, three consumer-based methods have recently been reported, CATA, projective mapping (PM) and polarised sensory positioning (PSP) (Antúnez et al. 2017; Fleming et al. 2015). Although these three methods were not being used to specifically focus on astringent sensations, astringency (intensity) was measured in their studies. Using naïve consumers instead of experts can save training time for the study of astringent sensations, yet as they are untrained, it is highly probable that larger numbers of people would be required. Unfortunately, the majority of consumers found it difficult to describe different sub-qualities of astringency perception (Vidal et al. 2015). Hence, using consumer-based methods to measure astringent sub-quality remains to be addressed in future. Furthermore, as astringent compounds tend to associate with bitterness sensations (Fleming et al. 2016), bitterness versus astringency perception in wines may confuse consumers to some extent (Vidal et al. 2015). A new methodology based on preselection of 'bitter blind' consumers could be developed in the future to achieve a better experimental result.

1.2.2 Instrumental analysis methods

Although crucial to advancing our understanding of astringency perception, sensory evaluation methods based on both experts and consumers are expensive to conduct and assessors of sensory analyses are variable among themselves and over time, and likely to bias results unless well trained (Meilgaard et al. 2006). Given the complexity of wine phenolic chemistry and the possible matrix interactions, wine astringency and its sub-qualities are likely to be much easier to detect in general by individuals, than define in a logical and reproducible way. As such, defining wine's astringency and mouthfeel by chemical measures of wine polyphenolic composition alone will never be more than partially successful. There are various different phenolic compounds in wines, but it is not easy to separate them from each other, and wine phenolic compounds are difficult to analyse because they are modified by exposure to

oxygen. Therefore, a combination of sensory and chemical analyses is probably the most appropriate and powerful way to conduct astringency research. Thus, a number of chemical assays and methods have been developed. The majority of chemical methods measure either phenolic content in wine, or the extent to which phenolics may combine with and/or precipitate proteins. The results from those measurements have been aligned with sensory findings in an attempt to predict astringency perception.

In terms of measurement of phenolic content, tannin concentration is the most common. Tannins are usually quantified by Methyl Cellulose Precipitable (MCP) assay (Mercurio and Smith 2006), Bovine Serum Albumin (BSA) precipitation (Hagerman and Butler 1978), Bate-Smith assay (Bate-Smith 1973), Vanillin assay (Price et al. 1978) or High Performance Liquid Chromatography (HPLC) (Peng et al. 2001) etc. After quantification of total tannin concentration, tannins may be extracted from wines by different selective adsorption chromatography methods achieved by different materials such as Solid Phase Extraction (SPE) cartridges (Jeffery et al. 2008a; Oszmianski et al. 1988), Toyopearl (Vidal et al. 2003) or Sephadex (Kantz and Singleton 1991) etc. Extracted tannins may then be fractionated to separate them from each other or directly analysed for other attributes. For example, the mean degree of polymerization (mDP, mean average length of the tannin chains) and by inference the molecular weight (MW) of tannins can be tested by HPLC phloroglucinolysis (Kennedy and Jones 2001) or Nuclear Magnetic Resonance spectroscopy (NMR) (Guyot et al. 1999). The MW can also be determined by Gel Permeation Chromatography (GPC) (Kennedy and Taylor 2003). The relative proportion of the different sub-units in tannins can be measured by HPLC phloroglucinolysis (Kennedy and Jones 2001), NMR (Hagerman et al. 1997) and Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Pasch et al. 2001). The NMR can detect the binding sites (4-6 or 4-8) between subunits, and the diameter of tannin particles analysed by Nano-particle Tracking analysis (NTA) (Bindon et

al. 2016). In addition, the Somers assay is often used to detect other phenolic attributes such as total phenolics etc. (Somers and Evans 1974, 1977).

In terms of examination of polyphenol and salivary protein interactions, Saliva Precipitation Index (SPI) and Astringency Mucin Index (AMI) are two indices which forecast astringency intensity measured by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and turbidity change, respectively (Gambuti et al. 2006; Monteleone et al. 2004). In addition to this, Payne et al. (2009) developed a method using 4-(dimethylamino) cinnamaldehyde (DMACA) to measure the binding of procyanidins to monolayers of oral epithelial cells. DMACA reacts with catechins and procyanidins to form a blue-green product that can be quantified according to absorbance measurement at 620-640 nm (Treutter 1989). Thus, the principle of this method is, to incubate the procyanidins and monolayers for a period to allow the molecules to react, and then remove any unbound material by washing with phosphate-buffered saline (PBS), and finally measure the absorbance to obtain the combined quantity.

At present, instrumental analysis methods were primarily utilised to predict overall astringency intensity. To date, there is a lack of understanding about the relationships between specific phenolic fractions/compounds and their respective astringent sub-qualities. As such, limited sensory studies have examined astringent sub-quality perception.

1.3 Wine astringency modification

Astringency is considered one of the most important factors driving wine quality (Peynaud and Blouin 1996), thus a large number of wine making techniques are applied to obtain optimal wine astringency levels (Smith et al. 2015). Too much astringency may render

the wine difficult to drink, whilst too little may make the wine insipid, i.e. lacking good structure and lower in complexity. One way to manipulate astringency in wine is to alter the maceration process (which is a process of soaking crushed grapes to extract colour, aroma and/or phenolic components into wine), ultimately affecting the extent or rate of transfer of tannins from the cap to the must/wine. This could involve pre-ferment, cold maceration, which extracts sufficient colour and flavour into wine but minimises the extraction of large-size tannins (Heredia et al. 2010; Parenti et al. 2004). This minimisation of large-size tannins can achieve less astringency intensity in wine, because higher polymerisation leads to higher astringency intensity (McRae et al. 2013). Alternatively, extended, post-ferment, and Accentuated-Cut-Edges (ACE) maceration can increase tannin extraction into wine (Smith et al. 2015; Sparrow et al. 2016). When astringency is too low, winemakers might even simply add grape tannin extract (e.g. grape seed extract, GSE) to increase the astringency level. Conversely, if the wine astringency is unacceptably high, processes such as ageing or micro-oxygenation can be used to decrease or 'soften' the astringency, which is now generally believed to be due to precipitation of large size tannins (Schmidtke et al. 2011). However, the ageing process can take long periods, and despite being faster, micro-oxygenation is high in capital investment. Thus, a convenient method widely utilised by the wine industry to modify astringency is a process known as fining (see 3.1 and 3.2). Fining involves an addition of agents in order to reduce unwanted components in wine such as too many astringent phenolics (Rankine 2007). Fining can reduce the astringency intensity but also might modify the astringent sub-quality as well.

1.3.1 Traditional fining agents for the modification of wine astringency

Wine astringent phenolic components are traditionally removed by protein or "protein-like" fining agents (proteinaceous and non-proteinaceous) because of their propensity for hydrophobic interactions and hydrogen-bonding (Figure 7) (Le Bourvellec and Renard 2012;

Margalit 2012; Scollary et al. 2012). Larger phenolic compounds with more available hydroxyl groups result in preferential bonding with proteins (Zoecklein et al. 1995), but the interactions are influenced by the characteristics of the fining agents (Maury et al. 2003; Sarni-Manchado et al. 1999). After binding, the conjugates of fining agents and astringent phenolic compounds precipitate resulting in a decrease in wine astringency.

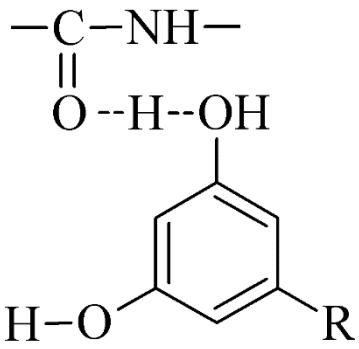


Figure 7 Structure of the H-bond where the ketone group of the fining agent shares a proton with the phenolic hydroxyl group.

Four traditional proteinaceous fining agents (Table 3) are commonly used in the wine industry; gelatin, egg albumen, isinglass and casein (Margalit 2012; Zoecklein et al. 1995). Although the efficacy of the fining agents are dependent on the internal conditions of wine (such as wine pH, temperature, polyphenol types etc.) and MW of the individual agent, overall, gelatin is the most efficient in tannin removal, followed by egg albumen, isinglass and casein (Cosme et al. 2009; Zoecklein et al. 1995).

An important consideration for the use of gelatin and egg albumen is their potential for residual amounts to remain soluble in the wine, which can leave undesirable precipitates later in finished wine (Rankine 2007). In such cases, these fining agents at times require a co-fining agent such as Kieselsol or hydrolysable tannins to remove the residual dissolved protein (Hahn and Possmann 1977). In comparison, isinglass and casein are insoluble in wine at wine pH and thus do not have this issue.

A traditional non-proteinaceous fining agent (Table 3) used in the wine industry, Polyvinyl-poly-pyrrolidone (PVPP), is a synthetic high MW polymer of Polyvinylpyrrolidone (PVP) (Margalit 2012).

Table 3 Traditional fining agents for the modification of wine astringency

	Agent	Source	Use for	Dose range	Characteristic	Reference
Proteinaceous agents	Gelatin	Skin and bone of animals	Both white and red wine varieties	25-50 mg/L for white, 50-100 mg/L for red and 50-200 mg/L for pressed red wines	Minimal effect on the colour of young red wines	Margalit 2004; Stankovic et al. 2012; Somers and Verette 1988; Zoecklein et al. 1995
	Egg albumen	Egg	Red wines	1-2 eggs per 225L barrel	Offers a gentler alternative to gelatin and tends to retain fruit character in wine	Zoecklein et al. 1995
	Casein	Milk	White and fortified wines	50-250 mg/L	Lower in MW which may be a reason for its comparatively low efficiency compared to the other fining agent counterparts. It is also used to reduce flavour of white wines that are over-oaked or contain off-flavours	Margalit 2004; Rankine 2007; Zoecklein et al. 1995
	Isinglass	The swim bladder of sturgeon, can also be derived from skins and bones	White wines	10-50 mg/L	The lees of isinglass is less than that of other proteinaceous agents, which can decrease the loss of fining. However, a more expensive alternative and may give wines a fishy character.	Margalit 2004; Rankine 2007
Non-proteinaceous	Polyvinyl-poly-pyrrolidone (PVPP)	Polyvinylpyrroli done (PVP)	Red, white and fortified wines	Typical dosage rates used are 100-700 mg/L for white wines and 100-200 mg/L for red; however these rates are more specific for the removal of bitterness and browning. It can also reduce astringency but their dosage rates are less well reported.	Its rigid tertiary structure gives it low flexibility and limits interactions with large polyphenols, thus preferentially binding with low MW phenolic compounds. It has a high capacity to remove total and flavonoid phenols in wines. A main advantage of PVPP is its relative inertness against the removal of desirable wine aromas.	Baron et al. 1997; Laborde et al. 2006; Margalit 2004; Ribéreau-Gayon et al. 2006; Sims et al. 1995; Zoecklein et al. 1995

In summary, the exact amount of fining agent required to fine wines depend upon the type of wine, the type of fining agent, and the chemical environment of the wine amongst other considerations. These factors all affect the fining efficiency and require careful consideration by wine makers. Thus, it is common in the wine industry to conduct bench top trials before fining to determine the right amounts for each batch of wine. Yet at the same time, the effect of traditional agents on astringent sub-quality is not yet fully understood, and there are several limitations of traditional fining agents. One more recent challenge facing the wine industry is to cater for a wide range of consumer dietary requirements; and while some are by choice (e.g. vegetarians and vegans) others are by necessity (such as food allergies). Use of animal-based fining agents may deter vegetarian/vegan consumers. Any residual allergens in wine can be a potential risk for allergic reactions such as alpha S1-casein (Cases et al. 2011), although evidence of this is currently inconclusive (Weber et al. 2007). In addition, as consumers become more educated, they are becoming more concerned with the use of additives; consumers have been shown to prefer natural over synthetic additives in wines (Saltman et al. 2015). Moreover, some traditional fining agents are expensive; isinglass ($180/ kg from Laffort Australia, Australia, April 2020) is almost double the price of vegetable protein alternatives such as potato proteins ($86/ kg from Laffort Australia, Australia, April 2020). Finally, usage of traditional fining agents potentially results in wine quality reduction (e.g. colour, aroma or flavour aspects) (Margalit 2012; Sims et al. 1995; Stankovic et al. 2012; Voilley et al. 1990). The use of alternatives may be a solution to some of the issues outlined above.

1.3.2 Alternative fining agents for the modification of wine astringency

1.3.2.1 Vegetable proteins as alternative astringency fining agents

The Food Standards of Australia and New Zealand permitted the use of plant proteins as wine fining agents in 2004 (Food Standards Australia New Zealand 2004,

https://www.foodstandards.gov.au/publications/documents/FLM_Complete_report_Phase%201%20Report.pdf). Due to the modification of this production law, the number of studies investigating vegetable proteins as potential wine fining agent alternatives has increased. However, knowledge up to April 2017 was still limited to a small number of studies that had examined ten types of vegetable proteins in a small number of red or model wines (Table 4). The plant sources from which vegetable fining proteins have been derived for these studies include; corn/maize (Simonato et al. 2013; Simonato et al. 2009; Tschiersch et al. 2010; Tschiersch et al. 2008), potato (Gambuti et al. 2012; Tschiersch et al. 2010), grape seed (Vincenzi et al. 2013), wheat (Granato et al. 2014; Maury et al. 2003; Tschiersch et al. 2010), white lupin (Maury et al. 2003), rice (Tschiersch et al. 2010), pea (Cosme et al. 2012; Granato et al. 2014), lentil, soy (Granato et al. 2014) and sorghum (Hagerman and Butler 1980). All of these proteins were demonstrated to have the capacity to reduce phenolic content in wine. The reduction of phenolic compounds in wine however does not necessarily translate to reduction in perceived astringency. The only studies within the literature that have conducted astringency evaluation of wine after fining with vegetable based proteins were limited to corn, potato, grape seed and pea proteins (Cosme et al. 2012; Gambuti et al. 2012; Simonato et al. 2013; Vincenzi et al. 2013). Further, the relative astringency fining capabilities of vegetable proteins plus their comparison to traditional fining agents and their impact on colour and flavour have not been fully examined. Thus, a study was conducted in 2018 to compare the ability of alternative vegetable proteins (derived from rice, soy, pea and potato) to reduce astringent phenolic components and astringency intensity, relative to that of traditional fining agents (gelatin and PVPP) in a commercial wine with added grape seed extract (Kang et al. 2018). The chemical and sensory measures showed that rice and potato proteins have the potential to replace PVPP and gelatin, respectively.

Table 4 Summary of studies examining vegetable proteins as alternative astringency fining agents.

Vegetable agent	Raw material	Experimental media	Instrumental analysis	Sensory test?	Colour change?	Compared with traditional agent?
Zeins	**Corn gluten or flour**	**Wines** (Cabernet Sauvignon,Merlot,Valpolicella,Samtrot and Lemberger)	**Spectro-photometry**	√	**None**	Gelatin
Patatin P	**Potato**	**Wines** (Agliancia,Samtrot and Lemberger)	**HPLC+SPI**	√	**None**	Gelatin, egg albumin and casein
Grape seed proteins	**Grape seed flour**	**Wine** (Cabernet Sauvignon)	**HPLC+AMI**	√	**Unknown**	Gelatin
Pea proteins	**Pea**	**Wines** (Blend wine of 60% Trajadura and 40% Loureiro, and Catalanesca)	**Spectro-photometry+LC/MS +LC/ESI-MS**	√	**Yes**	PVPP
Wheat proteins	**Wheat gluten**	**Wines** (Syrah, Samtrot and Lemberger)	**HPLC**	×	**Yes**	Gelatin
Hydrolyzed rice proteins	**Commercial rice proteins**	**Wines** (Samtrot and Lemberger)	**HPLC**	×	**None**	**None**
Preparation of white lupin	**White lupin**	**Wine** (Syrah)	**HPLC**	×	**Unknown**	Gelatin
Lentil proteins	**Lentil**	**Wine** (Catalanesca)	**LC/MS+LC/ESI-MS**	×	**Unknown**	**None**
Soy proteins	**Soy**	**Wine** (Catalanesca)	**LC/MS+LC/ESI-MS**	×	**Unknown**	**None**
Sorghum proteins	**Sorghum**	**Model wines**	**Radioiodinated trace**	×	**Unknown**	**None**

Note: LC/MS is Liquid chromatography–mass, and ESI is Electrospray ionization.

Overall, the research progress of astringency reduction by vegetable agents is less advanced compared to clarification studies, possibly because turbidity is more easily measured (Cosme et al. 2012; Iturmendi et al. 2010; Marchal et al. 2002; Simonato et al. 2013; Simonato et al. 2009). Many vegetable proteins for astringency modification are still at a theoretical study stage speculated upon by chemical analysis, with only a limited number of agents having gone through rigorous sensory tests. Potato proteins have been used to fine wines just 6 months after fermentation. What happens in older wine is unclear. Thus, further research is required to screen potential vegetable proteins by both instrumental and sensory analyses, to verify the effectiveness of vegetable proteins at an industry level, and to understand the impacts of vegetable proteins on wine astringent sub-quality, aroma, flavour, and colour aspects. A fining approach where different vegetable proteins used in combination at the same time for astringency modification represents a possible future research direction.

1.3.2.2 Other fining agents to reduce astringency

The polysaccharides from grape cell walls of three different varieties (Syrah, Cabernet Sauvignon, and Monastrell; *V. vinifera*) have recently been studied as alternative astringency fining agents (Bautista-Ortin et al. 2015). Cell wall polysaccharides have hydroxyl groups and aromatic and glycosidic oxygen atoms, which have the capacity to have hydrophobic interactions and form hydrogen bonds with tannins (Figure 9) (Le Bourvellec et al. 2004). Using inherently derived materials from grapes as a fining agent does not require labelling declarations. Earlier, Adams and Scholz (2008) had used Cabernet Sauvignon in their experiments and found that grape berry cell wall material could bind approximately 70% of tannin in the grape berry. These findings mean that using grape cell walls as an alternative

astringency fining agent has good prospects, but a more efficient extraction method and the practical application of grape cell walls requires further research.

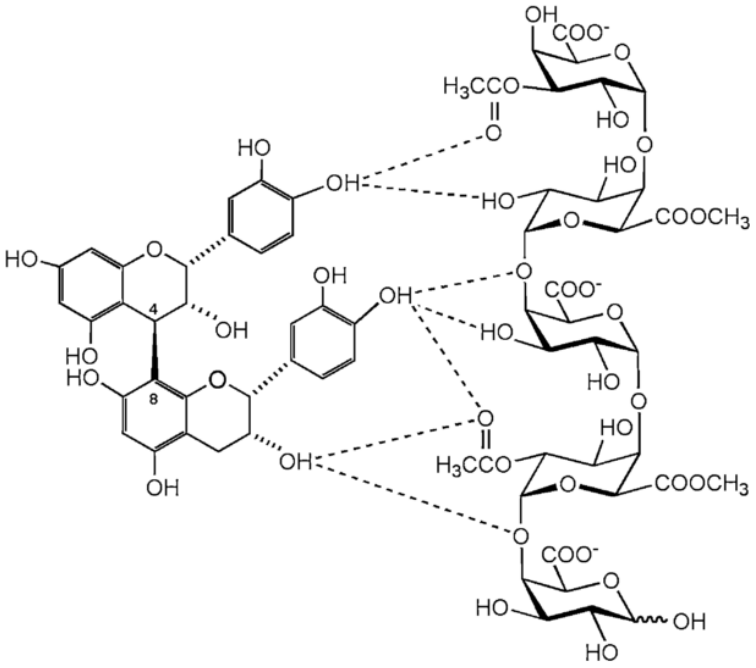


Figure 8 A schematic diagram to demonstrate the hydrogen bond (dashed lines) between hydroxyl groups of a tannin molecule and the oxygen atoms of the hydroxyl, acetyl groups, and glycosidic linkages of a polysaccharide (Hanlin et al. 2010).

Insoluble fibres (derived from apple and grape) can possibly act as alternative fining agents as well, since their capacity to adsorb red wine tannins has been proven (Guerrero et al. 2013). These fibres can simultaneously reduce total phenolics, anthocyanins as well as colour density of wine, but slightly change wine hue. Notably, grape fibres reduced both high and low MW phenolics, but apple fibre selectively decreased high MW phenolics (Guerrero et al. 2013).

Whey, a by-product of the cheese industry, has demonstrated its potential for astringency reduction by instrumental analyses. Jauregi et al. (2016) found that whey removes

polyphenols similarly to gelatin but removes less catechin. This research suggested that although whey interacts with tannins by hydrophobic interactions and hydrogen bonding, according to the results of fluorescence, size measurements and isothermal titration calorimetry (ITC), this interaction is weaker than that between gelatin and tannins.

Additional studies are required to assess alternatives from other sources too, such as ocean derived algal proteins. Since algal proteins consist of up to 10.5% proline (Fleurence 1999) compared to gelatin's approximately 30% proline content (Payne and Veis 1988), relative to other vegetable based proteins, algal proteins might possess a greater ability to remove those polyphenols which target salivary PRPs (Poncet-Legrand et al. 2007).

1.3.3 Alternative fining technologies to improve fining efficiency

There has been development of several alternative fining procedures using continuous processing to enhance time efficiency of fining for the wine industry. For example, Pashova et al. (2004) used a packed column (loaded with powder and pellets of zirconium oxides) to replace conventional bentonite fining. Verification of the efficiency of another method using in-line dosing with bentonite, followed by centrifugation is now widely used in the wine industry (Muhlack et al. 2006; Nordestgaard et al. 2007). However, these technologies have been utilised for protein stability management rather than astringency modification.

In terms of potential alternatives for wine astringency modification, an ultrafiltration process using polyethersulfone (PES)/PVP membranes could be applied (Borneman et al. 2001). This method has been established to reduce brown colour and polyphenols in apple juice. However, use of this method for wines still requires further research, especially its impact on aroma and flavour. In addition, in-line dosing with astringency fining agents (e.g.

PVPP) has been used in wineries, but simply for the industry convenience of mixing between wine and agent. These fining agents/wine mixtures still need to be stored in containers, and settled and racked later (the same as batch fining). Developing an alternative fining technology using in-line or a column system to modify wine astringency has research prospects. However, the suitable agents (i.e. the agents' adsorption of the target compound(s) must be reversible for column systems to be feasible), the kinetics of astringent phenolics removal as well as the overall total removal, engineering arrangements, and the most efficient separation method have to be elucidated beforehand. In addition, co-fining by mixtures of different proportions of conventional and/or alternative fining agents could be studied to develop updated techniques with improved fining efficiency.

Summary of research aims

The literature review identified several research gaps, such as little research into other/better methods to measure dynamic and quantitative perception of astringent sub-qualities, and poor understanding of compounds in the wine matrix that affect sub-quality perception. In addition, a lack of understanding of the effects of vegetable proteins on wine chemical parameters and sensory attributes, optimisation of vegetable proteins in a commercial sense, and novel methods to modify wine astringency would also need to be addressed.

Therefore, this project attempted to elucidate alternative sensory methodologies to face the challenge of measuring and to gain advanced knowledge of the human perception of the more nuanced sub-qualities of wine astringency. Furthermore, in the context of a global wine industry endeavouring to accommodate consumer expectations and needs for natural and non-animal based wine processing aids, a deeper understanding of the use of vegetable protein fining agents and alternative polyphenolic extraction techniques, and in turn modification of wine astringency, were required. To address some of these issues, the project had the following specific aims:

1. Develop methodologies for improved sensory assessment and measurement of wine astringency and astringent sub-quality perception.
2. Understand the drivers of different astringency ‘texture or mouthfeel’ qualities using chemistry and sensory evaluations.
3. Investigate alternative fining agents (natural & non-animal derived proteins) which have the capacity to modify wine astringency, and gain insights into how to optimise their use.
4. Examine a novel wine process technology for the modification of wine astringency and astringent sub-quality perception.

Aims 1 and 2: Develop methodologies for improved sensory assessment and measurement of wine astringency, and understand the drivers of different astringency 'texture or mouthfeel' sub-qualities using chemistry and sensory evaluations.

Aims 1 and 2 were addressed in Chapter 2. A modified progressive profiling method was investigated to examine wine astringency perception. The dynamic astringency profiles of 13 Australian commercial red wines and 2 rosés made from 11 grape varieties were evaluated by a trained sensory panel (n=8) using this method. Seven attributes (overall astringent intensity and 6 sub-qualities: pucker, mouth coat, dry, grippy, adhesive and graininess) generated and defined by the panel were scored across six different evaluation periods. Additionally, the wine composition and phenolic profiles were also determined to establish correlations between mouthfeel attributes and chemical measures. Details of this study can be found in the publication presented in Chapter 2.

Aim 3: Investigate alternative fining agents (natural & non-animal derived proteins) which have the capacity to modify wine astringency, and gain insights into how to optimise their use.

Potato proteins was investigated in our previous experiments that screened different vegetable proteins as alternative fining agents. The earlier chemical and sensory measurements indicated that potato proteins have the potential to replace gelatin. Therefore to meet aim 3, the alternative fining agent potato proteins was further studied. This study elucidated the time-dependent kinetics of fining with potato proteins for two unfined Cabernet Sauvignon wines at different fining doses. Insights into the steps required for the optimisation were also gained from this study by using a fractional factorial design (resolving three wine

matrix components and two physical factors). Details of this study can be found in the publication presented in Chapter 3.

Aim 4: Examine a novel wine process technology for the modification of wine astringency and astringent sub-quality perception.

Finally, aim 4 was addressed with the further examination of a new grape must processing technique (Accentuated Cut Edges, i.e. ACE) designed to extract phenolic compounds. This was investigated to examine the ability of ACE technique for modifying wine astringency and astringent sub-quality perception. This study was the first to elucidate the effect of the ACE technique on Shiraz wine non-volatile chemical compositions (basic wine compositions, colour, phenolic components and polysaccharides) and sensory profiles (by using rate-all-that-apply and modified progressive profiling). Additionally, any potential improvement provided by ACE for the pre-fermentative water addition to must was studied. In addition, two pilot commercial wines were produced by a winery at industry scale in the vintage of 2018 and analysed in 2019. It was a preliminary experiment investigating the feasibility of ACE grape extraction technique on Shiraz wines at an industry scale. Details of these studies can be found in the publication presented in Chapter 4 and 5.

Reference:

Adams, D., and R. Scholz. Tannins–the problem of extraction. *In* Proceedings of the Proceedings of the 13th Australian Wine Industry Technical Conference. 2008. pp. 160-164.

Adams, D.O. 2006. Phenolics and ripening in grape berries. Am. J. Enol. Viticult. 57:249-256.

Antúnez, L., L. Vidal, L. de Saldamando, A. Giménez, and G. Ares. 2017. Comparison of consumer-based methodologies for sensory characterization: Case study with four sample sets of powdered drinks. Food Qual. Prefer. 56:149-163.

Baron, R., M. Mayen, J. Merida, and M. Medina. 1997. Changes in phenolic compounds and colour in pale Sherry wines subjected to fining treatments. Z. Lebensm. Unters. F. A. 205:474-478.

Bate-Smith, E. 1973. Haemanalysis of tannins: the concept of relative astringency. Phytochemistry 12:907-912.

Bautista-Ortin, A.B., Y. Ruiz-Garcia, F. Marin, N. Molero, R. Apolinar-Valiente, and E. Gomez-Plaza. 2015. Remarkable proanthocyanidin adsorption properties of monastrell pomace cell wall material highlight its potential use as an alternative fining agent in red wine production. J. Agr. Food Chem. 63:620-33.

Bennick, A. 2002. Interaction of plant polyphenols with salivary proteins. Critical Reviews in Oral Biology & Medicine 13:184-196.

Bindon, K.A., A.L. Carew, A. Mierczynska-Vasilev, S. Kassara, F. Kerslake, and P.A. Smith. 2016. Characterization of macromolecular complexes in red wine: composition, molecular mass distribution and particle size. Food Chem. 199:838-846.

Bindon, K.A., S. Kassara, and P.A. Smith. 2017. Towards a model of grape tannin extraction under wine-like conditions: the role of suspended mesocarp material and anthocyanin concentration. Aust. J. Grape Wine Res. 23:22-32.

Borneman, Z., V. Gökmen, and H.H. Nijhuis. 2001. Selective removal of polyphenols and brown colour in apple juices using PES/PVP membranes in a single ultrafiltration process. Separation and Purification Technology 22:53-61.

Breslin, P.A.S., M.M. Gilmore, G.K. Beauchamp, and B.G. Green. 1993. Psychophysical evidence that oral astringency is a tactile sensation. Chemical Senses 18:405-417.

Burns, J., P.T. Gardner, D. Matthews, G.G. Duthie, J. Lean, and A. Crozier. 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J. Agr. Food Chem. 49:5797-5808.

Casassa, L.F., and J.F. Harbertson. 2014. Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. Annu. Rev. Food Sci. Technol. 5:83-109.

Casassa, L.F., R.C. Larsen, C.W. Beaver, M.S. Mireles, M. Keller, W.R. Riley, R. Smithyman, and J.F. Harbertson. 2013. Impact of extended maceration and regulated deficit irrigation (RDI) in Cabernet Sauvignon wines: characterization of proanthocyanidin distribution, anthocyanin extraction, and chromatic properties. J. Agr. Food Chem. 61:6446-6457.

Cases, B., C. Garcia-Ara, M. Boyano, M. Pérez-Gordo, M. Pedrosa, F. Vivanco, S. Quirce, and C. Pastor-Vargas. 2011. Phosphorylation reduces the allergenicity of cow casein in children with selective allergy to goat and sheep milk. J. Investig. Allergol. Clin. Immunol. 21:398-400.

Castillo-Muñoz, N., S. Gómez-Alonso, E. García-Romero, and I. Hermosín-Gutiérrez. 2007. Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. J. Agr. Food Chem. 55:992-1002.

Castura, J.C., L. Antúnez, A. Giménez, and G. Ares. 2016. Temporal Check-All-That-Apply (TCATA): A novel dynamic method for characterizing products. Food Qual. Prefer. 47:79-90.

Charlton, A.J., N.J. Baxter, M.L. Khan, A.J. Moir, E. Haslam, A.P. Davies, and M.P. Williamson. 2002. Polyphenol/peptide binding and precipitation. J. Agr. Food Chem. 50:1593-1601.

Cheynier, V. 2005. Polyphenols in foods are more complex than often thought. Am. J. Clin. Nutr. 81:223S-229S.

Cheynier, V., and J. Rigaud. 1986. HPLC separation and characterization of flavonols in the skins of Vitis vinifera var. Cinsault. Am. J. Enol. Viticult. 37:248-252.

Chira, K., and P.-L. Teissedre. 2015. Chemical and sensory evaluation of wine matured in oak barrel: effect of oak species involved and toasting process. Eur. Food Res. Technol. 240:533-547.

Chong, H.H., M.T. Cleary, N. Dokoozlian, C.M. Ford, and G.B. Fincher. 2019. Soluble cell wall carbohydrates and their relationship with sensory attributes in Cabernet Sauvignon wine. Food Chem.

Cliff, M.A., K. Stanich, J.E. Edwards, and C.T. Saucier. 2012. Adding grape seed extract to wine affects astringency and other sensory attributes. J. Food Qual. 35:263-271.

Condelli, N., C. Dinnella, A. Cerone, E. Monteleone, and M. Bertuccioli. 2006. Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection

and preliminary application on wine samples. Food Qual. Prefer. 17:96-107.

Cosme, F., I. Capão, L. Filipe-Ribeiro, R.N. Bennett, and A. Mendes-Faia. 2012. Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics. LWT - Food Sci. Technol. 46:382-387.

Cosme, F., J.M. Ricardo-Da-Silva, and O. Laureano. 2009. Effect of various proteins on different molecular weight proanthocyanidin fractions of red wine during wine fining. Am. J. Enol. Viticult. 60:74-81.

Courregelongue, S., P. Schlich, and A.C. Noble. 1999. Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. Food Qual. Prefer. 10:273-279.

Czochanska, Z., L.Y. Foo, R.H. Newman, and L.J. Porter. 1980. Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. J. Chem. Soc. Perkin Trans. I:2278-2286.

de Freitas, V., and N. Mateus. 2012. Protein/polyphenol interactions: past and present contributions. Mechanisms of astringency perception. Curr. Org. Chem. 16:724-746.

Demiglio, P., and G.J. Pickering. 2008. The influence of ethanol and pH on the taste and mouthfeel sensations elicited by red wine. J. Food Agric. Environ. 6:143-150.

DeMiglio, P., G.J. Pickering, and A.G. Reynolds. Astringent sub-qualities elicited by red wine: the role of ethanol and pH. *In* Proceedings of the Proceedings of the international Bacchus to the future conference, St Catharines, Ontario. pp. 2002.

Downey, M.O., J.S. Harvey, and S.P. Robinson. 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust. J. Grape Wine Res. 9:15-27.

Downey, M.O., J.S. Harvey, and S.P. Robinson. 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust. J. Grape Wine Res. 10:55-73.

Duizer, L., K. Bloom, and C. Findlay. 1996. Dual-attribute Time-intensity Measurement of sweetness and peppermint perception of chewing gum. J. Food Sci. 61:636-638.

Escribano-Bailón, M.T., M.T. Guerra, J.C. Rivas-Gonzalo, and C. Santos-Buelga. 1995. Proanthocyanidins in skins from different grape varieties. Z. Lebensm. Unters. Forsch. 200:221-224.

Ferrer-Gallego, R., J.M. Hernández-Hierro, J.C. Rivas-Gonzalo, and M.T. Escribano-Bailón. 2014. Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. Food Res. Int. 62:1100-1107.

Fischer, U., R. Boulton, and A. Noble. 1994. Physiological factors contributing to the variability

of sensory assessments: Relationship between salivary flow rate and temporal perception of gustatory stimuli. Food Qual. Prefer. 5:55-64.

Flamini, R., and P. Traldi. 2010. Mass spectrometry in grape and wine chemistry. Wiley-Interscience Series on Mass Spectrometry:45-76.

Fleming, E.E., G.R. Ziegler, and J.E. Hayes. 2015. Check-All-That-Apply (CATA), Sorting, and Polarized Sensory Positioning (PSP) with Astringent Stimuli. Food Qual. Prefer. 45:41-49.

Fleming, E.E., G.R. Ziegler, and J.E. Hayes. 2016. Investigating Mixture Interactions of Astringent Stimuli Using the Isobole Approach. Chemical senses 41:601-610.

Fleurence, J. 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends Food Sci. Technol. 10:25-28.

Fontoin, H., C. Saucier, P.-L. Teissedre, and Y. Glories. 2008. Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. Food Qual. Prefer. 19:286-291.

Furlan, A.l.L., A. Castets, F.d.r. Nallet, I. Pianet, A. Grélard, E.J. Dufourc, and J. Géan. 2014. Red wine tannins fluidify and precipitate lipid liposomes and bicelles. A role for lipids in wine tasting? Langmuir 30:5518-5526.

Gambuti, A., A. Rinaldi, and L. Moio. 2012. Use of patatin, a protein extracted from potato, as alternative to animal proteins in fining of red wine. Eur. Food Res. Technol. 235:753-765.

Gambuti, A., A. Rinaldi, R. Pessina, and L. Moio. 2006. Evaluation of aglianico grape skin and seed polyphenol astringency by SDS–PAGE electrophoresis of salivary proteins after the binding reaction. Food Chem. 97:614-620.

Gawel, R. 1998. Red wine astringency: a review. Aust. J. Grape Wine Res. 4:74-95.

Gawel, R., P.G. Iland, and I.L. Francis. 2001. Characterizing the astringency of red wine: a case study. Food Qual. Prefer. 12:83-94.

Gawel, R., A. Oberholster, and I.L. Francis. 2000. A 'Mouth-feel Wheel': terminology for communicating the mouth-feel characteristics of red wine. Aust. J. Grape Wine Res. 6:203-207.

Gibbins, H., and G. Carpenter. 2013. Alternative mechanisms of astringency–what is the role of saliva? J. Texture Stud. 44:364-375.

Glabasnia, A., and T. Hofmann. 2006. Sensory-directed identification of taste-active ellagitannins in American (Quercus alba L.) and European oak wood (Quercus robur L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. J. Agr.

Food Chem. 54:3380-3390.

González-Manzano, S., J.C. Rivas-Gonzalo, and C. Santos-Buelga. 2004. Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal. Chim. Acta 513:283-289.

González-Manzano, S., C. Santos-Buelga, J. Pérez-Alonso, J. Rivas-Gonzalo, and M. Escribano-Bailón. 2006. Characterization of the mean degree of polymerization of proanthocyanidins in red wines using liquid chromatography-mass spectrometry (LC-MS). J. Agr. Food Chem. 54:4326-4332.

González-Neves, G., G. Gil, G. Favre, and M. Ferrer. 2012. Influence of grape composition and winemaking on the anthocyanin composition of red wines of Tannat. J. Food Sci. Technol. 47:900-909.

Granato, T.M., A. Nasi, P. Ferranti, S. Iametti, and F. Bonomi. 2014. Fining white wine with plant proteins: effects of fining on proanthocyanidins and aroma components. Eur. Food Res. Technol. 238:265-274.

Guerrero, R.F., A. Liazid, M. Palma, B. Puertas, R. González-Barrio, Á. Gil-Izquierdo, C. García-Barroso, and E. Cantos-Villar. 2009. Phenolic characterisation of red grapes autochthonous to Andalusia. Food Chem. 112:949-955.

Guerrero, R.l.F., P. Smith, and K.A. Bindon. 2013. Application of insoluble fibers in the fining of wine phenolics. J. Agr. Food Chem. 61:4424-4432.

Guinard, J.-X., R.M. Pangborn, and M.J. Lewis. 1986. The time-course of astringency in wine upon repeated ingestion. Am. J. Enol. Viticult. 37:184-189.

Guyot, S., C. Le Guernevé, N. Marnet, and J.-F. Drilleau. 1999. Methods for determining the degree of polymerization of condensed tannins: A new 1H-NMR procedure applied to cider apple procyanidins. *In* Plant Polyphenols 2. pp. 211-222. Springer.

Hagerman, A.E., and L.G. Butler. 1978. Protein precipitation method for the quantitative determination of tannins. J. Agr. Food Chem. 26:809-812.

Hagerman, A.E., and L.G. Butler. 1980. Condensed tannin purification and characterization of tannin-associated proteins. J. Agr. Food Chem. 28:947-952.

Hagerman, A.E., Y. Zhao, and S. Johnson. 1997. Methods for determination of condensed and hydrolyzable tannins. *In*. ACS Publications.

Hahn, G.D., and P. Possmann. 1977. Colloidal silicon dioxide as a fining agent for wine. Am. J. Enol. Viticult. 28:108-112.

Hanlin, R.L., M. Hrmova, J.F. Harbertson, and M.O. Downey. 2010. Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aust.

J. Grape Wine Res. 16:173-188.

Harbertson, J.F., M.S. Mireles, E.D. Harwood, K.M. Weller, and C.F. Ross. 2009. Chemical and sensory effects of saignée, water addition, and extended maceration on high brix must. Am. J. Enol. Viticult. 60:450-460.

Hayashi, N., T. Ujihara, R. Chen, K. Irie, and H. Ikezaki. 2013. Objective evaluation methods for the bitter and astringent taste intensities of black and oolong teas by a taste sensor. Food Res. Int. 53:816-821.

Herderich, M., and P. Smith. 2005. Analysis of grape and wine tannins: Methods, applications and challenges. Aust. J. Grape Wine Res. 11:205-214.

Heredia, F., M. Escudero-Gilete, D. Hernanz, B. Gordillo, A. Meléndez-Martínez, I. Vicario, and M. González-Miret. 2010. Influence of the refrigeration technique on the colour and phenolic composition of Syrah red wines obtained by pre-fermentative cold maceration. Food Chem. 118:377-383.

Hernández-Jiménez, A., J.A. Kennedy, A.B. Bautista-Ortín, and E. Gómez-Plaza. 2011. Effect of ethanol on grape seed proanthocyanidin extraction. Am. J. Enol. Viticult.:11-53.

Hufnagel, J.C., and T. Hofmann. 2008a. Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. J. Agr. Food Chem. 56:1376-1386.

Hufnagel, J.C., and T. Hofmann. 2008b. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. J. Agr. Food Chem. 56:9190-9199.

Ishikawa, T., and A. Noble. 1995. Temporal perception of astringency and sweetness in red wine. Food Qual. Prefer. 6:27-33.

Iturmendi, N., D. Durán, and M.R. Marín-Arroyo. 2010. Fining of red wines with gluten or yeast extract protein. J. Food Sci. Technol. 45:200-207.

Jauregi, P., J.B. Olatujoye, I. Cabezudo, R.A. Frazier, and M.H. Gordon. 2016. Astringency reduction in red wine by whey proteins. Food Chem. 199:547-555.

Jeffery, D., M.D. Mercurio, M.J. Herderich, Y. Hayasaka, and P.A. Smith. 2008a. Rapid isolation of red wine polymeric polyphenols by solid-phase extraction. J. Agr. Food Chem. 56:2571-2580.

Jeffery, D., M. Parker, and P. Smith. 2008b. Flavonol composition of Australian red and white wines determined by high-performance liquid chromatography. Aust. J. Grape Wine Res. 14:153-161.

Jöbstl, E., J. O'Connell, J.P.A. Fairclough, and M.P. Williamson. 2004. Molecular model for astringency produced by polyphenol/protein interactions. Biomacromolecules 5:942-949.

Kallithraka, S., J. Bakker, and M. Clifford. 1997. Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. J. Sens. 12:25-37.

Kallithraka, S., J. Bakker, M. Clifford, and L. Vallis. 2001. Correlations between saliva protein composition and some T–I parameters of astringency. Food Qual. Prefer. 12:145-152.

Kaneda, H., J. Watari, M. Takashio, and Y. Okahata. 2002. Adsorption of tannins on lipid membrane in the presence of peptides as related to astringency. J. Food Sci. 67:3489-3492.

Kang, W., J. Niimi, and S.E.P. Bastian. 2018. Reduction of red wine astringency perception using vegetable protein fining agents. Am. J. Enol. Viticult. 69:22-31.

Kantz, K., and V. Singleton. 1991. Isolation and determination of polymeric polyphenols in wines using Sephadex LH-20. Am. J. Enol. Viticult. 42:309-316.

Kemp, B., S. Trussler, J. Willwerth, and D. Inglis. 2019. Applying temporal check-all-that-apply (TCATA) to mouthfeel and texture properties of red wines. J. Sens.:e12503.

Kennedy, J.A., and G.P. Jones. 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J. Agr. Food Chem. 49:1740-1746.

Kennedy, J.A., and A.W. Taylor. 2003. Analysis of proanthocyanidins by high-performance gel permeation chromatography. J. Chromatogr. A 995:99-107.

Koyama, K., N. Goto-Yamamoto, and K. Hashizume. 2007. Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (Vitis vinifera). Biosci. Biotechnol. Biochem. 71:958-965.

Laborde, B., V. Moine-Ledoux, T. Richard, C. Saucier, D. Dubourdieu, and J.P. Monti. 2006. PVPP-polyphenol complexes: a molecular approach. J Agric Food Chem 54:4383-9.

Lawless, H.T., and H. Heymann. 2010. *Sensory evaluation of food: principles and practices*. Springer Science & Business Media, New York.

Le Bourvellec, C., S. Guyot, and C. Renard. 2004. Non-covalent interaction between procyanidins and apple cell wall material: Part I. Effect of some environmental parameters. Biochimica et Biophysica Acta (BBA)-General Subjects 1672:192-202.

Le Bourvellec, C., and C. Renard. 2012. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. Crit. Rev. Food Sci. Nutr. 52:213-248.

Lee, C.A., B. Ismail, and Z.M. Vickers. 2012. The role of salivary proteins in the mechanism of astringency. J. Food Sci. 77:C381-C387.

Lee, C.B., and H.T. Lawless. 1991. Time-course of astringent sensations. Chemical senses

16:225-238.

Li, S., K. Bindon, S.E. Bastian, V. Jiranek, and K.L. Wilkinson. 2017. Use of winemaking supplements to modify the composition and sensory properties of Shiraz wine. J. Agr. Food Chem. 65:1353-1364.

Lyman, B.J., and B.G. Green. 1990. Oral astringency: effects of repeated exposure and interactions with sweeteners. Chemical Senses 15:151-164.

Macheix, J.-J., and A. Fleuriet. 1990. *Fruit phenolics*. CRC press.

Marchal, R., L. Marchal-Delahaut, F. Michels, M. Parmentier, A. Lallement, and P. Jeandet. 2002. Use of wheat gluten as clarifying agent of musts and white wines. Am. J. Enol. Viticult. 53:308-314.

Margalit, Y. 2012. Concepts in wine chemistry. The wine appreciation guild:101-110.

Maury, C., P. Sarni-Manchado, S. Lefebvre, V. Cheynier, and M. Moutounet. 2003. Influence of fining with plant proteins on proanthocyanidin composition of red wines. Am. J. Enol. Viticult. 54:105-111.

McRae, J.M., R.G. Dambergs, S. Kassara, M. Parker, D.W. Jeffery, M.J. Herderich, and P.A. Smith. 2012. Phenolic compositions of 50 and 30 year sequences of Australian red wines: the impact of wine age. J. Agr. Food Chem. 60:10093-10102.

McRae, J.M., and J.A. Kennedy. 2011. Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. Molecules 16:2348-2364.

McRae, J.M., A. Schulkin, S. Kassara, H.E. Holt, and P.A. Smith. 2013. Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. J. Agr. Food Chem. 61:719-727.

Mehansho, H., L.G. Butler, and D.M. Carlson. 1987. Dietary tannins and salivary proline-rich proteins: interactions, induction, and defense mechanisms. Annu. Rev. Nutr 7:423-440.

Meilgaard, M.C., B.T. Carr, and G.V. Civille. 2006. *Sensory evaluation techniques*. CRC press.

Melis, M., N.Y. Yousaf, M.Z. Mattes, T. Cabras, I. Messana, R. Crnjar, I.T. Barbarossa, and B.J. Tepper. 2017. Sensory perception of and salivary protein response to astringency as a function of the 6-n-propylthioural (PROP) bitter-taste phenotype. Physiol. Behav.

Mercurio, M., and P.A. Smith. 2006. New formats for the methyl cellulose precipitable (MCP) tannin assay allow high throughput measurement of grape and wine tannin by industry. Technical Review 164:1-10.

Mills, E., A.I. Sancho, N.M. Rigby, J.A. Jenkins, and A.R. Mackie. 2009. Impact of food processing on the structural and allergenic properties of food allergens. Mol. Nutr. Food Res. 53:963-969.

Monteleone, E., N. Condelli, C. Dinnella, and M. Bertuccioli. 2004. Prediction of perceived astringency induced by phenolic compounds. Food Qual. Prefer. 15:761-769.

Muhlack, R., S. Nordestgaard, E.J. Waters, B. O'NEILL, A. Lim, and C. Colby. 2006. In-line dosing for bentonite fining of wine or juice: Contact time, clarification, product recovery and sensory effects. Aust. J. Grape Wine Res. 12:221-234.

Naczk, M., D. Oickle, D. Pink, and F. Shahidi. 1996. Protein precipitating capacity of crude canola tannins: effect of pH, tannin, and protein concentrations. J. Agr. Food Chem. 44:2144-2148.

Narukawa, M., C. Noga, Y. Ueno, T. Sato, T. Misaka, and T. Watanabe. 2011. Evaluation of the bitterness of green tea catechins by a cell-based assay with the human bitter taste receptor hTAS2R39. Biochem. Biophys. Res. Commun. 405:620-625.

Nayak, A., and G. Carpenter. 2008. A physiological model of tea-induced astringency. Physiol. Behav 95:290-294.

Noble, A. 1994. Bitterness in wine. Physiol. Behav 56:1251-1255.

Nordestgaard, S., Y.P. Chuan, B. O'Neill, E. Waters, L. Deans, P. Policki, and C. Colby. 2007. In-line dosing of white wine for bentonite fining with centrifugal clarification. Am. J. Enol. Viticult. 58:283-285.

Oszmianski, J., T. Ramos, and M. Bourzeix. 1988. Fractionation of phenolic compounds in red wine. Am. J. Enol. Viticult. 39:259-262.

Parenti, A., P. Spugnoli, L. Calamai, S. Ferrari, and C. Gori. 2004. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. Eur. Food Res. Technol. 218:360-366.

Pasch, H., A. Pizzi, and K. Rode. 2001. MALDI–TOF mass spectrometry of polyflavonoid tannins. Polymer 42:7531-7539.

Pashova, V., C. Güell, and F. López. 2004. White wine continuous protein stabilization by packed column. J. Agr. Food Chem. 52:1558-1563.

Payne, C., P.K. Bowyer, M. Herderich, and S.E.P. Bastian. 2009. Interaction of astringent grape seed procyanidins with oral epithelial cells. Food Chem. 115:551-557.

Payne, K., and A. Veis. 1988. Fourier transform IR spectroscopy of collagen and gelatin solutions: deconvolution of the amide I band for conformational studies. Biopolymers 27:1749-1760.

Peleg, H., K.K. Bodine, and A.C. Noble. 1998. The influence of acid on astringency of alum and phenolic compounds. Chemical senses 23:371-378.

Peleg, H., K. Gacon, P. Schlich, and A.C. Noble. 1999. Bitterness and astringency of flavan-3-

ol monomers, dimers and trimers. J. Sci.I Food Agr. 79:1123-1128.

Peleg, H., and A.C. Noble. 1999a. Effect of viscosity, temperature and pH on astringency in cranberry juice. Food Qual. Prefer. 10:343-347.

Peleg, H., and A.C. Noble. 1999b. Effect of viscosity, temperature and pH on astringency in cranberry juice. Food Qual. Prefer. 10:343-347.

Peng, Z., Y. Hayasaka, P.G. Iland, M. Sefton, P. Høj, and E.J. Waters. 2001. Quantitative analysis of polymeric procyanidins (tannins) from grape (Vitis vinifera) seeds by reverse phase high-performance liquid chromatography. J. Agr. Food Chem. 49:26-31.

Peynaud, E., and J. Blouin. 1996. *The taste of wine: The art science of wine appreciation*. John Wiley & Sons, New Jersey.

Pickering, G.J., and P. Demiglio. 2008. The White Wine Mouthfeel Wheel: A Lexicon for Describing the Oral Sensations Elicited by White Wine. Journal of Wine Research 19:51-67.

Pickering, G.J., and G. Robert. 2006. Perception of mouthfeel sensations elicited by red wine are associated with sensitivity to 6-n-propylthiouracil. J. Sens. 21:249-265.

Pickering, G.J., K. Simunkova, and D. DiBattista. 2004. Intensity of taste and astringency sensations elicited by red wines is associated with sensitivity to PROP (6-n-propylthiouracil). Food Qual. Prefer. 15:147-154.

Piggott, J.R., S.J. Simpson, and S.A. Williams. 1998. Sensory analysis. J. Food Sci. Technol. 33:7-12.

Pineau, N., P. Schlich, S. Cordelle, C. Mathonnière, S. Issanchou, A. Imbert, M. Rogeaux, P. Etiévant, and E. Köster. 2009. Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. Food Qual. Prefer. 20:450-455.

Pocock, K., M. Sefton, and P. Williams. 1994. Taste thresholds of phenolic extracts of French and American oakwood: the influence of oak phenols on wine flavor. Am. J. Enol. Viticult. 45:429-434.

Poncet-Legrand, C., C. Gautier, V. Cheynier, and A. Imberty. 2007. Interactions between flavan-3-ols and poly (L-proline) studied by isothermal titration calorimetry: effect of the tannin structure. J. Agr. Food Chem. 55:9235-9240.

Price, M.L., S. Van Scoyoc, and L.G. Butler. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agr. Food Chem. 26:1214-1218.

Price, S., P. Breen, M. Valladao, and B. Watson. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. Am. J. Enol. Viticult. 46:187-194.

Puech, J.L., F. Feuillat, and J.R. Mosedale. 1999. The tannins of oak heartwood: Structure,

properties, and their influence on wine flavor. Am. J. Enol. Viticult. 50:469-478.

Quijada-Morín, N., P. Williams, J.C. Rivas-Gonzalo, T. Doco, and M.T. Escribano-Bailón. 2014. Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. Food Chem. 154:44-51.

Rankine, B. 2007. Making good wine. Macmillan Publishers, Australia:137-144.

Ribéreau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. 2006. Clarification and Stabilization Treatments: Fining Wine. *In* Handbook of Enology. pp. 301-331. John Wiley & Sons, Ltd.

Rodriguez, M., L. Albertengo, I. Vitale, and E. Agullo. 2003. Relationship Between Astringency and Chitosan-Saliva Solutions Turbidity at Different pH. J. Food Sci. 68:665-667.

Rubico, S.M., and M.R. McDaniel. 1992. Sensory evaluation of acids by free-choice profiling. Chemical Senses 17:273-289.

Sáenz-Navajas, M.P., V. Ferreira, M. Dizy, and P. Fernández-Zurbano. 2010. Characterization of taste-active fractions in red wine combining HPLC fractionation, sensory analysis and ultra performance liquid chromatography coupled with mass spectrometry detection. Anal. Chim. Acta 673:151-159.

Saltman, Y., T.E. Johnson, K.L. Wilkinson, and S.E.P. Bastian. 2015. Australian wine consumers' acceptance of and attitudes toward the use of additives in wine and food production. Int. J. Wine Res. 7:83-92.

Sarneckis, C.J., R. Dambergs, P. Jones, M. Mercurio, M.J. Herderich, and P. Smith. 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Aust. J. Grape Wine Res. 12:39-49.

Sarni-Manchado, P., A. Deleris, S. Avallone, V. Cheynier, and M. Moutounet. 1999. Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agent in enology. Am. J. Enol. Viticult. 50:81-86.

Scharbert, S., N. Holzmann, and T. Hofmann. 2004. Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. J. Agr. Food Chem. 52:3498-3508.

Schmidtke, L.M., A.C. Clark, and G.R. Scollary. 2011. Micro-oxygenation of red wine: techniques, applications, and outcomes. Crit. Rev. Food Sci. Nutr. 51:115-131.

Schöbel, N., D. Radtke, J. Kyereme, N. Wollmann, A. Cichy, K. Obst, K. Kallweit, O. Kletke, A. Minovi, and S. Dazert. 2014. Astringency is a trigeminal sensation that involves the

activation of G protein–coupled signaling by phenolic compounds. Chemical senses:bju014.

Scollary, G.R., G. Pásti, M. Kállay, J. Blackman, and A.C. Clark. 2012. Astringency response of red wines: Potential role of molecular assembly. Trends Food Sci. Technol. 27:25-36.

Simonato, B., F. Mainente, E. Selvatico, M. Violoni, and G. Pasini. 2013. Assessment of the fining efficiency of zeins extracted from commercial corn gluten and sensory analysis of the treated wine. LWT - Food Sci. Technol. 54:549-556.

Simonato, B., F. Mainente, I. Suglia, A. Curioni, and G. Pasini. 2009. Evaluation of fining efficiency of corn Zeins in red wine: A preliminary study. Ital. J. Food Sci. 21:97-105.

Sims, C.A., J.S. Eastridge, and R.P. Bates. 1995. Changes in phenols, color, and sensory characteristics of muscadine wines by pre-and post-fermentation additions of PVPP, casein, and gelatin. Am. J. Enol. Viticult. 46:155-158.

Sipiora, M.J., and M.-J.G. Granda. 1998. Effects of pre-veraison irrigation cutoff and skin contact time on the composition, color, and phenolic content of young Cabernet Sauvignon wines in Spain. Am. J. Enol. Viticult. 49:152-162.

Smith, A.K., H. June, and A.C. Noble. 1996. Effects of viscosity on the bitterness and astringency of grape seed tannin. Food Qual. Prefer. 7:161-166.

Smith, P.A., J.M. McRae, and K.A. Bindon. 2015. Impact of winemaking practices on the concentration and composition of tannins in red wine. Aust. J. Grape Wine Res. 21:601-614.

Somers, T. 1971. The polymeric nature of wine pigments. Phytochemistry 10:2175-2186.

Somers, T.C., and M.E. Evans. 1974. Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. J. Sci.I Food Agr. 25:1369-1379.

Somers, T.C., and M.E. Evans. 1977. Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO2,“chemical age”. J. Sci.I Food Agr. 28:279-287.

Somers, T.C., and E. Verette. 1988. *Phenolic composition of natural wine types*. Springer.

Souquet, J.M., B. Labarbe, C. Le Guerneve, V. Cheynier, and M. Moutounet. 2000. Phenolic composition of grape stems. J. Agr. Food Chem. 48:1076-80.

Sparrow, A.M., R.E. Smart, R.G. Dambergs, and D.C. Close. 2016. Skin particle size affects the phenolic attributes of Pinot noir wine: Proof of concept. Am. J. Enol. Viticult. 67:29-37.

Spayd, S.E., J.M. Tarara, D.L. Mee, and J. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. Am. J. Enol.

Viticult. 53:171-182.

Stankovic, S., S. Jovic, J. Zivkovic, and R. Pavlovic. 2012. Influence of age on red wine colour during fining with bentonite and gelatin. Int. J. Food Prop. 15:326-335.

Stark, T., S. Bareuther, and T. Hofmann. 2005. Sensory-guided decomposition of roasted cocoa nibs (Theobroma cacao) and structure determination of taste-active polyphenols. J. Agr. Food Chem. 53:5407-5418.

Su, C.T., and V. Singleton. 1969. Identification of three flavan-3-ols from grapes. Phytochemistry 8:1553-1558.

Sun, B., M.d. Sá, C.a.o. Leandro, I. Caldeira, F.L. Duarte, and I. Spranger. 2013. Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. J. Agr. Food Chem. 61:939-946.

Thorngate, J., and A. Noble. 1995. Sensory evaluation of bitterness and astringency of 3R (−)-epicatechin and 3S (+)-catechin. J. Sci.I Food Agr. 67:531-535.

Treutter, D. 1989. Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. J. Chromatogr. A 467:185-193.

Tschiersch, C., M.P. Nikfardjam, O. Schmidt, and W. Schwack. 2010. Degree of hydrolysis of some vegetable proteins used as fining agents and its influence on polyphenol removal from red wine. Eur. Food Res. Technol. 231:65-74.

Tschiersch, C., M. Pour Nikfardjam, O. Schmidt, and W. Schwack. 2008. Comparison of seventeen different fining agents used with wine. Mitt Klosterneuburg 4:123-131.

Valentová, H., S. Skrovánková, Z. Panovská, and J. Pokorný. 2002. Time–intensity studies of astringent taste. Food Chem. 78:29-37.

Vidal, L., L. Antúnez, A. Giménez, K. Medina, E. Boido, and G. Ares. 2016. Dynamic characterization of red wine astringency: Case study with Uruguayan Tannat wines. Food Res. Int. 82:128-135.

Vidal, L., A. Giménez, K. Medina, E. Boido, and G. Ares. 2015. How do consumers describe wine astringency? Food Res. Int. 78:321-326.

Vidal, S., L. Francis, S. Guyot, N. Marnet, M. Kwiatkowski, R. Gawel, V. Cheynier, and E.J. Waters. 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J. Sci.I Food Agr. 83:564-573.

Vidal, S., L. Francis, A. Noble, M. Kwiatkowski, V. Cheynier, and E. Waters. 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal. Chim. Acta 513:57-65.

Vidal, S., L. Francis, P. Williams, M. Kwiatkowski, R. Gawel, W. Cheynier, and E. Waters.

2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chem. 85:519-525.

Vincenzi, S., C. Dinnella, A. Recchia, E. Monteleone, D. Gazzola, G. Pasini, and A. Curioni. 2013. Grape seed proteins: a new fining agent for astringency reduction in red wine. Aust. J. Grape Wine Res. 19:153-160.

Voilley, A., C. Lamer, P. Dubois, and M. Feuillat. 1990. Influence of macromolecules and treatments on the behavior of aroma compounds in a model wine. J. Agr. Food Chem. 38:248-251.

Weber, P., H. Steinhart, and A. Paschke. 2007. Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA. J. Agr. Food Chem. 55:3127-3133.

Zhu, M., J.D. Phillipson, P.M. Greengrass, N.E. Bowery, and Y. Cai. 1997. Plant polyphenols: biologically active compounds or non-selective binders to protein? Phytochemistry 44:441-447.

Zoecklein, B.W., K.C. Fugelsang, B.H. Gump, and F.S. Nury. 1995. Production wine analysis. Van Nostrand Reinhold, New York:249-263.

CHAPTER 2

Dynamic characterization of wine astringency profiles using modified progressive profiling

Wenyu Kang [a], Jun Niimi [a], Richard A. Muhlack [a], Paul A. Smith [b] and Susan E.P. Bastian [a,*]

[a] School of Agriculture, Food & Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA, Australia, 5064.

[b] Wine Australia, P.O. Box 660, Kent Town, SA, Australia, 5071.

[*] Corresponding author, sue.bastian@adelaide.edu.au

Food Research International – 2019, 120, 244–254.

Statement of Authorship

Title of Paper	Dynamic characterization of wine astringency profiles using modified progressive profiling
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Kang, W., Niimi, J., Muhlack, R. A., Smith, P. A., & Bastian, S. E. P. (2019). Dynamic characterization of wine astringency profiles using modified progressive profiling. Food Research International, 120, 244-254.

Principal Author

Name of Principal Author (Candidate)	Wenyu Kang		
Contribution to the Paper	Designed experiments, performed experimental works, conducted sensory trails with expert tasters, analysed and interpreted data, drafted and constructed the manuscript.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	17/04/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jun Niimi		
Contribution to the Paper	Contributed to the research idea, sensory analysis design, interpretation of data and editing of the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Richard A. Muhlack		
Contribution to the Paper	Contributed to the research idea, interpretation of data and editing of the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Paul A. Smith		
Contribution to the Paper	Contributed to the research idea, interpretation of data and editing of the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Susan E.P. Bastian		
Contribution to the Paper	Contributed to the research idea, sensory analysis design, interpretation of data and editing of the manuscript, and acted as corresponding author.		
Signature		Date	21/05/2020

Food Research International 120 (2019) 244–254

Contents lists available at ScienceDirect

Food Research International

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

Dynamic characterization of wine astringency profiles using modified progressive profiling

Wenyu Kang[a], Jun Niimi[a], Richard A. Muhlack[a], Paul A. Smith[b], Susan E.P. Bastian[a,*]

[a] School of Agriculture, Food & Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia
[b] Wine Australia, P.O. Box 660, Kent Town, SA 5071, Australia

ARTICLE INFO

Keywords:
Astringent sub-quality
Progressive profiling
Temporal profiling
Texture
Wine tannin
Mouthfeel

ABSTRACT

Wine astringency is important for quality and consumer acceptance. Perception of this mouthfeel is temporal and can be separated further into unique textural sub-qualities. Quantitative data on these astringent sub-qualities in wine however are poorly understood. The aim of this study was to characterize the dynamic astringency profiles of 13 Australian commercial red wines and 2 rosés made from 11 grape varieties using modified progressive profiling by a trained sensory panel (n = 8). Seven attributes generated and defined by the panel (overall astringent intensity and 6 sub-qualities: pucker, mouth coat, dry, grippy, adhesive and graininess) were scored at six time periods (each lasting 10 s), with 20 s gap between each time period. Attributes were rated on 15 cm scales with anchors at 10 and 90% and samples were evaluated in duplicate. The wine composition as well as phenolic profiles were determined. Intensities of astringent sub-qualities were correlated with overall intensity, but the sub-quality profiles at a specific evaluation period and the progression of an attribute varied differently depending on the wine. The discrimination of wines at each time interval was dependent on attribute, and the relative importance of each astringent sub-quality varied at different evaluation periods. Correlations between mouthfeel attributes and chemical measures were established. This study demonstrated the utilisation of modified progressive profiling for wine astringency evaluation, providing a tool to capture quantitative data on astringent sub-qualities in wine.

1. Introduction

Astringency is a tactile sensation that occurs on the mouth surfaces (Breslin, Gilmore, Beauchamp, & Green, 1993) primarily due to the lack of lubrication of the oral epithelium (Bennick 2002; Kallithraka et al. 2001). Astringency is one of the key factors in wine products, which drives wine quality and consumer preference (Harrison, 2018; Lattey, Bramley, & Francis, 2010; Peynaud & Blouin, 1996). Wine astringency is believed to consist of two percepts (i.e. cognitive impressions of perceiving astringency); astringency intensity and a number of sub-qualities (textures or mouthfeels). Astringency intensity can be regarded as the overall level of roughing, drying, shrinking or drawing in of mouth surfaces. Astringency sensation can gradually increase after repeated ingestion and the perception can last up to six minutes after expectoration or swallowing (Ishikawa & Noble, 1995). However, the intensity alone is insufficient to fully characterize astringent sensations during wine consumption (Bajec & Pickering, 2008), as some wines (particularly made by different varieties) may have a similar astringency intensity but diverse textures. Therefore, a number of astringent sub-qualities have been characterized and suggested for use in the description of wines, such as drying, velvety, puckering, dusty, adhesive and chamois amongst others (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Gawel, Iland, & Francis, 2001; Gawel, Oberholster, & Francis, 2000; Pickering & Demiglio, 2008; Vidal et al., 2016). Astringency sensation is dynamic (both the intensity and sub-qualities), that is, the percepts change over time (Guinard, Pangborn, & Lewis, 1986; Lawless & Heymann, 2010). The literature on astringency sub-quality of wines have either reported time averaged quantitative intensity data or the qualitative changes in the presence of the astringent sub-qualities as a function of time but with no quantitative intensity measures. However, no study has been reported that measures intensity of sub-qualities and follows them with time.

Several stimuli elicit astringency including various phenolic components (Kallithraka, Bakker, & Clifford, 1997), multivalent salts like alum (Peleg, Bodine, & Noble, 1998), organic acids (Rubico & McDaniel, 1992), and charged polysaccharides such as chitosan (Rodriguez, Albertengo, Vitale, & Agullo, 2003). It is widely accepted

* Corresponding author.
E-mail address: sue.bastian@adelaide.edu.au (S.E.P. Bastian).

https://doi.org/10.1016/j.foodres.2019.02.041
Received 7 November 2018; Received in revised form 20 February 2019; Accepted 21 February 2019
Available online 22 February 2019

that in wine a strong and positive relationship exists between astringency intensity and concentration of phenolic components. Grape-derived tannins (condensed tannins or proanthocyanidins), polymeric pigments, flavonols, and possibly flavan-3-ols as well as oak-derived tannins (hydrolysable tannins) cause astringent sensations (Harrison, 2018; Pocock, Sefton, & Williams, 1994; Soares, Brandão, Mateus, & De Freitas, 2017). During wine consumption, the astringency can also be influenced by a number of other indirect factors. For instance, the pH, alcohol level (Payne, Bowyer, Herderich, & Bastian, 2009), viscosity, sweetness (Peleg & Noble, 1999), temperature, acidity, number of repeated exposures, resonance time on the palate (Lyman & Green, 1990; Payne et al., 2009; Valentová, Skrovánková, Panovská, & Pokorný, 2002) and physiological factors of the taster (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006; Melis et al., 2017; Naczk, Oickle, Pink, & Shahidi, 1996).

The generally accepted mechanism of astringency perception is primarily due to the interactions of astringent stimuli with salivary proteins, such as proline-rich proteins (PRP) and histatins and the subsequent precipitation of these complexes (Jöbstl, O'Connell, Fairclough, & Williamson, 2004; Mehansho, Butler, & Carlson, 1987; Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007; Scollary, Pásti, Kállay, Blackman, & Clark, 2012). However, the mechanism of astringency perception is not yet fully understood, especially the principle behind sub-quality sensations (Ma, Waffo-Teguo, Jourdes, Li, & Teissedre, 2016). Therefore, to advance our understanding of perception of astringency and its sub-qualities, chemosensory experiments (i.e. those that create models of chemical measures or compositional prediction of sensations) remain a necessary approach. Nevertheless, this type of research provides significant challenges for both chemists and sensory scientists, as astringent compounds interact with many components simultaneously, temporally and spatially in the mouth. Thus, to the moment, sensory experiments for evaluating astringency sensation should not (or cannot) be replaced completely by chemical measurements.

A variety of sensory methods have been utilised to study astringency. For example, discrimination testing (e.g. triangle test or duo-trio test) (Piggott, Simpson, & Williams, 1998) and scalar methods (Peleg & Noble, 1999) are simple methods which are widely used. However, a limitation of these methods is that they only capture the astringency sensation at a single time point; astringency perception on the contrary is dynamic. To monitor the change in astringency intensity with time, Time Intensity (TI) has been used (Lee & Lawless, 1991). The difficulty with TI however is that it only allows intensity measurements of a maximum of two attributes in each round of measurement (Castura, Antúnez, Giménez, & Ares, 2016; Duizer, Bloom, & Findlay, 1996) and if the measurement of more than two attributes is required, each sample requires several rounds of evaluation. This is a particular problem for evaluating astringency because of the build-up of astringent sensation. In order to avoid evaluating samples in many rounds, two methods were developed. Temporal Dominance of Sensations (TDS), a method that can measure the dominance of attributes as a function of time (Pineau et al. 2009), and which has been recently used in the study of astringent sub-qualities of wines (Vidal, Antúnez, Giménez, Medina, et al., 2016). Another method is called Temporal Check-All-That-Apply (TCATA). TCATA and TDS are similar but they differ in that TDS involves choosing a single and dominant attribute at each moment of the evaluation, while TCATA allows selection of multiple attributes at each moment. Furthermore, it is important to note that these two methods alone are qualitative only and capture the frequency of a chosen attribute/s. The intensity of the attributes within the samples as a function of time are not measured. Further, Vidal, Antúnez, Giménez, Medina, et al. (2016) examined the wine astringency profiles by a two-step process, where TDS (for wine astringent sub-qualities) and time averaged intensity evaluation (but only for overall astringency intensity) were done separately and then the data analysed in combination.

A method that enables dynamic measurement of attribute intensities is progressive profiling (PP) where multiple attributes can be measured at once. It was first used to monitor changes in cheese texture at chosen discrete time points during the course of oral food processing (Jack, Piggott, & Paterson, 1994). Since the evaluation is not based on contiguous measures (i.e. blocks of measures that are related or connected across time rather than measuring continuously at each point in time as time progresses), PP can measure more attributes than TI (nine attributes were evaluated in the original paper). PP has been used in texture studies of food products such as bread (De Lavergne, van Delft, van de Velde, van Boekel, & Stieger, 2015; Jourdren et al., 2016; Jourdren et al., 2017). Since wines have multiple dynamic attributes that can vary over time, PP has the capacity to be applied for sensory evaluation of wine texture, such as astringency and its sub-qualities too.

The objective of this study was to describe the dynamic sensory properties of wine astringent sub-qualities using PP. Fifteen Australian, commercially available wines were selected from a larger group to encompass wines with as many different astringency properties as possible. The dynamic astringency sensory profiles were described using terms defined by the trained PP panel. These wines were chemically characterized by measuring wine composition, including phenolic profiles, to assist in the interpretation of any identified sensory differences.

2. Materials and methods

2.1. Wine samples

In this study, 15 commercial wines (13 red and two Rosés) from eleven Australian wine regions were used (Table 1). These wines were selected by bench top tasting of a larger group of 52 wines using six experts (3 male and 3 female, all had > 5-years of wine industry experience), so as to have a set of wines with as many differences in astringency properties as possible and covered eleven different grape varieties, including two blends.

2.2. Chemicals

Reagents used for the methyl cellulose precipitable (MCP) tannin method and high performance liquid chromatography (HPLC) including (−)-epicatechin ≥97% which was used as the quantitation standard for both MCP and HPLC were from Sigma-Aldrich (Castle Hill, NSW, Australia). HPLC solvents were from Merck Pty. Ltd. (Kilsyth, VIC,

Table 1
The 15 Australian commercial wines used to assess astringency sub-quality by Progressive Profiling.

Wine No.	Category	Region	Variety	Vintage
1	Red	Langhorne Creek	Malbec	2015
2	Red	Heathcote	Sagrantino	2012
3	Red	McLaren Vale	Grenache	2014
4	Red	McLaren Vale	Tempranillo	2015
5	Red	Yarra Valley	Pinot Noir	2015
6	Red	Yarra Valley	Cabernet Sauvignon and Merlot blend	2014
7	Red	South Eastern Australia	Merlot	2016
8	Red	Adelaide Hills	Nebbiolo	2016
9	Red	Tumbarumba	Pinot Noir	2014
10	Red	Barossa Valley	Shiraz	2012
11	Red	Riverland	Lagrein	2016
12	Red	Barossa Valley	Graciano	2015
13	Red	Margaret River	Cabernet Sauvignon	2014
14	Rosé	Clare Valley	Grenache, Cabernet Sauvignon, Malbec and Merlot blend	2016
15	Rosé	McLaren Vale	Grenache	2016

Australia), and tartaric acid (H_2T) and grape seed extract (GSE) were purchased from Tarac Technologies (Nuriootpa, SA, Australia). Milli-Q water (Millipore, North Ryde, NSW, Australia) was used for the preparation of solutions.

2.3. Sensory assessment using modified progressive profiling (PP)

A conventional PP method (Jack et al., 1994) was modified for this study to characterize the dynamic astringency profile of wines. The PP sensory experiment comprised of ten 2 h training and four 2 h formal evaluation sessions, held twice weekly commencing at 10 am at the University of Adelaide's Waite campus sensory facility. A progressive profiling sensory panel (n = 8, 2 male and 6 female; age range 28–70 years, average age 52 years) was assembled from our laboratory's ISO screened external panel who had extensive red wine descriptive analysis panel experience and based on their availability for testing. Training is crucial because astringency is a complex sensation for untrained individuals to comprehend, and normal consumers also find it hard to describe different sub-qualities of astringency perception (Vidal, Giménez, Medina, Boido, & Ares, 2015). During the training sessions, a list covering a wide range of attributes regarding astringency sensation were initially gathered by the researchers based on the previous literature and provided to the panel (Ferrer-Gallego et al., 2014; Gawel et al., 2000; Gawel et al., 2001; Pickering & Demiglio, 2008; Sáenz-Navajas et al., 2017; Vidal et al., 2004; Vidal, Antúnez, Giménez, Medina, et al., 2016). The panel evaluated the 15 wines (13 red table wines and 2 rosés) twice to select relevant astringency sensory terms and defined them with reference standards to generate a final list of seven astringency related attributes (Table 2). All reference standards were assessed with panellists wearing nose clips so as to keep the conditions the same as the final wine evaluations (avoid assessors' being biased by wine aromas). The assessors were trained in the use of 15 cm line scales (anchored at 10 and 90% with 'low' and 'high' intensity, respectively) on all reference standards and 15 samples via the data collection software (RedJade, Silicon Valley, CA, USA) for PP. The panel's performance was gathered by RedJade and monitored with Panel Check (Ver.1.4.0, Matforsk, Oslo, Norway), and once the judge by sample interaction for discrimination of attributes across 3 wine samples in duplicate was not significant, formal wine evaluations commenced.

The PP timeline used in the current study was partially based on the evaluation protocol reported in a previous wine astringency characterization paper (Vidal, Antúnez, Giménez, Medina, et al., 2016) (Fig. 1). The panellists were required to click the start button of the software and ingest a mouthful of palate cleanser (plain sweetened yogurt drink) at the same time. After 20 s, a sip of distilled water was taken to remove any residual yogurt, followed by putting on a nose clip. Drinkable plain sweetened yogurt followed by a water rinse has been demonstrated to provide better sample discrimination compared to the usual alternatives (e.g. water, pectin solutions or plain crackers) (Vidal, Antúnez, Giménez, & Ares, 2016). At 40s post palate cleanser, the panellists took the entire wine sample (15 mL) in their mouth, swirled the sample gently for 5 s with a standardized vertical tongue movement and at the 45th sec, undertook a 10-sec evaluation period before expectoration. A cycle of a 20 s period break followed by a 10 s period of evaluation was repeated five times to capture the contiguous, dynamic changes in astringency over time, resulting in 6 data points per sample over the course of 205 s. For ease of assessment, dynamic assessment of the 7 attributes per sample were split into two rounds. The attributes of pucker, OAI and mouth coat were assessed in the first round, and dry, grippy, adhesive and graininess in the second round ('mouth coat' required longer time to be estimated according to the feedback from the panel). Therefore, in each replicate, every wine sample was prepared identically in two glasses for these two rounds of evaluation. A break of two minutes was provided along with water and the same yogurt cleansers between rounds.

All wines were evaluated in duplicate across four sessions (seven or eight samples were evaluated in a session), with replicates presented on different days. Prior to each session, the standards were used to refresh the panellists' memory of each attribute, and a warm-up wine was also provided. Wine samples were served in black ISO wine tasting glasses with four digit codes and in a randomized order, under red light, at constant ambient temperature (20 °C). Wines were evaluated in individual booths, with inter-sample breaks of two minutes and a 10-min break between every four samples. Water and drinkable plain sweetened yogurt (Vidal, Antúnez, Giménez, & Ares, 2016) were provided as palate cleansers to minimize astringency carry-over in the breaks.

Table 2
Definition of the overall astringency and sub-quality attributes with corresponding reference standards.

Attribute	Definition	Reference	Method for evaluation
Overall astringent intensity (OAI)	The level of shrinking or drawing in of all mouth surfaces.	0.3 g/L GSE[a] + 0.5 g/L H_2T[b] solutions→Low 0.9 g/L GSE + 0.5 g/L H_2T solutions→Medium (represented the 50% point on the scale) 1.5 g/L GSE + 0.5 g/L H_2T solutions→High	NA
Pucker	Reflex action of front mouth surfaces being brought together	0.7% Alum solution	Involuntary pouting.
Mouth coat	Coating of astringent film that adheres to all mouth surfaces	Banana peel	Move the cheeks & lips, and run them against the gums. Meanwhile, use tongue to feel all parts of mouth surface.
Dry	Lack of lubrication on the tongue mainly	Black tea (served at room temperature, 150 ml boiled water per tea bag, dipped for 15 s)	The tongue is dried out, but not the other parts of palate.
Grippy	Distinct lack of slip between mouth surfaces resulting in the inability to easily drag tongue against the roof of mouth.	0.5% Alum solution	Inability to easily drag tongue against the roof of palate.
Adhesive	A sensation that mouth surfaces (notably front lips and gums) are sticking or adhering to one another.	1.5 g/L GSE + 0.5 g/L H_2T solution	Front lips are sticking or adhering to gums.
Graininess	A sensation of particulate matter that can be detected on the mouth surface, with the particles being fine, medium or coarse in size.	Corn flour →Fine grain (represented the 'low' on the scale) Semolina →Medium (represented the 50% on the scale) Polenta →Coarse (represented the 'high' on the scale)	Use tongue against the whole mouth surfaces.

[a] GSE - grape seed extract.
[b] H_2T - tartaric acid.

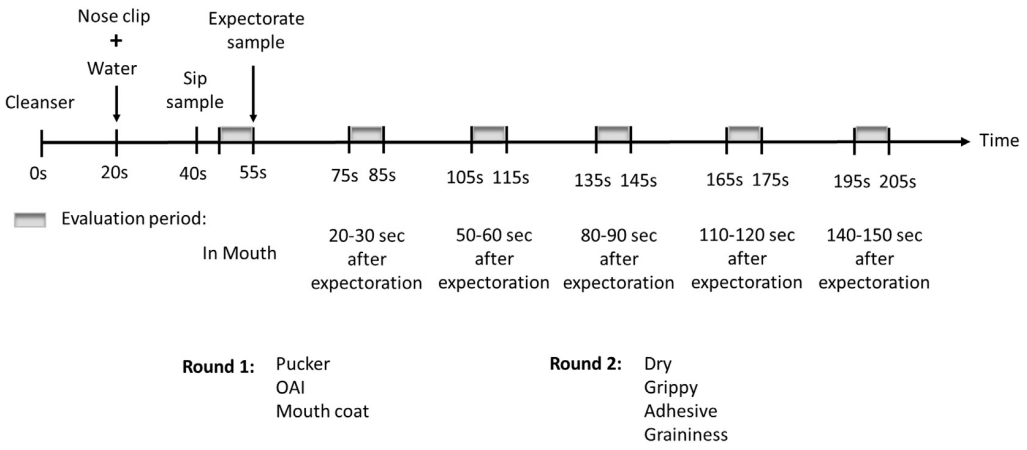


Fig. 1. Schematic representation of the modified Progressive Profiling protocol.

2.4. Wine composition

Wine samples were measured for pH and titratable acidity (TA, equivalent to tartaric acid) using a Mettler Toledo T50 Autotitrator (the pH end point for TA measurement was 8.2, Port Melbourne, VIC, Australia). The alcohol concentration and density of the samples were determined with the Anton Paar Alcolyzer Wine ME and DMA 4500 M (North Ryde, NSW, Australia). The content of residual sugars (glucose and total) were analysed by Chemwell® 2910 Automated EIA and Chemistry Analyser (Awareness Technology, Palm City, FL, USA) with the Megazyme K-FRUGL test kits (Chicago, Illinois, USA). All measurements were conducted in duplicate.

2.5. Phenolics analyses

Total tannin concentration for samples was determined by the high throughput MCP tannin method, and total phenolics through the modified Somers assay (Mercurio, Dambergs, Herderich, & Smith, 2007). Tannins from wine samples were isolated by the modified solid phase extraction (Kassara & Kennedy, 2011), and analysed using HPLC phloroglucinolysis with modifications (Kennedy & Jones, 2001; Kennedy & Taylor, 2003) (Agilent 1100 (Melbourne, VIC, Australia)) for the subunit composition, mean degree of polymerization (mDP), and molecular mass (MM (phloro)). The molecular mass of tannins was also measured by gel permeation chromatography (MM (GPC)) (Kennedy & Taylor, 2003) on an Agilent 1200 HPLC. All measurements were conducted in duplicate.

2.6. Data analyses

The chemical measures were analysed by one-way analysis of variance (ANOVA) at an alpha level of 5% with Fisher's least significant difference (LSD) post hoc test in XLSTAT (ver. 2016; Addinsoft SARL, Paris, France). The sensory data were firstly analysed by repeated measures ANOVA (RMANOVA) in SPSS statistics (ver. 23; IBM Corporation, Chicago, IL, USA). RMANOVA was conducted respectively on each attribute using periods of time as the within subjects factor and sample as between subject factor, the interaction between time and samples were also included in the model. In case of violation of the sphericity assumption of RMANOVA, Greenhouse–Geisser correction was applied. This was followed by a univariate ANOVA for each attribute at a single given time period, with sample and replicate as fixed factors, and assessor as random factor using SPSS. The effect sizes were determined by calculating partial omega squared (ω_p^2) values (Lakens, 2013) as a measure of the size of discrimination across means to determine the relative importance. The correlation (Pearson) between sensory and chemical data was calculated by XLSTAT. In order to account for the scaling effect caused by different assessors, the mixed assessor model canonical variate analysis (MAM-CVA) was analysed by RStudio (R ver. 3.5.1, Boston, MA, USA) with the software package CVAS (Version 1.0, written by Caroline Peltier on 2014-11-03, < caroline.peltier@dijon.inra.fr >), and the figure of samples and attributes loadings was presented.

3. Results and discussion

3.1. Astringency profiles of wines measured using modified progressive profiling

Examination of the sensory data of all 15 wines indicated high repeatability performance for the panel as there were no significant differences between replicates for each attribute at all six time intervals. All attribute intensities were significant for the sample effect at every time interval (Supplemental Fig. 1). The significantly different astringency profiles perceived by the panel confirmed the observations from bench top tests. An initial exploration of the sensory data was carried out through a Multiple Factor Analysis based on the seven astringency attributes of 15 wines, across the six time intervals (Supplemental Fig. 2), which showed that the wine samples clustered into two groups, either rosé or red wine. Thus, in order to further investigate the subtlety of astringency sub-quality profiles, the sensory data of the two rosés were excluded from further data analysis. All 13 red wines were analysed by RMANOVA. The results indicated that time was a significant factor influencing the sensory attributes and all seven attributes showed significantly reduced intensities ($p < 0.001$) with time (Fig. 2). The intensity of astringent sub-qualities were highly, positively related to the overall astringency intensity (OAI) as shown in the figure. This was not so surprising, since OAI is inherently composed of astringent sub-qualities. Samples averaged by time were significantly different ($p < 0.001$) for the intensity of attributes of OAI, mouth coat, dry, grippy and graininess. Only the attributes of OAI, mouth coat and grippy displayed significant time by sample interactions ($p < 0.001$, 0.05 and 0.001, respectively) (Fig. 3). This indicated that the perception of wine samples changed differently across time for these three attributes. Only a sub-sample of wines are presented in Fig. 3 for ease of interpretation, but intensities of all astringency attributes for the 13 wines are presented in Fig. 4. In terms of the attribute OAI (Fig. 3a), wine 2 and 5 showed a similarly high intensity when the samples were evaluated initially in mouth. However, the intensity of wine 5 decreased immediately after wine expectoration, but wine 2 did not. Wine 6 had a relatively low OAI when the sample was evaluated in mouth, but the intensity rose at the 2nd assessment period. A similar pattern

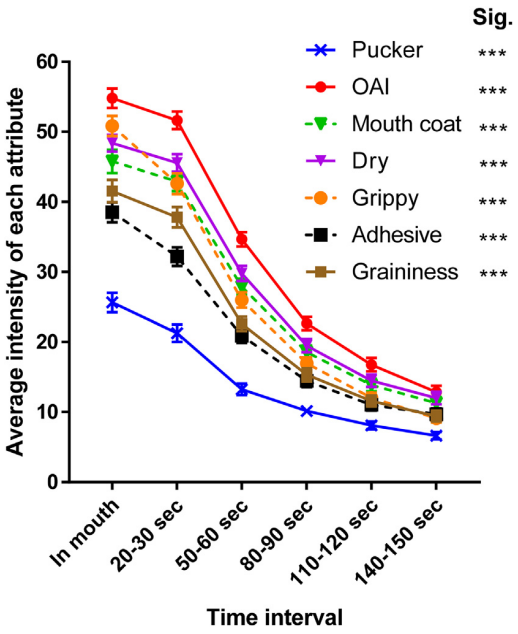


Fig. 2. Intensities (± standard error) of each attribute averaged across wines by time intervals, symbols *** denote a p value < 0.001 for RMANOVA.

with mouth coat intensity rising from in-mouth to the 20–30 s interval assessment also occurred for wine 10 (Fig. 3b). When it comes to wine 6 vs. 12, the wines illustrated a similar intensity of mouth coat at the initial evaluation period. However, the wine 12 seem to have more persistence on mouth coat. In terms of grippy (Fig. 3c), although the initial intensities of all displayed wines were different, wine 8 and 12 decay initially more rapidly than others on this attribute.

In order to account for the effect of assessors that were not included in RMANOVA, individual attributes at each time point were further analysed with univariate ANOVA (with sample and replicate as fixed factor, and assessor as random factor). There was no significant difference between two evaluated replicates for all attributes, but a significant influence of assessor was found in this study. This was especially notable, at the later period of wine assessment, where the difference between assessors trended to be larger. This observation is probably due to genetic differences between the panellists, such as saliva flow rate, types of saliva proteins and haze developing capacity etc. (Condelli et al., 2006; Fischer, Boulton, & Noble, 1994; Lee, Ismail, & Vickers, 2012). The assessors with higher saliva flow rates may recover more quickly from the astringency sensation (Condelli et al., 2006) thus resulting in the greater difference between panellists over time. The results indicated that, within red wine samples, all attributes were still significant for the sample effect when in mouth and just after expectoration (Fig. 4). However, the discrimination of wine samples became less distinct for several attributes with time. For instance, the wine samples were not differentiated in terms of adhesive, mouth coat and dry at the 4th, 5th and 6th time period, respectively. The sub-quality of pucker showed a distinctive phenomenon where it could be significantly differentiated at the initial two and the last time periods, but not in the middle three. This phenomenon might be caused by different acid conditions (e.g. pH) in wines, because the acids could result in an involuntary pouting (Lawless, Horne, & Giasi, 1996). The involuntary pouting was the definition and perception of pucker in this study. As a result, the involuntary pouting was clearly different across samples at the initial two evaluation periods, but the pucker sensation was possibly suppressed by other sub-qualities until other astringency sensations weaken at the end. However, this speculation needs to be confirmed.

To further understand the different astringent sub-qualities, effect

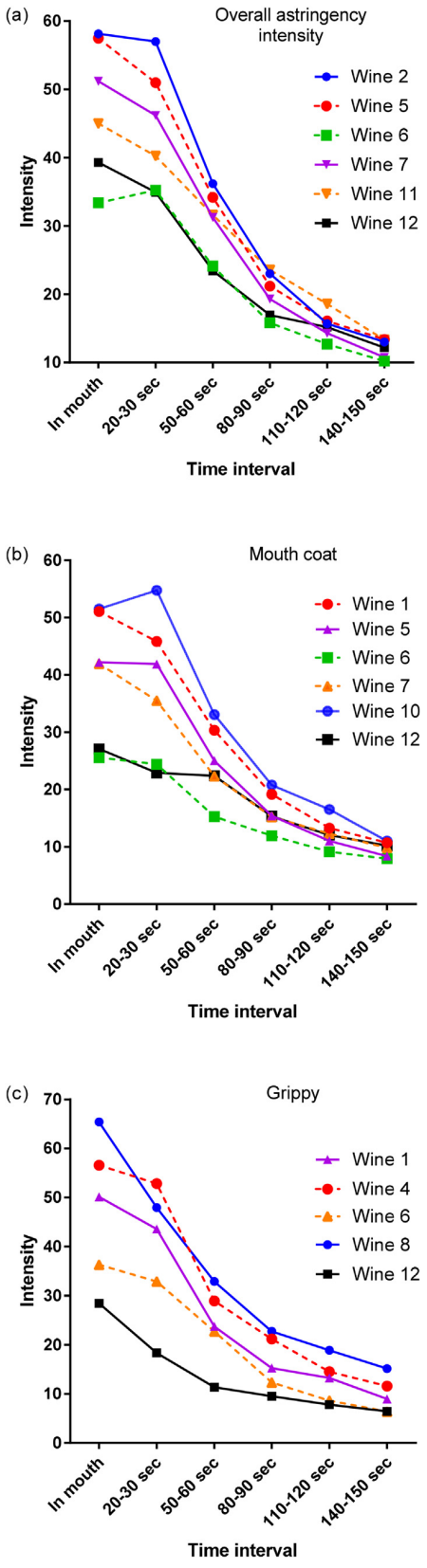
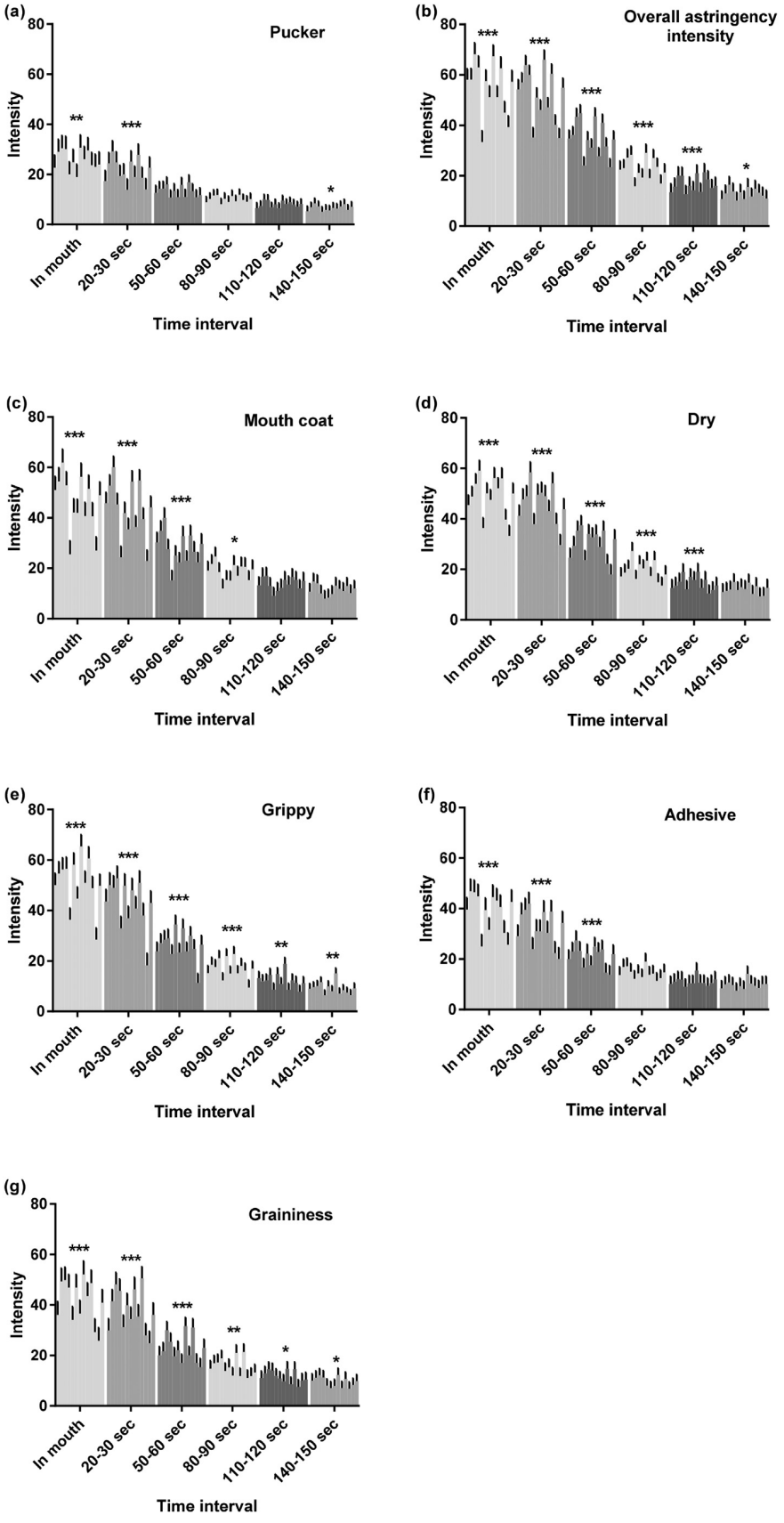


Fig. 3. Dynamic intensity of wines on three attributes which had a significant difference in RMANOVA (Time * Sample). (a) Overall astringency intensity (b) Mouth Coat (c) Grippy.



(caption on next page)

Fig. 4. The intensities of all 13 red wines across time on each attribute (wine number 1–7 and then 9–14 from left to right). Symbols *, ** and *** denote for p value < 0.05, 0.01 and 0.001 respectively from the analyses of univariate ANOVA. (a) Pucker (b) Overall astringency intensity (c) Mouth Coat (d) Dry (e) Grippy (f) Adhesive (g) Graininess. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

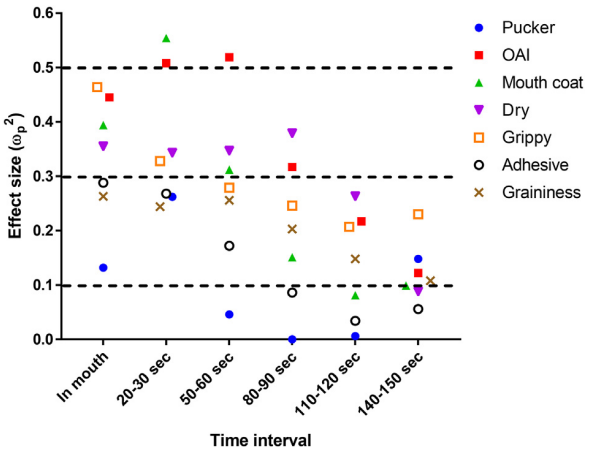


Fig. 5. The effect sizes calculated as partial omega-squared (ω_p^2) for each sensory attribute at every single evaluation period of all 13 red wines. The dotted lines at the values of 0.1, 0.3 and 0.5 interpreted small, moderate and large effect sizes, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sizes were computed as ω_p^2 values to show the relative importance of these sub-qualities at different time intervals, because ω_p^2 is more conservative and less biased than partial-eta squared value (Lakens, 2013). Lakens suggests "that eta squared is an uncorrected effect size estimate that estimates the amount of variance explained based on the sample, and not based on the entire population". It has been suggested that omega squared (ω^2) will correct for this bias, even though it is, at best, only a less biased estimate. The general guide to interpret effect sizes are the corresponding values of 0.1, 0.3 and 0.5 to small, moderate and large effect sizes, respectively (Khalilzadeh & Tasci, 2017). As shown in Fig. 5, the relative importance of each astringent sub-quality varied at different evaluation periods. The period "in mouth" showed the largest discrimination of wines based on the sub-qualities of grippy, adhesive and graininess relative to the six other time periods. The largest effect size of pucker and mouth coat was at the period between 20 and 30 s after wine expectoration, by OAI between 50 and 60 s, and by dry between 80 and 90 s, respectively. Additionally, as the effect sizes were calculated within a single sensory panel, the effect sizes across attributes can be compared to each other. The sub-qualities of pucker, adhesive and graininess solely had small effect sizes at every evaluation period, which means they were relatively less important to discriminate the 13 red wine samples compared to other attributes. Furthermore, greatest discrimination of wine at each time interval differed by attribute. In mouth, grippy had most impact, followed by mouth coat 20 s after expectoration, then OAI, drying and then grippy at the final time period. Finally, it would be worth comparing the results from modified PP and TDS in the future, to investigate whether the attribute with the highest effect size from modified PP is the same as the dominant attribute from TDS at each time interval (Esmerino et al., 2017).

In terms of wine sample distribution, a multidimensional product map of the CVA was used in this study to visualise how the wines differed in their astringency profile with time interval. CVA shows the product discrimination based on the ratio between product variability and dispersion around the product means (while no consideration of dispersion around those means occurs in the principal component analysis). Meanwhile, the MAM accounts for the scaling effect and results in an increase of power (Brockhoff, Schlich, & Skovgaard, 2015; Peltier, Visalli, & Schlich, 2018). A MAM-CVA of the 13 red wines (Fig. 6) displays all significantly different sensory attributes from the six time intervals. This figure provides a broad look of wine sample profiles which are more difficult to view from Fig. 4, and demonstrates that the red wines are distinguished from each other based on subtle texture differences. For instance, wine 8 in the bottom right hand corner of the plot was set apart from other wine samples according to mouth coat at the second time interval, while wine 12, in the top left hand quadrant, was also clearly distinguished from others based on its lower intensity of mouth coat. Wine 4 and 10 appearing in the top right hand quadrant were similar to each other, but different to other wines owing to the perceived attributes dry (2nd and 4th time intervals) and adhesive (2nd time interval). However, this MAM-CVA figure verified previous literatures suggesting that the astringency sub-quality differences of wines were subtle, because numerous samples were not highly discriminated (the sample loading of these were close together and the explanation of the second dimension of the figure was around 15%).

This study was the first to utilise modified PP to evaluate the astringent sub-qualities of wines. Compared to other existing sensory methods, modified PP enables the evaluation of more attributes at the same time than TI, which is important for reducing times (rounds) of evaluations and thus avoid fatiguing the assessors. The modified PP has the ability to capture the quantitative intensity data of wine astringency profiles rather than categorical data measured by TDS or TCATA. It may provide a better understanding of the relationships between the subtle differences of wine astringency sub-qualities and chemical composition, because the data of chemical measurement are normally quantitative as well. However, modified PP is still limited by two-rounds of evaluation per sample for 7 attributes, because the astringency sensations (especially its sub-qualities) are complex and require more time to evaluate. In addition, as this study did not actually carry out a comparison between different sensory methods on characterizing wine astringency profiles, further investigations are required. As modified PP showed the capacity to generate the intensity measurements of astringency profiles in wine products, its use in further studies could examine a larger number of wines made from the same grape variety to understand typical varietal textural characters. It would be of interest to examine whether modifications to the current protocol i.e. reducing the time interval length and/or time between evaluations provides enhanced information about subtle wine astringent sub-quality.

3.2. Chemical analyses

The wine composition and phenolic profiles are shown in Tables 3 and 4, respectively. For each chemical parameter, the wine samples were significantly different, where p values of every column in both table were < 0.001. In Table 4, the total tannin concentration of the two rosé wines were close to 0 g/L which was quite distinct from the red wines. Tannins in wine are a crucial contributor to astringency sensation (Monteleone, Condelli, Dinnella, & Bertuccioli, 2004), thus the astringent profiles of rosés in this study were obviously different to red wines (Supplementary Fig. 1 and 2). This is not surprising as rosés are a distinct wine style from dry red table wines and are produced with minimal skin and seed contact. To establish any correlations between the subtle red wine mouthfeel attribute differences and chemical measures, the two rosés were excluded in the following analysis. A correlation matrix was plotted to illustrate the relationship between sensory and chemical data of the 13 red wines (Fig. 7). For simplicity, for each of the 7 attributes only one sensory evaluation time interval that represented the largest perceived difference between wine samples was selected to analyse with the wine's chemical measures. In other words, attributes at time intervals with the highest effect sizes were

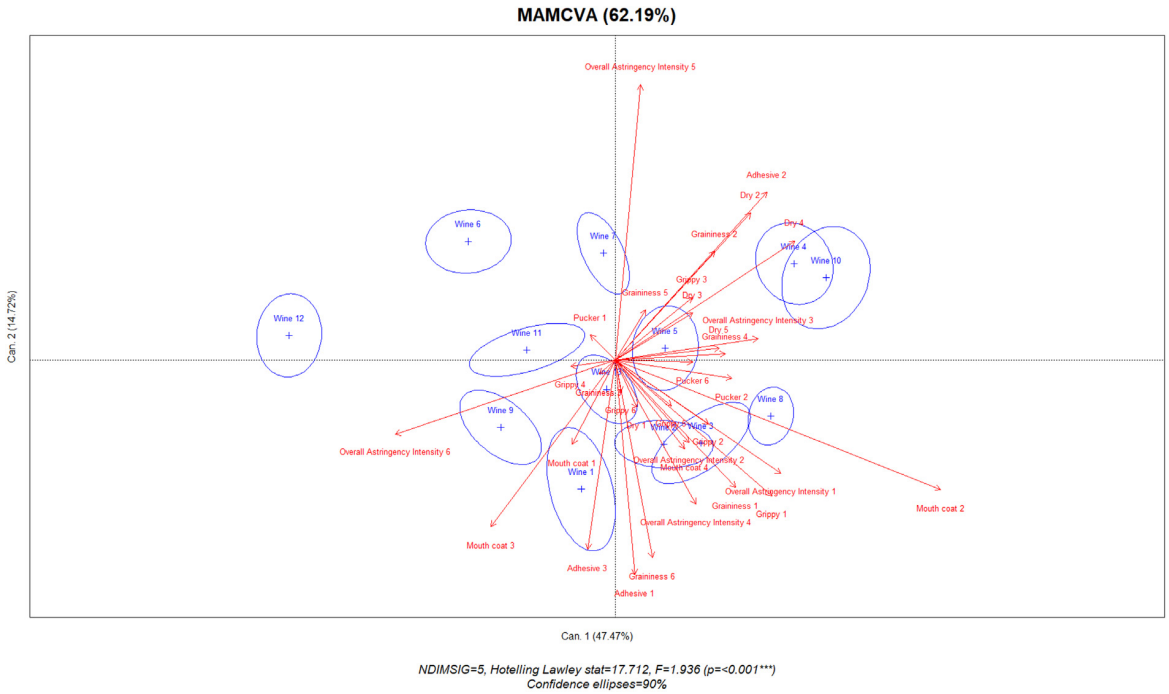


Fig. 6. The MAM-CVA of 13 red wines for all significantly different sensory attributes from six time intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correlated with chemical measures of the wine. Wine tannins (including total concentration, percentage of epigallocatechin, tannin mDP and molecular mass) were highly correlated with OAI. Tannin mDP indicates the average length of depolymerisable tannin chains and molecular mass the size of the whole tannin population, respectively. Tannin from wine is generally less depolymerisable than grape tannin, thus the compositional insights relate only to that portion which is depolymerised (19–47% in these wines), but nonetheless this depolymerisable sub-class of tannins provides compositional insights. Generally, larger condensed tannins have been found to have stronger binding capacity for saliva proteins (Ma et al., 2016), which can often result in a higher astringency sensation. Epigallocatechin is a sub-unit of tannins which is derived from grape skin (Adams, 2006). A higher percentage of epigallocatechin was associated with higher overall astringency intensity in this study, possibly because more exposed hydroxyls provided more binding sites for saliva proteins (by hydrogen bonding). The intensity of pucker was negatively correlated to wine sugars, but not influenced significantly by acids as has been speculated previously. The literature has demonstrated that sugars can suppress the perception of acid and astringency (Courregelongue, Schlich, & Noble, 1999) supporting this observation. The correlation analysis also indicated that intensity of dry was positively correlated with total tannin concentration, tannin mDP and molecular mass, supporting previous reports (McRae, Schulkin, Kassara, Holt, & Smith, 2013; Vidal, Francis, et al., 2004). The intensity of dry positively associated with pH, which differed to the work from Gawel et al. (2014). This difference was possibly caused by different wine samples (white wines used in their study rather than red wines) and different evaluation approaches (time-averaged drying intensity in their work rather than the dynamic profile). The alcohol concentration positively correlated to the sub-

Table 3
Composition of wines in the current study. [1]

Wine No.	Density (g/cm3)	Alcohol% (V/V)	pH	TA (g/L)	Glucose (g/L)	Total residual sugar (g/L)
1	0.99099 ± 0.00002 i	14.49 ± 0.00 e	3.75 ± 0.00 b	6.01 ± 0.03 h	0.22 ± 0.01 ij	0.44 ± 0.01 gh
2	0.99047 ± 0.00002 k	14.77 ± 0.03 b	3.36 ± 0.01 i	6.25 ± 0.03 ef	0.22 ± 0.00 ij	0.43 ± 0.01 h
3	0.99205 ± 0.00004 f	14.88 ± 0.01 a	3.43 ± 0.01 h	6.36 ± 0.04 cd	0.48 ± 0.00 e	0.97 ± 0.02 e
4	0.99269 ± 0.00001 c	14.08 ± 0.01 f	3.94 ± 0.00 a	6.36 ± 0.02 cd	0.07 ± 0.00 kl	0.18 ± 0.01 ij
5	0.99354 ± 0.00000 a	12.95 ± 0.01 l	3.49 ± 0.00 ef	6.18 ± 0.05 fg	0.95 ± 0.01 c	1.47 ± 0.01 d
6	0.99155 ± 0.00000 g	13.57 ± 0.00 i	3.52 ± 0.00 d	5.88 ± 0.06 i	0.34 ± 0.01 g	0.74 ± 0.03 f
7	0.99329 ± 0.00002 b	13.97 ± 0.03 g	3.49 ± 0.00 ef	6.32 ± 0.01 de	1.41 ± 0.06 b	2.91 ± 0.07 c
8	0.98983 ± 0.00003 l	14.76 ± 0.02 b	3.62 ± 0.00 c	6.28 ± 0.03 de	0.08 ± 0.00 k	0.34 ± 0.01 hi
9	0.99116 ± 0.00000 h	13.55 ± 0.00 i	3.53 ± 0.01 de	5.66 ± 0.02 j	0.43 ± 0.02 f	0.69 ± 0.00 f
10	0.99246 ± 0.00003 e	14.65 ± 0.01 c	3.53 ± 0.01 d	6.41 ± 0.04 bc	0.27 ± 0.00 h	0.59 ± 0.04 fg
11	0.99261 ± 0.00004 d	12.99 ± 0.02 k	3.34 ± 0.01 i	7.17 ± 0.00 a	0.02 ± 0.00 l	0.13 ± 0.05 j
12	0.99093 ± 0.00001 i	14.59 ± 0.00 d	3.45 ± 0.01 gh	6.17 ± 0.06 g	0.59 ± 0.04 d	0.96 ± 0.02 e
13	0.99097 ± 0.00003 i	14.50 ± 0.02 e	3.47 ± 0.00 fg	6.48 ± 0.03 b	0.19 ± 0.00 j	0.46 ± 0.00 gh
14	0.99151 ± 0.00007 g	13.23 ± 0.01 j	2.98 ± 0.04 j	7.16 ± 0.06 a	1.94 ± 0.03 a	6.80 ± 0.27 a
15	0.99055 ± 0.00001 j	13.77 ± 0.03 h	2.96 ± 0.01 j	7.23 ± 0.03 a	0.24 ± 0.00 hi	3.65 ± 0.00 b
F Value	4468.270	3454.942	2301.306	185.890	741.846	1281.413
p Value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] Data are means (± standard deviation), analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. A post-hoc test was run across wines within each column; values followed by the same letter in a column are not significantly different.

Table 4
The phenolic profiles of wines in the current study. [1]

Wine No.	Total phenolics (absorbance unit)[2]	Total tannin (g/L)[3]	epigallocatechin(%)[4]	epicatechin gallate (%)[4]	Tannin mDP[4]	Tannin MM (phloro) (g/mol)[4]	Mass conversion (%) of phloroglucinolysis[4]	Tannin MM (GPC) (g/mol)[5]
1	51.80 ± 2.99 d	1.58 ± 0.52 def	31.3 ± 0.7 c	2.1 ± 0.1 e	8.11 ± 0.11 bc	2419 ± 30 b	31.0 ± 0.0	1909 ± 29 f
2	61.12 ± 3.18 b	2.81 ± 0.11 bc	23.9 ± 1.1 fg	2.7 ± 0.2 c	5.38 ± 0.077 ef	1604 ± 22 de	26.4 ± 0.1	2346 ± 2 b
3	58.42 ± 2.10 bc	2.48 ± 0.30 bcd	29.4 ± 0.0 d	2.0 ± 0.0 e	7.91 ± 0.54 bc	2356 ± 160 b	29.1 ± 5.0	2200 ± 40 c
4	57.35 ± 2.64 c	4.38 ± 1.41 a	30 ± 1.2 cd	2.3 ± 0.0 de	14.30 ± 1.95 a	4268 ± 583 a	22.2 ± 1.6	2404 ± 15 a
5	50.98 ± 1.86 d	1.89 ± 0.13 cdef	9.5 ± 0.4 i	3.1 ± 0.1 b	4.59 ± 0.19 f	1360 ± 58 e	43.9 ± 8.3	1916 ± 15 f
6	40.00 ± 1.35 f	1.26 ± 0.15 ef	24.5 ± 0.4 f	1.5 ± 0.0 f	4.06 ± 0.18 f	1204 ± 54 e	19 ± 0.9	1744 ± 0 g
7	51.60 ± 1.82 d	2.12 ± 0.37 cde	22.4 ± 0.4 g	3.2 ± 0.3 b	6.44 ± 0.35 de	1923 ± 108 cd	38.4 ± 0.0	1974 ± 17 e
8	41.10 ± 1.78 f	2.17 ± 0.25 cde	31.3 ± 1.0 c	2.4 ± 0.2 d	7.37 ± 0.21 bcd	2204 ± 62 bc	47.4 ± 5.4	2443 ± 23 a
9	48.88 ± 1.94 d	1.70 ± 0.27 def	9.4 ± 0.4 i	1.4 ± 0.0 f	4.31 ± 0.14 f	1266 ± 43 e	38.3 ± 4.9	1945 ± 5 ef
10	71.13 ± 3.57 a	3.28 ± 0.53 b	27.4 ± 0.4 e	3.7 ± 0.2 a	6.83 ± 0.46 cd	2052 ± 141 bc	19.6 ± 0.7	2187 ± 20 c
11	45.23 ± 1.23 e	1.94 ± 0.03 cdef	34.2 ± 0.1 b	2.0 ± 0.1 e	8.20 ± 0.56 b	2451 ± 169 b	21.6 ± 4.5	1924 ± 20 f
12	38.13 ± 1.43 f	1.05 ± 0.23 f	19.4 ± 1.3 h	2.5 ± 0.2 cd	4.38 ± 0.22 f	1300 ± 63 e	21.4 ± 0.7	1560 ± 2 h
13	50.37 ± 2.15 d	2.05 ± 0.01 cde	36.2 ± 0.7 a	1.7 ± 0.1 f	7.61 ± 0.36 bcd	2272 ± 104 bc	23.2 ± 0.4	2047 ± 17 d
14	5.97 ± 0.48 h	0.00 ± 0.05 g	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
15	12.95 ± 1.00 g	0.01 ± 0.16 g	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
F Value	49.867	13.180	244.954	45.852	37.971	367.743	N/A	38.070
p Value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	N/A	< 0.001

[1] Data are mean (± standard deviation), analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. A post-hoc test was run across wines within each column; values followed by the same letter in a column are not significantly different.
[2] Determined by modified Somers assay.
[3] Determined by high throughput MCP tannin method.
[4] Determined by phloroglucinolysis.
[5] Determined by gel permeation chromatography at 50% elution.

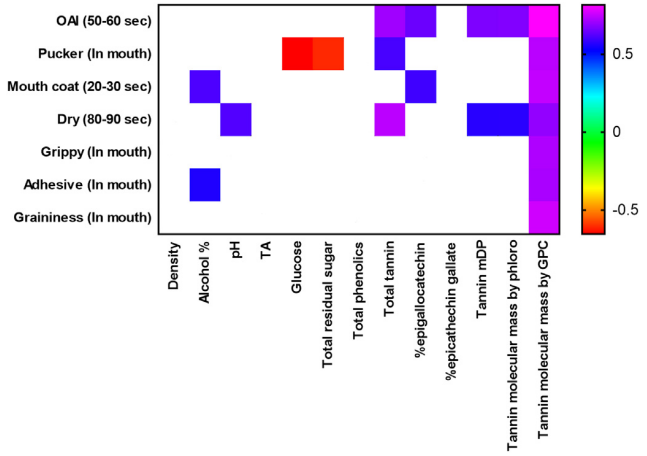


Fig. 7. Correlation matrix (Pearson) between sensory and chemical data of 13 red wines. Only correlation with a significance level alpha < 0.05 were included in the figure. No colour for a value means no significant correlation was found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

qualities of mouth coat and adhesive. The tannin MM (GPC) had significant positive correlation (the correlation coefficient range was from 0.69 to 0.82) to all sensory attributes, but MM (phloro) only related to two attributes. The data from the measurement of GPC was more accurate than phloroglucinolysis, because GPC measured average size of all tannins by hydrodynamic volume. As discussed, the tannin MM (phloro) was calculated by summing tannin subunits, and the low mass conversion of wine tannins by phloroglucinolysis means that the molecular mass by this method only relates to a sub-portion of all tannin.

Overall, the chemical analyses have provided an insight into the compositional factors affecting the sensory assessment by modified PP in this study. Some correlations between chemical factors and sub-quality sensations have been indicated. However, wine is a very complex matrix, and consists of other components (such as proteins and polysaccharides), and the exploration of relationships between other matrix composition and astringency sub-qualities would be warranted.

4. Conclusions

This study was the first to utilise modified PP to evaluate the astringent sub-qualities in a set of commercial red wines made from different grape varieties. Modified PP provided a tool for the intensity measurements of astringency profiles and showed the wines differed in astringency sub-quality (mouth coat and grippy) intensity development over time and that these were positively related to the overall astringency intensity. Greatest discrimination of wine at each time interval differed by attribute. For example, at the in mouth time interval, grippy had most impact, while mouth coat did at 20 s after expectoration. Furthermore, the relative importance of each astringent sub-quality varied at different evaluation periods. As such, progressive profiling has helped provide a better understanding of the relationships between the subtle differences of wine astringency sub-qualities and chemical composition. This knowledge on wine astringent sub-qualities may provide insights for the wine industry on how to evaluate and modify wine texture.

Acknowledgements

The authors appreciate the funding of Wine Australia (AGW Ph1605). Wine Australia invests in and manages research, development, and extension on behalf of Australia's grape growers and winemakers and the Australian government. The sensory assessment was approved by the Human Research Ethics Committee of the University of Adelaide (project number: H-2016-084). We are thankful for the assistance from The Australian Wine Research Institute given by Keren Bindon, Stella Kassara, and Bo Teng, Lukas Danner, Victorien Onillon and all members of the sensory panel. We also thank Bleasdale, d'Arenberg, Helen & Joey, Jacobs Creek, Jim Barry, King and Country, Rock of Wisdom, Tscharke, Vasse Felix and Wirra Wirra for the donation of wine samples (in alphabetical order).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.02.041.

References

Adams, D. O. (2006). Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture, 57*(3), 249–256.

Bajec, M. R., & Pickering, G. J. (2008). Astringency: Mechanisms and perception. *Critical Reviews in Food Science and Nutrition, 48*(9), 858–875.

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology & Medicine, 13*(2), 184–196.

Breslin, P. A. S., Gilmore, M. M., Beauchamp, G. K., & Green, B. G. (1993). Psychophysical evidence that oral astringency is a tactile sensation. *Chemical Senses, 18*(4), 405–417.

Brockhoff, P. B., Schlich, P., & Skovgaard, I. (2015). Taking individual scaling differences into account by analyzing profile data with the mixed assessor model. *Food Quality and Preference, 39*, 156–166.

Castura, J. C., Antúnez, L., Giménez, A., & Ares, G. (2016). Temporal check-all-that-apply (TCATA): A novel dynamic method for characterizing products. *Food Quality and Preference, 47*, 79–90.

Condelli, N., Dinnella, C., Cerone, A., Monteleone, E., & Bertuccioli, M. (2006). Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine samples. *Food Quality and Preference, 17*(1), 96–107.

Courregelongue, S., Schlich, P., & Noble, A. C. (1999). Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food Quality and Preference, 10*(4), 273–279.

De Lavergne, M. D., van Delft, M., van de Velde, F., van Boekel, M. A., & Stieger, M. (2015). Dynamic texture perception and oral processing of semi-solid food gels: Part 1: Comparison between QDA, progressive profiling and TDS. *Food Hydrocolloids, 43*, 207–217.

Duizer, L., Bloom, K., & Findlay, C. (1996). Dual-attribute time-intensity measurement of sweetness and peppermint perception of chewing gum. *Journal of Food Science, 61*(3), 636–638.

Esmerino, E. A., Castura, J. C., Ferraz, J. P., Tavares Filho, E. R., Silva, R., Cruz, A. G., et al. (2017). Dynamic profiling of different ready-to-drink fermented dairy products: A comparative study using temporal check-all-that-apply (TCATA), temporal dominance of sensations (TDS) and progressive profile (PP). *Food Research International, 101*, 249–258.

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. *Food Research International, 62*, 1100–1107.

Kallithraka, S., Bakker, J., Clifford, M. N., & Vallis, L. (2001). Correlations between saliva protein composition and some T–I parameters of astringency. *Food Quality and Preference, 12*(2), 145–152.

Fischer, U., Boulton, R., & Noble, A. (1994). Physiological factors contributing to the variability of sensory assessments: Relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Quality and Preference, 5*(1–2), 55–64.

Gawel, R., Day, M., Van Sluyter, S. C., Holt, H., Waters, E. J., & Smith, P. A. (2014). White wine taste and mouthfeel as affected by juice extraction and processing. *Journal of Agricultural and Food Chemistry, 62*(41), 10008–10014.

Gawel, R., Iland, P. G., & Francis, I. L. (2001). Characterizing the astringency of red wine: A case study. *Food Quality and Preference, 12*(1), 83–94.

Gawel, R., Oberholster, A., & Francis, I. L. (2000). A 'Mouth-feel Wheel': Terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research, 6*(3), 203–207.

Guinard, J.-X., Pangborn, R. M., & Lewis, M. J. (1986). The time-course of astringency in wine upon repeated ingestion. *American Journal of Enology and Viticulture, 37*(3), 184–189.

Harrison, R. (2018). Practical interventions that influence the sensory attributes of red wines related to the phenolic composition of grapes: A review. *International Journal of Food Science and Technology, 53*(1), 3–18.

Ishikawa, T., & Noble, A. (1995). Temporal perception of astringency and sweetness in red wine. *Food Quality and Preference, 6*(1), 27–33.

Jack, F. R., Piggott, J. R., & Paterson, A. (1994). Analysis of textural changes in hard cheese during mastication by progressive profiling. *Journal of Food Science, 59*(3), 539–543.

Jöbstl, E., O'Connell, J., Fairclough, J. P. A., & Williamson, M. P. (2004). Molecular model for astringency produced by polyphenol/protein interactions. *Biomacromolecules, 5*(3), 942–949.

Jourdren, S., Saint-Eve, A., Panouille, M., Lejeune, P., Deleris, I., & Souchon, I. (2016). Respective impact of bread structure and oral processing on dynamic texture perceptions through statistical multiblock analysis. *Food Research International, 87*, 142–151.

Jourdren, S., Saint-Eve, A., Pollet, B., Panouillé, M., Lejeune, P., Guichard, E., et al. (2017). Gaining deeper insight into aroma perception: An integrative study of the oral processing of breads with different structures. *Food Research International, 92*, 119–127.

Kallithraka, S., Bakker, J., & Clifford, M. (1997). Evaluation of bitterness and astringency of (+)-catechin and (−)-epicatechin in red wine and in model solution. *Journal of Sensory Studies, 12*(1), 25–37.

Kassara, S., & Kennedy, J. A. (2011). Relationship between red wine grade and phenolics. 2. Tannin composition and size. *Journal of Agricultural and Food Chemistry, 59*(15), 8409–8412.

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), 99–107.

Khalilzadeh, J., & Tasci, A. D. (2017). Large sample size, significance level, and the effect size: Solutions to perils of using big data for academic research. *Tourism Management, 62*, 89–96.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology, 4*, 863.

Lattey, K. A., Bramley, B. R., & Francis, I. L. (2010). Consumer acceptability, sensory properties and expert quality judgements of Australian cabernet sauvignon and shiraz wines. *Australian Journal of Grape and Wine Research, 16*(1), 189–202.

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices.* New York: Springer Science & Business Media.

Lawless, H. T., Horne, J., & Giasi, P. (1996). Astringency of organic acids is related to pH. *Chemical Senses, 21*(4), 397–403.

Lee, C. A., Ismail, B., & Vickers, Z. M. (2012). The role of salivary proteins in the mechanism of astringency. *Journal of Food Science, 77*(4), C381–C387.

Lee, C. B., & Lawless, H. T. (1991). Time-course of astringent sensations. *Chemical Senses, 16*(3), 225–238.

Lyman, B. J., & Green, B. G. (1990). Oral astringency: Effects of repeated exposure and interactions with sweeteners. *Chemical Senses, 15*(2), 151–164.

Ma, W., Waffo-Teguo, P., Jourdes, M., Li, H., & Teissedre, P. L. (2016). Chemical affinity between tannin size and salivary protein binding abilities: Implications for wine astringency. *PLoS ONE, 11*(8), e0161095.

McRae, J. M., Schulkin, A., Kassara, S., Holt, H. E., & Smith, P. A. (2013). Sensory properties of wine tannin fractions: Implications for in-mouth sensory properties. *Journal of Agricultural and Food Chemistry, 61*(3), 719–727.

Mehansho, H., Butler, L. G., & Carlson, D. M. (1987). Dietary tannins and salivary proline-rich proteins: Interactions, induction, and defense mechanisms. *Annual Review of Nutrition, 7*(1), 423–440.

Melis, M., Yousaf, N. Y., Mattes, M. Z., Cabras, T., Messana, I., Crnjar, R., et al. (2017). Sensory perception of and salivary protein response to astringency as a function of the 6-n-propylthioural (PROP) bitter-taste phenotype. *Physiology & Behavior, 173*, 163–173.

Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry, 55*(12), 4651–4657.

Monteleone, E., Condelli, N., Dinnella, C., & Bertuccioli, M. (2004). Prediction of perceived astringency induced by phenolic compounds. *Food Quality and Preference, 15*(7), 761–769.

Naczk, M., Oickle, D., Pink, D., & Shahidi, F. (1996). Protein precipitating capacity of crude canola tannins: Effect of pH, tannin, and protein concentrations. *Journal of Agricultural and Food Chemistry, 44*(8), 2144–2148.

Payne, C., Bowyer, P. K., Herderich, M., & Bastian, S. E. P. (2009). Interaction of astringent grape seed procyanidins with oral epithelial cells. *Food Chemistry, 115*(2), 551–557.

Peleg, H., Bodine, K. K., & Noble, A. C. (1998). The influence of acid on astringency of alum and phenolic compounds. *Chemical Senses, 23*(3), 371–378.

Peleg, H., & Noble, A. C. (1999). Effect of viscosity, temperature and pH on astringency in cranberry juice. *Food Quality and Preference, 10*(4–5), 343–347.

Peltier, C., Visalli, M., & Schlich, P. (2018). Enhancing canonical variate analysis by taking the scaling effect into account. *Food Quality and Preference, 64*, 88–93.

Peynaud, E., & Blouin, J. (1996). *The taste of wine: The art science of wine appreciation.* New Jersey: John Wiley & Sons.

Pickering, G. J., & Demiglio, P. (2008). The white wine mouthfeel wheel: A lexicon for describing the oral sensations elicited by white wine. *Journal of Wine Research, 19*(1), 51–67.

Piggott, J. R., Simpson, S. J., & Williams, S. A. (1998). Sensory analysis. *International Journal of Food Science and Technology, 33*(1), 7–12.

Pocock, K., Sefton, M., & Williams, P. (1994). Taste thresholds of phenolic extracts of French and American Oakwood: The influence of oak phenols on wine flavor. *American Journal of Enology and Viticulture, 45*(4), 429–434.

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., Rogeaux, M., Etiévant, P., & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Quality and Preference, 20*(6), 450–455.

Poncet-Legrand, C., Gautier, C., Cheynier, V., & Imberty, A. (2007). Interactions between flavan-3-ols and poly (L-proline) studied by isothermal titration calorimetry: Effect of the tannin structure. *Journal of Agricultural and Food Chemistry, 55*(22), 9235–9240.

Rodriguez, M., Albertengo, L., Vitale, I., & Agullo, E. (2003). Relationship between astringency and chitosan-saliva solutions turbidity at different pH. *Journal of Food Science, 68*(2), 665–667.

Rubico, S. M., & McDaniel, M. R. (1992). Sensory evaluation of acids by free-choice profiling. *Chemical Senses, 17*(3), 273–289.

Sáenz-Navajas, M.-P., Avizcuri, J.-M., Ferrero-del-Teso, S., Valentin, D., Ferreira, V., & Fernández-Zurbano, P. (2017). Chemo-sensory characterization of fractions driving different mouthfeel properties in red wines. *Food Research International, 94*, 54–64.

Scollary, G. R., Pásti, G., Kállay, M., Blackman, J., & Clark, A. C. (2012). Astringency response of red wines: Potential role of molecular assembly. *Trends in Food Science and Technology, 27*(1), 25–36.

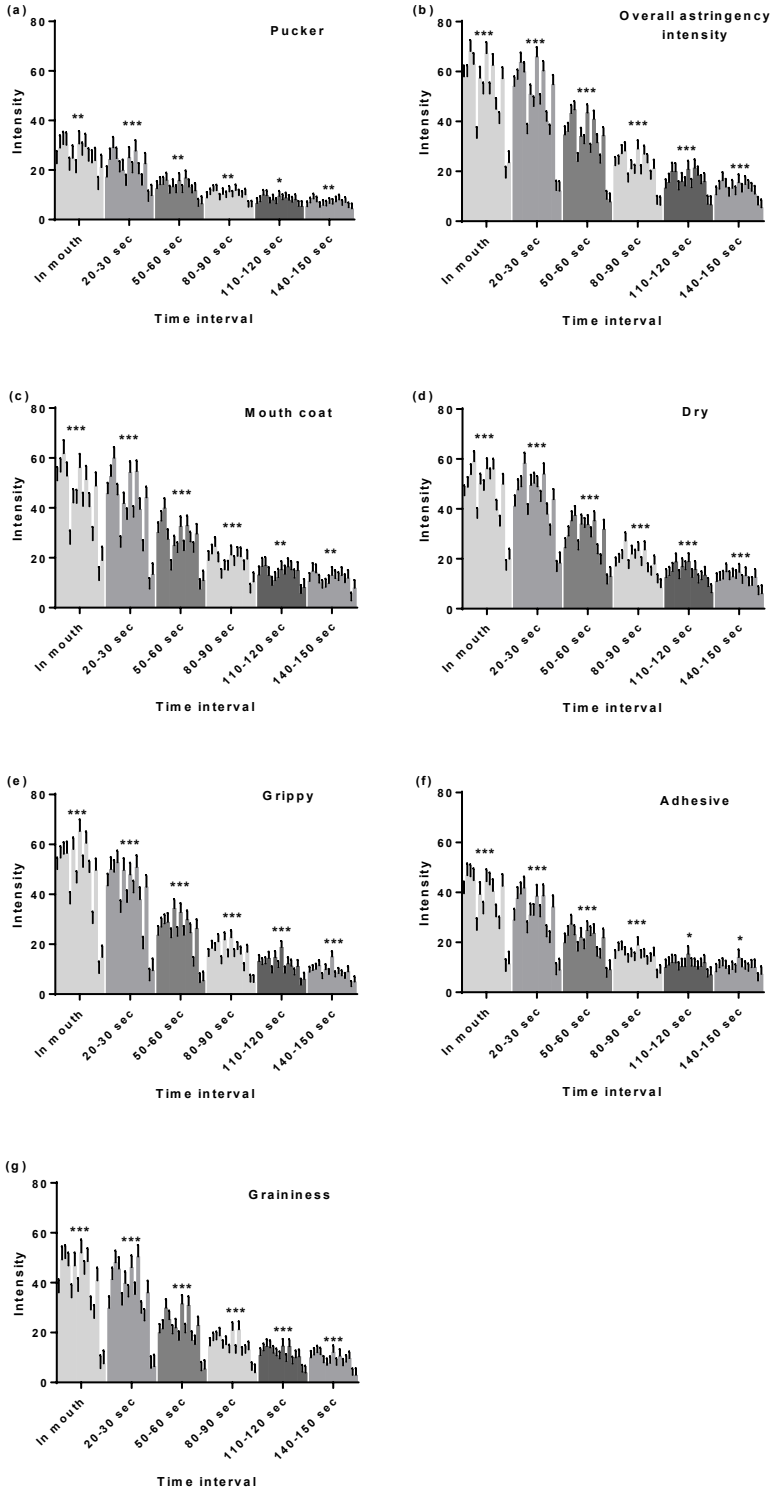
Soares, S., Brandão, E., Mateus, N., & De Freitas, V. (2017). Sensorial properties of red wine polyphenols: Astringency and bitterness. *Critical Reviews in Food Science and Nutrition, 57*(5), 937–948.

Valentová, H., Skrovánková, S., Panovská, Z., & Pokorný, J. (2002). Time-intensity studies of astringent taste. *Food Chemistry, 78*(1), 29–37.

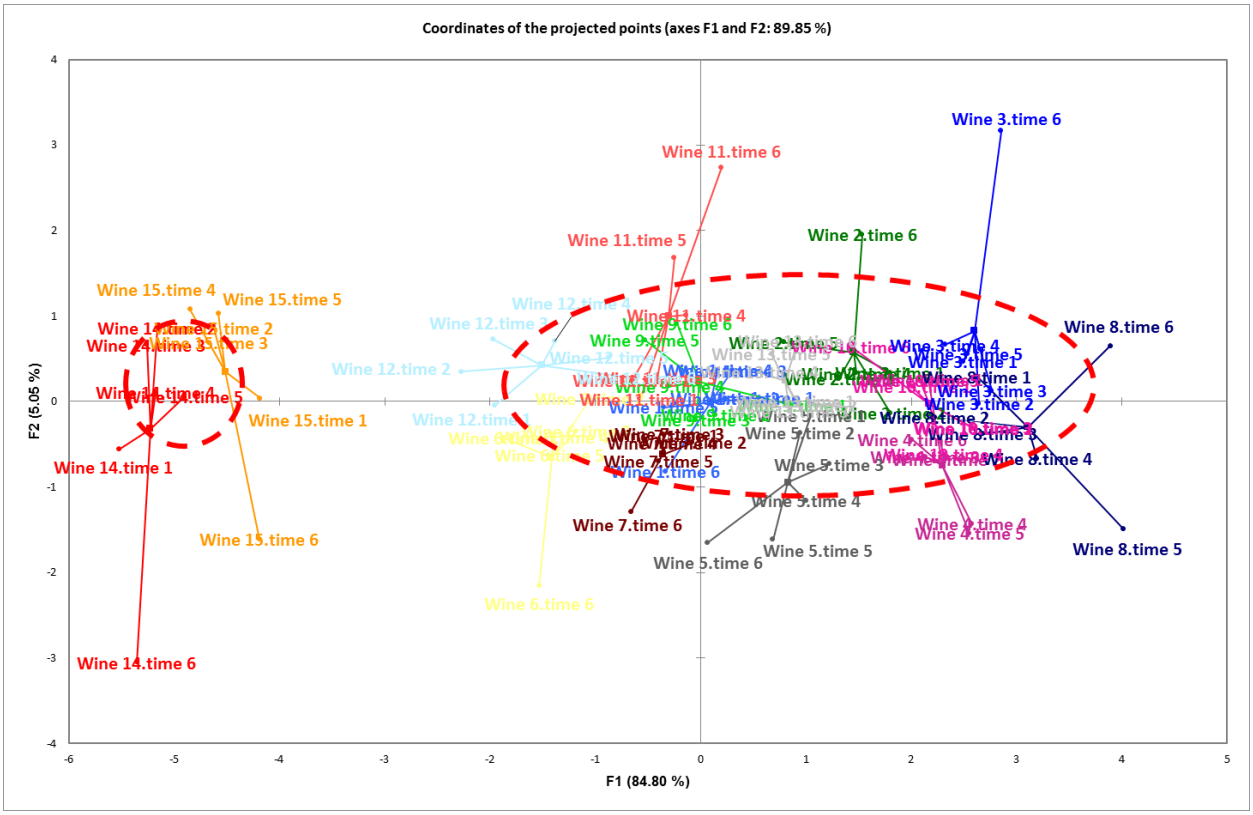
Vidal, L., Antúnez, L., Giménez, A., & Ares, G. (2016). Evaluation of palate cleansers for

astringency evaluation of red wines. *Journal of Sensory Studies, 31*(2), 93–100.
Vidal, L., Antúnez, L., Giménez, A., Medina, K., Boido, E., & Ares, G. (2016). Dynamic characterization of red wine astringency: Case study with Uruguayan Tannat wines. *Food Research International, 82*, 128–135.
Vidal, L., Giménez, A., Medina, K., Boido, E., & Ares, G. (2015). How do consumers describe wine astringency? *Food Research International, 78*, 321–326.
Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta, 513*(1), 57–65.

Supplementary Materials (Food Research International 2019, 120, 244–254):



Supplemental Figure 1. The intensities of each attribute for all 15 wines (in the wine order 1-15 from left to right) across six time intervals. Individual attributes at each time point were analysed with univariate ANOVA (with sample and replicate as fixed factor, and assessor as random factor). Symbols *, ** and *** denote the *p* values < 0.05, 0.01 and 0.001, respectively.



Supplemental Figure 2. The plot of the Multiple Factor Analysis (MFA) (calculated by XLSTAT ver. 2016; Addinsoft SARL, Paris, France) shows the sample configuration based on the seven astringency attributes of 15 wines across the six time intervals. For the calculations, each interval was considered as a separate group of variables encompassing the seven astringency attributes. In this MFA figure, the first dimension was the overall astringency intensity.

CHAPTER 3

Potato Protein Fining of Phenolic Compounds in Red Wine: A Study of the Kinetics and the Impact of Wine Matrix Components and Physical Factors

Wenyu Kang [1], Richard A. Muhlack [1], Keren A. Bindon [2], Paul A. Smith [3], Jun Niimi [1] and Susan E.P. Bastian [1,*]

[1] School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.

[2] The Australian Wine Research Institute, Hartley Grove, Urrbrae, Adelaide, SA 5064, Australia.

[3] Wine Australia, Industry House, Corner Hackney and Botanic Roads, Adelaide, SA 5000, Australia.

[*] Correspondence: sue.bastian@adelaide.edu.au; Tel.: +61-8-83136647

Molecules – 2019, 24 (24), 4578.

Statement of Authorship

Title of Paper	Potato Protein Fining of Phenolic Compounds in Red Wine: A Study of the Kinetics and the Impact of Wine Matrix Components and Physical Factors
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Kang, W., Muhlack, R. A., Bindon, K. A., Smith, P. A., Niimi, J., & Bastian, S. E. P. (2019). Potato protein fining of phenolic compounds in red wine: A study of the kinetics and the impact of wine matrix components and physical factors. Molecules, 24 (24), 4578.

Principal Author

Name of Principal Author (Candidate)	Wenyu Kang		
Contribution to the Paper	Conceived and designed the experiments, performed the experiments, analysed and interpreted the data, as well as drafted and edited the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	30/04/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Richard A. Muhlack		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Keren A. Bindon		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Paul A. Smith		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Jun Niimi		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Susan E.P. Bastian		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, reviewed and edited the manuscript, as well as acted as corresponding author.		
Signature		Date	21/05/2020

molecules

MDPI

Article

Potato Protein Fining of Phenolic Compounds in Red Wine: A Study of the Kinetics and the Impact of Wine Matrix Components and Physical Factors

Wenyu Kang [1], Richard A. Muhlack [1], Keren A. Bindon [2], Paul A. Smith [3], Jun Niimi [1] and Susan E.P. Bastian [1,*]

1 School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia; a1642412@adelaide.edu.au (W.K.); richard.muhlack@adelaide.edu.au (R.A.M.); jun.niimi@adelaide.edu.au (J.N.)
2 The Australian Wine Research Institute, Hartley Grove, Urrbrae, Adelaide, SA 5064, Australia; keren.bindon@awri.com.au
3 Wine Australia, Industry House, Corner Hackney and Botanic Roads, Adelaide, SA 5000, Australia; Paul.Smith@wineaustralia.com
* Correspondence: sue.bastian@adelaide.edu.au; Tel.: +61-8-83136647

Academic Editors: Encarna Gómez-Plaza and Rocio Gil-Muñoz
Received: 28 November 2019; Accepted: 12 December 2019; Published: 13 December 2019

Abstract: Producing wines within an acceptable range of astringency is important for quality and consumer acceptance. Astringency can be modified by fining during the winemaking process and the use of vegetable proteins (especially potato proteins) as fining agents has gained increasing interest due to consumers' requirements. The research presented was the first to investigate the effect of a potato protein dose on the kinetics of tannin and phenolic removal compared to gelatin for two unfined Cabernet Sauvignon wines. To further understand the results, the influence of the wine matrix and fining parameters (including pH, ethanol concentration, sugar concentration, temperature, and agitation) were tested according to a fractional 2^{5-1} factorial design on one of the Cabernet Sauvignon wines using potato proteins. The results from the factorial design indicate that potato protein fining was significantly influenced by wine pH, ethanol concentration, fining temperature as well as an interaction (pH × ethanol) but not by sugar content or agitation. Insights into the steps required for the optimisation of fining were gained from the study, revealing that potato protein fining efficiency could be increased by treating wines at higher temperatures (20 °C, rather than the conventional 10–15 °C), and at both a lower pH and/or alcohol concentration.

Keywords: wine; fining; potato proteins; gelatin; phenolics; tannin; Cabernet Sauvignon; design of experiments; factorial design; process optimisation

1. Introduction

Astringency (a drying and rough in-mouth sensation) is considered to be one of the most important factors driving wine quality [1] and winemaking techniques are frequently applied in order to modulate wine astringency [2]. Too much astringency may render the wine difficult to drink, whilst too little may make the wine insipid and lower in complexity [1]. One way to manipulate astringency in wine is to alter the maceration process, ultimately affecting the extent or rate of transfer of phenolic compounds from the cap to the must/wine. This could involve pre-ferment cold maceration, which can potentially extract sufficient colour and flavours into wine but minimize the extraction of larger tannins [3–5]. Alternatively, extended maceration can increase tannin extraction into wine [2] and enhance astringency and possibly body. Other methods used to control astringency are for winemakers

to make various additions to the ferments or finished wines. When astringency is lacking, winemakers may add oenological tannins (e.g., grape seed extract) to increase astringency perception. Conversely, if wine astringency is unacceptably high, astringency can be decreased or 'softened' by processes such as ageing or micro-oxygenation [6]. However, the ageing process can take long periods of time and despite being faster, micro-oxygenation is high in capital investment. Thus, a widely utilised convenient method to modify astringency is by a process known as fining. Fining involves an addition of agents in order to bind and remove phenolic components in wine in a targeted way, which, in turn, reduces astringency [7] and in doing so, possibly modifies the astringent sub-quality (nuanced differences in astringency texture perception) as well.

Traditionally, and to this day, winemakers use animal-based (i.e., gelatin, egg albumen, isinglass, and casein) and/or synthetic (i.e., polyvinylpolypyrrolidone, PVPP) products as fining agents to remove astringent compounds such as tannin in wine [7]. Nevertheless, using alternatives such as vegetable proteins has gained increased interest because of consumer demands due to the allergenic nature of animal-derived additives, or for ethical reasons [8]. One of the alternative vegetable-based fining products available on the market are potato proteins. Potatoes contain an active protein, patatin, which accounts for 40% of the total soluble potato protein and it is recovered from an aqueous by-product of potatoes [9]. Patatin ranges in molecular weight from 15 kDa to 120 kDa, with the majority around 40 kDa [10]. The patatin protein has a pI of 4.6, low solubility at wine pH [11] and has been demonstrated as a low risk for over-fining [12]. As one of the alternative agents, potato proteins have been shown to have a good capacity to fine wine phenolics and reduce grape must turbidity [12–16]. The fining efficiency of potato proteins was demonstrated to be similar to gelatin for phenolic removal and reduction of astringency sensation in commercial and model wine with added grape seed extract, but more effective than other traditional (casein, egg white, PVPP) and plant derived (pea, soy bean and rice) fining agents [12,16]. Overall flavour intensity and bitterness were not found to have been significantly affected by potato protein fining, but they can influence wine colour intensity and hue [16].

Currently, the fining efficiency of potato proteins (on phenols and turbidity) and the mechanism behind the interaction between potato proteins and components in wines are the focus of research in this field. Yet specifically, the time-dependent kinetics of fining with potato proteins have not yet been fully eludicated for red wines. In addition, wine matrixes vary greatly, but current knowledge on the use of potato proteins as fining agents for wine astringency modification has been limited to a small number of studies for a narrow range of red wine styles (Aglianico, Pinot Noir and Blaufränkisch) as well as a Cabernet Sauvignon unfined model wine [12,15,16]. Notably, the chemical environment of wine is very important for fining such as wine pH, polyphenol composition, and temperature [17]. These factors may influence the fining efficiency of potato proteins, either independently or cooperatively, and should therefore be considered for their potential influence on the efficacy of potato protein fining.

The intrinsic and extrinsic factors of wine can be investigated by the use of Design of Experiments (DoE). DoE (e.g., screening design, response surface design, and robust parameter designs, etc.) are very useful tools to examine complex processes because they allow the determination of the direct effects of each parameter and their interactions in a relatively small number of experiments [18]. Several studies to optimise vinification protocols using DoE techniques have been conducted. For instance, the optimization of using ultrasound to extract aroma compounds in white wine [19]; the sorption of wine volatile phenols by yeast lees [20]; the extraction of flavanols, phenolic acid and anthocyanin from Champagne grape varieties [21]; the control of haze-forming wine proteins by bentonite fining [22]; and anthocyanin transfer in simulated red wine fermentation [23] were all investigated by DoE. Thus, DoE can be a robust way to investigate the interactive processes which might occur in response to a technological intervention, in this case, the reduction of astringent compounds in wine by potato proteins.

The objectives of this research were firstly to investigate the kinetics of tannin and phenolic removal by fining using potato proteins at different doses on two real unfined Cabernet Sauvignon

Molecules **2019**, *24*, 4578

wines; and secondly, to investigate the interactive effects of key wine matrix variables on the fining of wine phenolics by potato protein using DoE.

2. Results and Discussion

2.1. Fining Kinetics of Potato Proteins Compared with Gelatin

Fining experiments were performed on two unfined red wines to study the changes in total phenolics and total tannin concentrations after the addition of potato proteins and gelatin (Figures 1 and 2). The data were analysed by repeated measures analysis of variance (RMANOVA) with the Huynh-Feldt correction applied (Table 1), as the values of ε were greater than 0.75 from Mauchly's sphericity test [24].

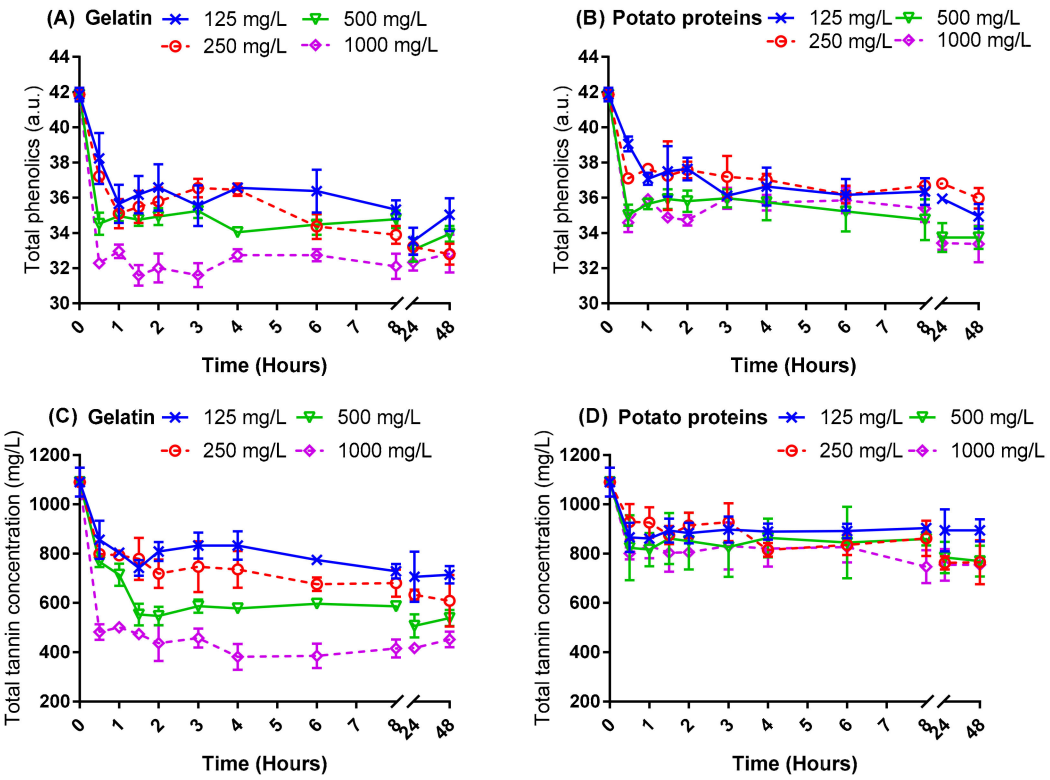


Figure 1. The fining kinetics of potato proteins compared with gelatin on unfined wine 1. (**A**,**B**) Total phenolics (absorbance units), and (**C**,**D**) total tannin (mg/L, epicatechin eq.).

Molecules **2019**, *24*, 4578

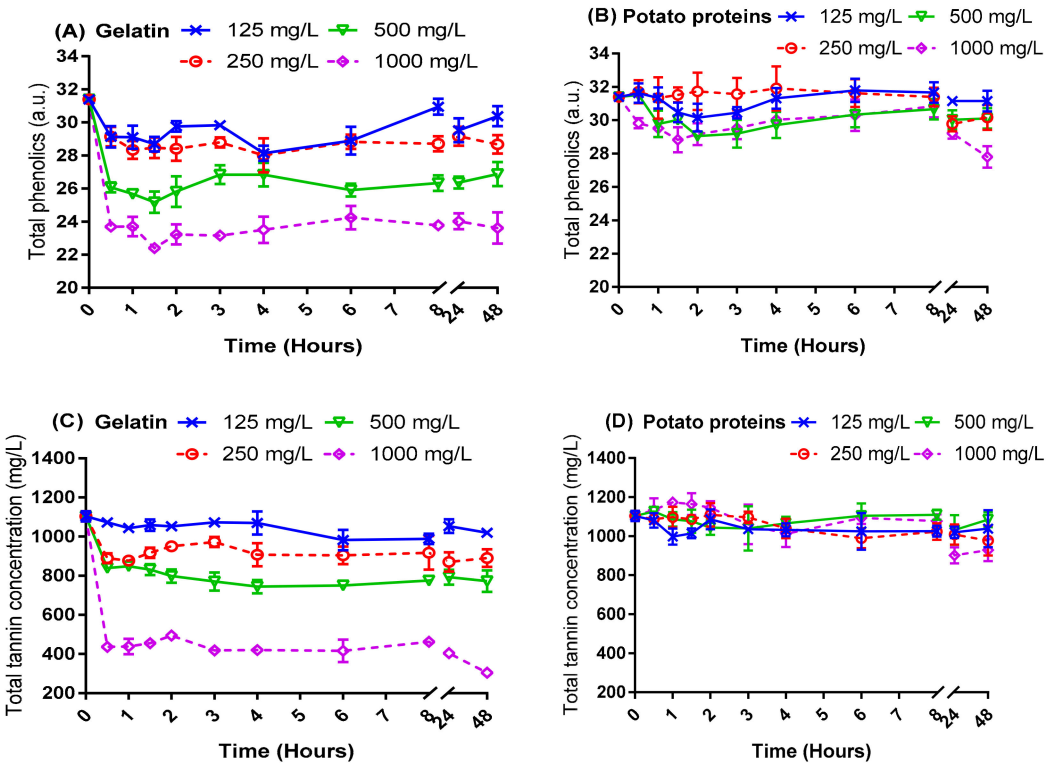


Figure 2. The fining kinetics of potato proteins compared with gelatin on unfined wine 2. (**A**,**B**) Total phenolics (absorbance units), and (**C**,**D**) total tannin (mg/L, epicatechin eq.).

Table 1. The results of the repeated measures ANOVA with the Huynh-Feldt correction.

			Concentration (mg/L)			
			125	**250**	**500**	**1000**
Wine 1	Gelatin	Total phenolics	*	***	***	***
		Total tannin	**	**	***	***
	Potato proteins	Total phenolics	***	*	***	***
		Total tannin	ns [a]	ns	ns	*
Wine 2	Gelatin	Total phenolics	**	**	***	***
		Total tannin	ns	ns	*	***
	Potato proteins	Total phenolics	ns	ns	*	**
		Total tannin	ns	ns	ns	*

[a] ns: no significant difference. Symbols *, ** and *** denoted for p value < 0.05, 0.01 and 0.001 respectively, showing a significant change was detected across the fining period (eleven time points across 48 h).

As shown in Figures 1 and 2 as well as Table 1, the concentration of total phenolics and tannin generally decreased as the dose of gelatin was increased. With the exception of total phenolics in wine 1, a concentration-dependent trend for phenolics reduction using potato protein was not as strong as that observed for gelatin. At the same dose of fining agent applied, gelatin consistently brought about a greater reduction in total phenolics and tannin than potato proteins, for both wines studied. This finding was consistent with previous observations [16] which investigated a Cabernet Sauvignon model, unfined wine. However, the fining response differed between the two wines for both protein types applied. For instance, the addition of 125 mg/L gelatin significantly reduced tannin concentration in wine 1, but to achieve a similar fining response, a 500 mg/L dose was required for wine 2. Similarly, total phenolics were significantly reduced by potato proteins at a dose of only 125 mg/L in wine 1 but required a dose equal to or greater than 500 mg/L in wine 2 to bring about a statistically significant fining effect.

Molecules **2019**, *24*, 4578

In addition, one hour of fining time was sufficient for both fining agents to achieve maximal adsorption of phenolics and tannin in wine 1. Furthermore, for gelatin, and less obviously for potato, an increased fining dose (i.e., 500 and 1000 mg/L) resulted in a maximum reduction of phenolics and tannins in a reduced time of 30 min. Given typical wine industry scale logistics, this bodes well for the use of in-line dosing of fining agents to remove phenols as opposed to batch fining and racking off fining lees.

Based on this kinetic study, it was discovered that wine conditions were important for fining, no matter which type of fining agent was used. As the fining efficacy observed in wine 1 was better than wine 2, hypotheses were made that the different fining efficacies were caused by the different phenolic profiles between the two wines and/or differences in basic chemical parameters (such as acid, sugar, and alcohol). Thus, the impact factors for potato proteins fining were further resolved in the current work.

2.2. Relevance of the Wine Phenolic Profile in the Response to Fining Agent Addition

As shown in Figures 1 and 2, wine 1 had a higher concentration of total phenolics than wine 2, at 41.86 and 31.39 absorbance units (a.u.), respectively, but had a similar initial tannin concentration. Based on this observation, it was considered that differences in phenolics other than tannin (non-polymeric material) might account for the differences in the efficacy of potato protein fining between the two wines. Furthermore, given that tannin concentration was similar between wines 1 and 2, but very different responses were found for potato protein fining efficacy between the two wines, it was hypothesised that differences in tannin composition might be useful to explain these effects.

Therefore, tannins were isolated from the two wines and analysed (Table 2). Generally, it was observed that the tannins from each wine were compositionally similar in terms of subunit composition. An important difference between the two wines was found for tannin molecular mass (MM), measured both by phloroglucinolyisis and gel permeation chromatography (GPC). In terms of three-dimensional tannin size, the GPC measurement is considered to be more accurate, as it accounts for the hydrodynamic volume of the tannin material and gives an estimate of relative polydispersity [25]. According to the GPC result, tannin size in wine 2 was larger than wine 1. Theoretically, for proteins are observed to have a stronger binding capacity for larger than smaller tannins, when other structural attributes are similar, as was the case in the current study [26]. However, the fining efficacy for tannin was higher for wine 1 relative to wine 2, which did not support this hypothesis. This result suggests that the different effectiveness of fining by proteins observed for the two wines was not primarily due to differences in tannin composition or size but was more likely to be due to the influence of other chemical parameters within the wine matrix.

Table 2. The tannin composition (mean ± standard deviation) of the two unfined wines in the current study.

	mDP [a]	Epigallocatechin (%) [a]	Epicatechin Gallate (%) [a]	Mass Conversion (%) of Phloroglucinolysis [a]	MM (phloro) (g/mol) [a]	MM (GPC) (g/mol) [b]
Wine 1	8.32 ± 0.06	38.7 ± 0.0	2.3 ± 0.0	45.5 ± 0.6	2495 ± 19	1628 ± 0
Wine 2	8.76 ± 0.25	36.4 ± 0.0	2.8 ± 0.0	44.0 ± 0.9	2631 ± 77	1935 ± 3

[a] Determined by phloroglucinolysis. [b] Determined by gel permeation chromatography at 50% elution.

It was therefore considered that while, in general, the phenolic profiles of wine affect the final outcome of a fining treatment, other wine compositional parameters should not be ignored. Hence, the impact of other basic factors (such as pH, ethanol concentration, and sugar concentration) which can differ within different wine matrices, were further investigated in this study for their potential impact on the efficacy of fining by potato protein.

Molecules **2019**, *24*, 4578

2.3. Experimental Design for Potato Proteins Fining

A 2^{5-1} fractional factorial experimental (one-half screening) design was used to determine the influence of basic wine and processing variables (during fining) on wine total phenolics and tannin concentration. Based on the results of the previously described kinetic study of fining by potato proteins where a reduction in polyphenols was observed more readily, wine 1 was selected for further experimentation via DoE. Furthermore, from the results of the kinetic study, 1 g/L and 48 h were chosen as the fining dose and contact time respectively, to ensure that a significant fining response would be observed.

The effects (factors and their interactions) from the 2^{5-1} design on both responses were displayed in a Pareto chart (Figure 3). By running the ANOVA on the 2^{5-1} fractional factorial design, the factors of pH, ethanol, and temperature were found to be significant for total phenolics removal, however, only pH and an interaction (pH × ethanol) were significant for the tannin adsorption (all p values < 0.01). As sugar concentration and agitation were not significant for either response, the fractional factorial design was consolidated into a full factorial design (2^3) of the remaining significant factors (with each remaining factor combination now consisting of six replicates), permitting the effect and significance of all two and three factor interactions between pH, temperature and ethanol concentration to be determined.

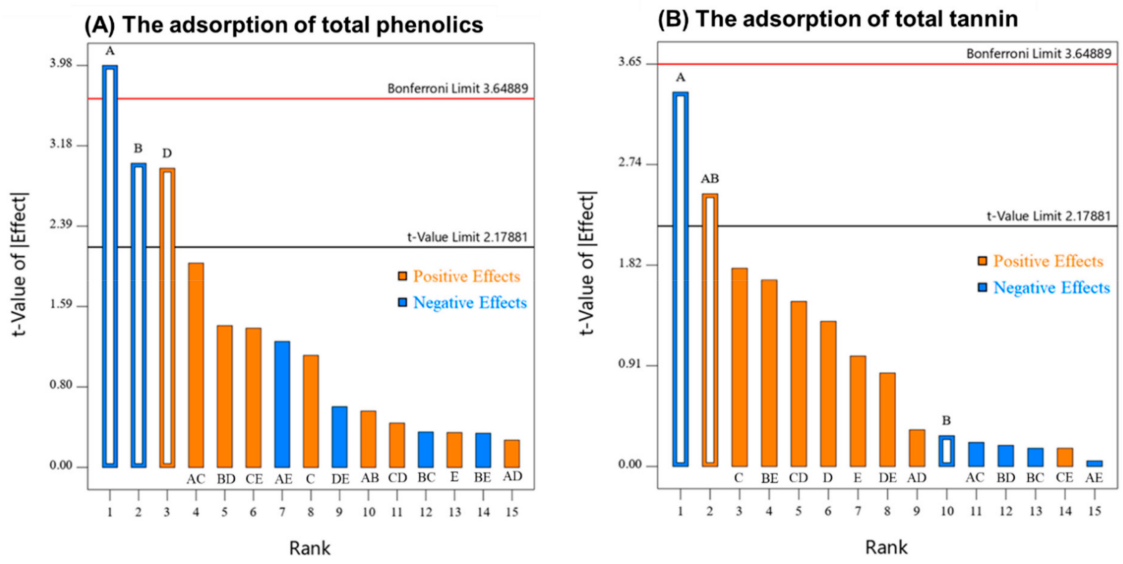


Figure 3. Pareto chart for the responses of (**A**) total phenolics adsorption and (**B**) total phenolics adsorption by potato proteins in the 2^{5-1} fractional factorial experimental. Factors A to E were pH, ethanol concentration, sugar concentration, temperature and agitation, respectively.

2.3.1. Modelling the Adsorption of Total Phenolics by Potato Protein Fining

The ANOVA on the consolidated design to 2^3 was determined, and the model F value was significant at 5.88 (p = 0.0001), while the "lack of fit" was not significant (F = 1.10, p = 0.3907). Meanwhile, the F values in this model for pH, ethanol concentration and temperature were 17.21, 9.85 and 9.52, respectively (all p values < 0.01). This confirmed the observation above that the factors pH, ethanol concentration and temperature were all relevant in determining the potato protein fining response for the removal of total wine phenolics, but their interaction (two-way and three-way) were not significantly important.

A three-dimensional response surface diagram for phenolics adsorption as a function of both pH and ethanol concentration at both low (Figure 4A) and high (Figure 4B) temperatures was visualised. Both pH and ethanol were found to exert negative effects, which indicates that higher pH and ethanol concentration diminished the efficacy for total phenolics removal by the specified potato protein dose. pH is known to have an influence on polyphenol-protein interactions [27], where pH can alter the ionic

Molecules **2019**, *24*, 4578

charge of proteins [28]. To interpret the current results, it is possible that an increased charge on the surface of the potato protein may have resulted at lower pH, which caused a stronger electrostatic interaction with polyphenols. Prior studies have demonstrated that there is generally a combination of both hydrophobic and hydrogen bonding between proteins and tannins, the degree of which depends on structure and matrix differences [29,30]. Ethanol concentration is also known to have an impact on the mechanism of interaction between wine phenols and poly(L-proline). A transition was found from a combination of hydrophobic and hydrogen bonding at 10% (*v*/*v*) ethanol, with a small increase to 15% (*v*/*v*) ethanol, resulting in interactions which were primarily via hydrogen bonding [31]. In light of these observations, to interpret the results of the present study, it may be hypothesised that since a higher ethanol concentration in wine was found to reduce phenolic adsorption and precipitation by potato proteins, that both hydrophobic and hydrogen bonding were important in driving the fining response.

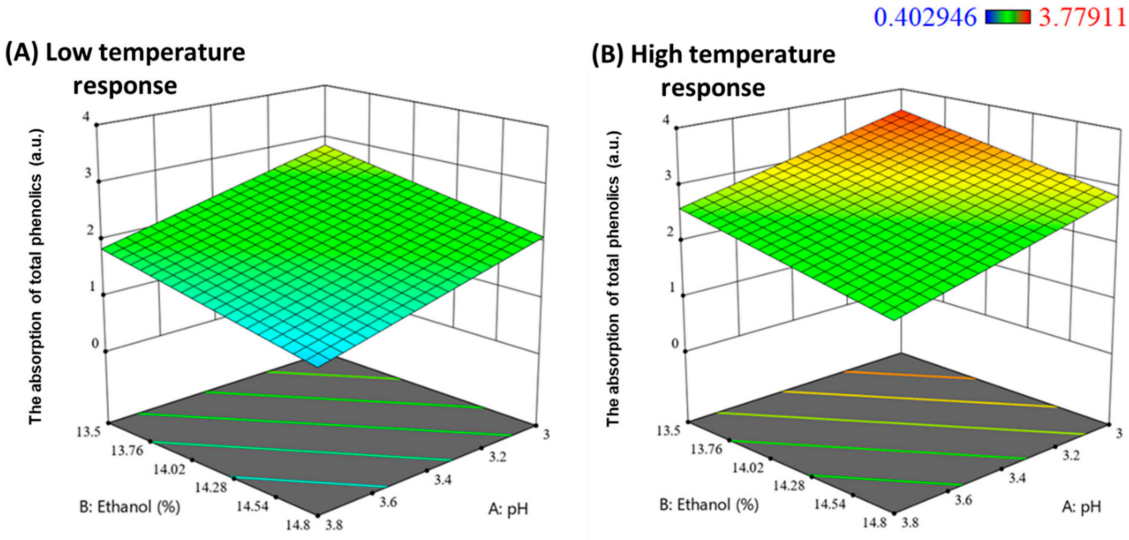


Figure 4. Response surface showing the adsorption of total phenolics as a function of pH and ethanol concentration for (**A**) 'low' and (**B**) 'high' temperature (10 °C and 20 °C respectively).

Conversely, another significant main effect, fining temperature, was found to positively influence the adsorption of total phenolics by potato protein fining. There was approximately a 1 a.u. difference between the low- and high-fining temperature responses when other factors were kept constant. This observation caused by different temperatures was also consistent with other wine fining literature with other fining agents [22,32].

From a processing perspective, the results determined by the DoE suggested potato proteins fining efficiency (on total phenolics adsorption) would be optimised by treating wines at higher temperatures rather than at normal storage temperatures for red wines (10 °C to 15 °C) Although this study did not vary the amount of fining agent, an adjustment of wine pH and alcohol to a lower level could possibly reduce the amount of fining agent required. In turn, this would reduce fining agent costs, whilst lowering risks of over-fining other wine components such as colour and aroma. However, manipulating alcohol through earlier harvest, water addition or reverse osmosis, might lead to reduced wine quality in itself [33–35], so wine producers would need to consider what the best option may be. Overall, pH adjustment is likely the best parameter to manipulate in order to enhance potato fining efficacy.

2.3.2. Modelling the Adsorption of Tannin by Potato Protein Fining

The tannin response model of the 2^3 full design was analysed, the model F value in ANOVA was 5.20, which indicated that there was only a 1% chance that differences were due to noise, hence the significance of the model was confirmed. The "lack of fit" was also not significant ($F = 0.04$, $p = 0.9861$).

Adsorption of total tannins after fining were significantly influenced by pH ($F = 11.52$, $p = 0.005$) likely due to its impact on protein-tannin interactions as discussed for phenolics in Section 2.3.1. Furthermore, the significant pH × ethanol interaction observed from the 2^{5-1} design analysis was confirmed in this 2^3 design analysis ($F = 6.11$, $p = 0.029$).

This interaction (pH × ethanol) positively influenced the removal of wine tannin, and it may be visualised in Figure 5. The fining efficacy was significantly decreased with increasing pH when the ethanol concentration was low. However, the pH change did not significantly influence the tannin removal efficacy when the wine ethanol was high. Insights of impact of various processing parameters on tannin removal were gained that treated wines with potato proteins at lower pH and ethanol concentration. However, there is no need for winemakers to modify wine pH in order to enhance fining efficiency, if the alcohol concentration is high.

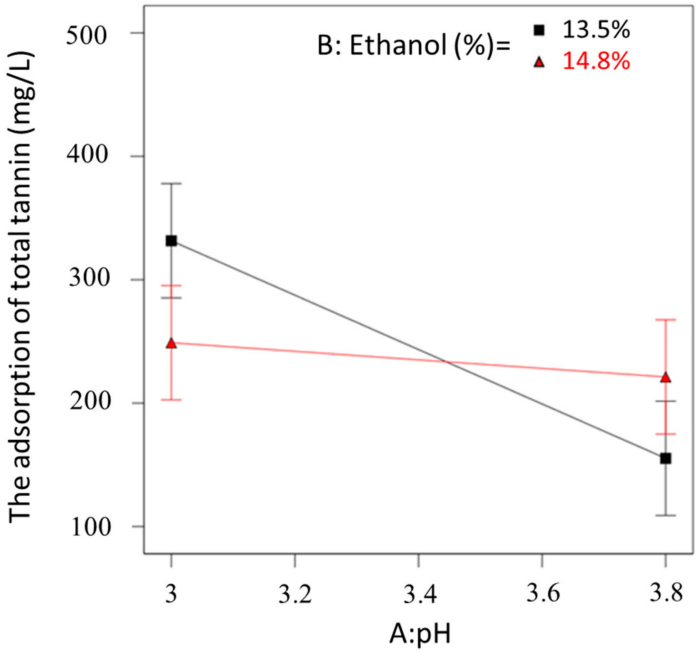


Figure 5. The interaction plot between the factors of pH and ethanol concentration on the adsorption of total tannin.

Importantly, in the current study, sugar concentration in wine was not a significant factor for the fining treatment with potato proteins. Similarly, agitation during fining did not significantly impact on either the adsorption of total phenolics or tannin. Based on the observations from the current study, future work could be expanded to apply the DoE methodology on a greater range of red wines, with greater compositional variation. Furthermore, wine is a very complex matrix and consists of other components such as metal ions and polysaccharides [36,37], the impact from other components should be deciphered in the future.

3. Materials and Methods

3.1. Wine Samples and Fining Agents

Two Australian Cabernet Sauvignon (*Vitis vinifera*) wines from vintage 2019 were used as the unfined wines (Table 3). The fining experiments were conducted immediately after wine fermentation and cross flow filtration for wine 1 and fermentation followed by cold settling and racking for wine 2.

Molecules **2019**, *24*, 4578

Table 3. The oenological parameters of the unfined wines used in the current study.

	Wine 1	**Wine 2**
Grape Source	Limestone Coast, Australia	McLaren Vale, Australia
Yeast strain	Maurivin AWRI 796	Enartis Ferm red fruit
Malo-lactic fermentation Strain	CHR Hansen CH16	LALLEMAND VP41
Oak influence	No	No
pH [a]	3.51	3.77
Tartaric acidity (g/L) [a]	6.67	5.90
Malic acid (g/L) [a]	<0.40	<0.40
Volatile acidity (g/L) [a]	0.44	0.76
Alcohol (%) [a]	13.73	15.60
Residual sugar (g/L) [a]	0.17	3.30
Free sulfur dioxide (mg/L) [b]	29	30
Total sulfur dioxide (mg/L) [b]	48	59

[a] Wines were measured by the Australian Wine Research Institute's (AWRI) Commercial Services Laboratory by Winescan method. [b] Wines were measured by the Australian Wine Research Institute's (AWRI) Commercial Services Laboratory by the method of sulfur dioxide free and total (Gallery).

The potato protein was purchased from Laffort Australia (VEGECOLL®, Lot 117. Woodville North, SA, Australia). In addition, a powdered gelatin that is conventionally used in the wine industry, was used as a reference standard fining agent for comparison (Lot 161129, purchased from Laffort Australia). Before the fining process was initiated, each agent was solubilized in Milli-Q water as a stock solution (50 g/L, stirred for 12 h at 20 °C), to ensure accurate additions [38].

3.2. Chemicals

Reagents and reference compounds (≥97% purity) used for the methyl cellulose precipitable (MCP) tannin assay, the modified Somers assay, and high-performance liquid chromatography (HPLC) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

Ethanol (96%), D-(+)-Glucose (≥99.5% purity), sodium hydroxide, and hydrochloric acid (37%) were purchased from Chem-supply (Gillman, SA, Australia), Sigma-Aldrich, Rowe Scientific (Lonsdale, SA, Australia), and Merck (Bayswater, VIC, Australia), respectively.

Milli-Q water (Millipore, North Ryde, NSW, Australia) was used for all solution preparations.

3.3. Fining Kinetics of Potato Proteins Compared with Gelatin

Fining experiments were performed on a 500 mL scale in both unfined wines (in 500 mL Schott bottles). The stock solutions of fining agents were serially diluted 1 in 2, to generate the following concentrations of 125, 250, 500 and 1000 mg/L (i.e., a total of eight treatments for each wine). After mixing (by Ratek OM11 orbital mixer, 150 rpm for 2 min at 20 °C), the headspace of each treated wine was filled with N_2 gas to avoid oxidation. Thereafter, all fining treatments were settled in the dark at 20 °C. After 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24 and 48 h of treatment with fining agents, 10 mL of each treated wine was sampled from the same position in the Schott bottle (midpoint of the bottle and 1.5 cm below the liquid surface). Aliquots were immediately transferred into 15 mL tubes and centrifuged at 4000 rpm for 5 min (by Eppendorf centrifuge 5810). The supernatants of each treated wine were recovered into new tubes. Total tannin concentration and total phenolics were measured for all samples. The unfined wine had an addition of the equivalent volume of Milli-Q water and was set as time point '0 h' in this kinetic study (1.25 mL water in 500 mL unfined wine, the same amount as fining agent addition). All fining trials were conducted in triplicate.

3.4. Phenolics Analyses

Total phenolics for samples was measured by the modified Somers assay [39] in technical triplicate. Total tannin concentration was measured through the high throughput MCP tannin method [39] in a

Molecules **2019**, *24*, 4578

technical duplicate. The tannin concentrations were calculated as epicatechin equivalents (g/L) from an epicatechin standard curve (Figure S1).

In addition, tannins from the two unfined wine samples were isolated using solid phase extraction [40] and analysed by HPLC following phloroglucinolysis (Agilent 1100 (Melbourne, VIC, Australia)) [41] to determine the subunit composition, mean degree of polymerization (mDP), and molecular mass (MM (phloro)). The molecular mass of tannins was also determined by gel permeation chromatography (MM (GPC)) [25] on an Agilent 1200. The tannin isolation and following measurements were performed in triplicate per sample.

3.5. Experimental Design for Potato Proteins Fining

A 2^{5-1} fractional factorial experimental design (one-half screening design) was used to determine the influence of the 5 variables (pH, ethanol concentration, sugar concentration, temperature, and agitation) at two levels each on the ability of potato proteins to remove total phenolics and tannin in wine. The factors and their associated levels (typical levels found in table wines) are summarised in Table 4. One of the two wines (Wine 1) was selected for the study. The pH of the wine was altered by using a few drops of sodium hydroxide (50%) and hydrochloric acid (37%), respectively. The wine ethanol concentration was manipulated by using the same volume (1 mL addition in every 75 mL Wine 1) of Milli-Q water and 96% ethanol. Although the dilution effect in wine from the water and ethanol addition might not be the same, the assumption was made that it was as the same volume was added of each. Glucose was used to modify the sugar concentration of the wine. The corresponding temperatures were achieved using temperature-controlled rooms, and the agitation was achieved by a Ratek mixer (at 100 rpm).

Table 4. Experimental factors for the two-level fractional factorial experimental (one-half).

Factor	Description	Low Level	High Level
A	pH [a]	3.00	3.80
B	Ethanol (%) [b]	13.5	14.8
C	Sugar (g/L) [c]	0.16	8.00
D	Temperature (°C)	10	20
E	Agitation	No	Yes

[a] Wine samples were measured for pH by using a Mettler Toledo T50 Autotitrator (Port Melbourne, VIC, Australia). [b] The ethanol concentration was determined with the Anton Paar Alcolyzer Wine ME and DMA 4500M (North Ryde, NSW, Australia). [c] The sugar content were determined via Chemwell® 2910 Automated EIA and Chemistry Analyser (Awareness Technology, Palm City, FL, USA) with the Megazyme K-FRUGL test kits (Chicago, Illinois, USA).

The experiment was performed with the treatment combinations generated by the Design Expert software (version 11, Minneapolis, MN, USA) shown in Table 5. The fining process in DoE was conducted on a 50 mL scale, but all other experimental details (such as the process of fining agent addition and centrifugation to remove fining agent) were kept the same as the kinetic study. A fining agent dose of 1000 mg/L and 48 h were chosen as the fining concentration and time for the DoE experiment, to ensure that a significant fining response would be observed. The adsorption of total phenolics and total tannin in wine was measured as two responses in the factorial design.

Table 5. Treatment combinations for the fractional factorial experimental (one-half) design. Factors A to E were pH, ethanol concentration, sugar concentration, temperature and agitation, respectively.

	Treatments *		Treatments
1	AbcDE	9	abCDE
2	AbCDe	10	ABcDe
3	ABCDE	11	aBCDe
4	Abcde	12	aBcDE
5	aBCdE	13	AbCdE
6	aBcde	14	ABCde
7	abCde	15	abcdE
8	ABcdE	16	abcDe

* A high level of any factor in the treatment combination is denoted by the capital letter and a low level of a factor is denoted by lowercase letter.

The experiments were performed in triplicate.

3.6. Data Analyses

The data of the fining kinetic study (including the '0 h' time point) were analysed by RMANOVA in SPSS statistics (ver. 26; IBM Corporation, Chicago, IL, USA). RMANOVA was conducted independently on the total phenolics and tannin measures from the two unfined wines. The Huynh–Feldt correction was applied due to the violation of the sphericity assumption of RMANOVA which was determined by Mauchly's test. The adsorption of total phenolics and tannin was obtained by subtracting the values of total phenolics and tannin before and after fining, and the data for the factorial design were processed via the Design Expert software (multiple linear regression).

4. Conclusions

This study was the first to investigate the fining kinetics of potato proteins on phenolic components in Cabernet Sauvignon wines. The fining performances were driven by different conditions in wines, including the phenolic profiles and basic chemical composition of the matrices. This work was also the first to resolve the main impact factors for reducing wine astringent compounds (total phenolics and total tannin) by using potato proteins. Potato protein fining was significantly influenced by wine pH, ethanol concentration, fining temperature as well as the pH × ethanol interaction, but not by sugar content or agitation. Winemaking optimisation insight was gained by the factorial design experiment in this work, in that reduction in the amount of potato protein required may be achieved by fining wines either at higher temperatures (rather than normal storage temperatures), at a lower pH, and/or lower alcohol concentration.

Supplementary Materials: The following are available online, Figure S1: The standard curve of tannin concentration (epicatechin eq.) of methyl cellulose precipitable method.

Author Contributions: W.K., R.A.M., K.A.B., P.A.S., J.N., S.E.P.B. conceived and designed the experiments. W.K. performed the experiments, analysed the data and drafted the manuscript. W.K., R.A.M., K.A.B., P.A.S., J.N., S.E.P.B. contributed to the data interpretation, as well as reviewed and edited the manuscript.

Funding: This study was funded by the University of Adelaide (The Adelaide Graduate Research Scholarship) and Wine Australia (AGW Ph1605). Wine Australia invests in and manages research, development, and extension on behalf of Australia's grape growers and winemakers and the Australian government.

Acknowledgments: The authors appreciate the funding of Wine Australia, the donations from Treasury Wine Estates and Laffort. We are thankful for the assistance from L.Q. and S.K. of the Australian Wine Research Institute.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Peynaud, E.; Blouin, J. *The Taste of Wine: The Art Science of Wine Appreciation*; John Wiley & Sons: New Jersey, NJ, USA, 1996; pp. 1–188.
2. Smith, P.A.; McRae, J.M.; Bindon, K.A. Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 601–614. [CrossRef]
3. Heredia, F.; Escudero-Gilete, M.; Hernanz, D.; Gordillo, B.; Meléndez-Martínez, A.; Vicario, I.; González-Miret, M. Influence of the refrigeration technique on the colour and phenolic composition of Syrah red wines obtained by pre-fermentative cold maceration. *Food Chem.* **2010**, *118*, 377–383. [CrossRef]
4. Parenti, A.; Spugnoli, P.; Calamai, L.; Ferrari, S.; Gori, C. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *Eur. Food Res. Technol.* **2004**, *218*, 360–366. [CrossRef]
5. Bindon, K.; Kassara, S.; Curtin, C.; Li, S.; Hixson, J.; Teng, B.; Wilkinson, K.; Smith, P. In Cap on red wine macromolecules? Updates on how winemaking interventions influence tannin and polysaccharide composition in Shiraz wines. In *Abstracts of papers of the American Chemical Society*; American Chemical Society: Washington, DC, USA, 2017.
6. Schmidtke, L.M.; Clark, A.C.; Scollary, G.R. Micro-oxygenation of red wine: Techniques, applications, and outcomes. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 115–131. [CrossRef]
7. Rankine, B. *Making Good Wine*; Macmillan Publishers: Sydney, Australia, 2007; pp. 137–144.
8. Marangon, M.; Vincenzi, S.; Curioni, A. Wine Fining with Plant Proteins. *Molecules* **2019**, *24*, 2186. [CrossRef] [PubMed]
9. Waglay, A.; Karboune, S.; Alli, I. Potato protein isolates: Recovery and characterization of their properties. *Food Chem.* **2014**, *142*, 373–382. [CrossRef] [PubMed]
10. Park, W.D.; Blackwood, C.; Mignery, G.A.; Hermodson, M.A.; Lister, R.M. Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH2-terminal sequence analysis. *Plant Physiol.* **1983**, *71*, 156–160. [CrossRef]
11. Løkra, S.; Helland, M.H.; Claussen, I.C.; Strætkvern, K.O.; Egelandsdal, B. Chemical characterization and functional properties of a potato protein concentrate prepared by large-scale expanded bed adsorption chromatography. *LWT-Food Sci. Technol.* **2008**, *41*, 1089–1099.
12. Gambuti, A.; Rinaldi, A.; Moio, L. Use of patatin, a protein extracted from potato, as alternative to animal proteins in fining of red wine. *Eur. Food Res. Technol.* **2012**, *235*, 753–765. [CrossRef]
13. Gambuti, A.; Rinaldi, A.; Romano, R.; Manzo, N.; Moio, L. Performance of a protein extracted from potatoes for fining of white musts. *Food Chem.* **2016**, *190*, 237–243. [CrossRef]
14. Iturmendi, N.; Moine, V.; O'Kennedy, K. Potato, a new source of vegetal protein for allergen-free fining of juice and wine. *Aust. N.Z. Grapegrow. Winemak.* **2013**, *598*, 67–70.
15. Tschiersch, C.; Nikfardjam, M.P.; Schmidt, O.; Schwack, W. Degree of hydrolysis of some vegetable proteins used as fining agents and its influence on polyphenol removal from red wine. *Eur. Food Res. Technol.* **2010**, *231*, 65–74. [CrossRef]
16. Kang, W.; Niimi, J.; Bastian, S.E.P. Reduction of red wine astringency perception using vegetable protein fining agents. *Am. J. Enol. Viticult.* **2018**, *69*, 22–31. [CrossRef]
17. Zoecklein, B.W.; Fugelsang, K.C.; Gump, B.H.; Nury, F.S. *Production Wine Analysis*; Van Nostrand Reinhold: New York, NY, USA, 1995; pp. 249–263.
18. Montgomery, D.C. *Design and Analysis of Experiments*; John Wiley Sons: Hoboken, NJ, USA, 2001; Volume 52, pp. 28–286.
19. Vila, D.H.; Mira, F.J.H.; Lucena, R.B.; Recamales, M.F. Optimization of an extraction method of aroma compounds in white wine using ultrasound. *Talanta* **1999**, *50*, 413–421. [CrossRef]
20. Chassagne, D.; Guilloux-Benatier, M.; Alexandre, H.; Voilley, A. Sorption of wine volatile phenols by yeast lees. *Food Chem.* **2005**, *91*, 39–44. [CrossRef]
21. Mané, C.; Souquet, J.; Olle, D.; Verries, C.; Veran, F.; Mazerolles, G.; Cheynier, V.; Fulcrand, H. Optimization of simultaneous flavanol, phenolic acid, and anthocyanin extraction from grapes using an experimental design: Application to the characterization of champagne grape varieties. *J. Agr. Food Chem.* **2007**, *55*, 7224–7233. [CrossRef]

22. Muhlack, R.A.; O'Neill, B.K.; Waters, E.J.; Colby, C.B. Optimal conditions for controlling haze-forming wine protein with bentonite treatment: Investigation of matrix effects and interactions using a factorial design. *Food Bioprocess Tech.* **2016**, *9*, 936–943. [CrossRef]
23. Setford, P.C.; Jeffery, D.W.; Grbin, P.R.; Muhlack, R.A. Mathematical modelling of anthocyanin mass transfer to predict extraction in simulated red wine fermentation scenarios. *Food Res. Int.* **2019**, *121*, 705–713. [CrossRef]
24. Girden, E.R. *ANOVA: Repeated Measures*; Sage Publications: Thousand Oaks, CA, USA, 1992.
25. Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A* **2003**, *995*, 99–107. [CrossRef]
26. Guerrero, R.L.F.; Smith, P.; Bindon, K.A. Application of insoluble fibers in the fining of wine phenolics. *J. Agr. Food Chem.* **2013**, *61*, 4424–4432. [CrossRef] [PubMed]
27. Hagerman, A.E.; Butler, L.G. Protein precipitation method for the quantitative determination of tannins. *J. Agr. Food Chem.* **1978**, *26*, 809–812. [CrossRef]
28. Hagerman, A.E. Fifty years of polyphenol-protein complexes. In *Recent Advances in Polyphenol Research*; John Wiley & Sons Publisher: Hoboken, NJ, USA, 2012; pp. 71–97.
29. Cala, O.; Pinaud, N.; Simon, C.; Fouquet, E.; Laguerre, M.; Dufourc, E.J.; Pianet, I. NMR and molecular modeling of wine tannins binding to saliva proteins: Revisiting astringency from molecular and colloidal prospects. *FASEB J.* **2010**, *24*, 4281–4290. [CrossRef] [PubMed]
30. Murray, N.J.; Williamson, M.P.; Lilley, T.H.; Haslam, E. Study of the interaction between salivary proline-rich proteins and a polyphenol by 1H-NMR spectroscopy. *Eur. J. Biochem.* **1994**, *219*, 923–935. [CrossRef]
31. McRae, J.M.; Ziora, Z.M.; Kassara, S.; Cooper, M.A.; Smith, P.A. Ethanol concentration influences the mechanisms of wine tannin interactions with poly (L-proline) in model wine. *J. Agr. Food Chem.* **2015**, *63*, 4345–4352. [CrossRef]
32. Yokotsuka, K.; Singleton, V.L. Interactive precipitation between phenolic fractions and peptides in wine-like model solutions: Turbidity, particle size, and residual content as influenced by pH, temperature and peptide concentration. *Am. J. Enol. Viticult.* **1995**, *46*, 329–338.
33. Petrie, P.R.; Teng, B.; Smith, P.A.; Bindon, K.A. Sugar reduction: Managing high Baume juice using dilution. *Wine Vitic. J.* **2019**, *34*, 36–37.
34. Schelezki, O.J.; Šuklje, K.; Boss, P.K.; Jeffery, D.W. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food Chem.* **2018**, *259*, 196–206. [CrossRef]
35. Longo, R.; Blackman, J.W.; Torley, P.J.; Rogiers, S.Y.; Schmidtke, L.M. Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *J. Sci. Food Agric.* **2017**, *97*, 8–16. [CrossRef]
36. Maury, C.; Sarni-Manchado, P.; Poinsaut, P.; Cheynier, V.; Moutounet, M. Influence of polysaccharides and glycerol on proanthocyanidin precipitation by protein fining agents. *Food Hydrocoll.* **2016**, *60*, 598–605. [CrossRef]
37. Berg, H.; Raul, T.; Akiyoshi, M. The effect of refrigeration, bentonite clarification and ion exchange on potassium behavior in wines. *Am. J. Enol. Viticult.* **1968**, *19*, 208–212.
38. Simonato, B.; Mainente, F.; Selvatico, E.; Violoni, M.; Pasini, G. Assessment of the fining efficiency of zeins extracted from commercial corn gluten and sensory analysis of the treated wine. *LWT-Food Sci. Technol.* **2013**, *54*, 549–556. [CrossRef]
39. Mercurio, M.D.; Dambergs, R.G.; Herderich, M.J.; Smith, P.A. High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. *J. Agr. Food Chem.* **2007**, *55*, 4651–4657. [CrossRef] [PubMed]
40. Kassara, S.; Kennedy, J.A. Relationship between red wine grade and phenolics. 2. Tannin composition and size. *J. Agr. Food Chem.* **2011**, *59*, 8409–8412. [CrossRef]
41. Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agr. Food Chem.* **2001**, *49*, 1740–1746. [CrossRef]

Sample Availability: Not available.

Supplementary Material (Molecules 2019, 24 (24), 4578):

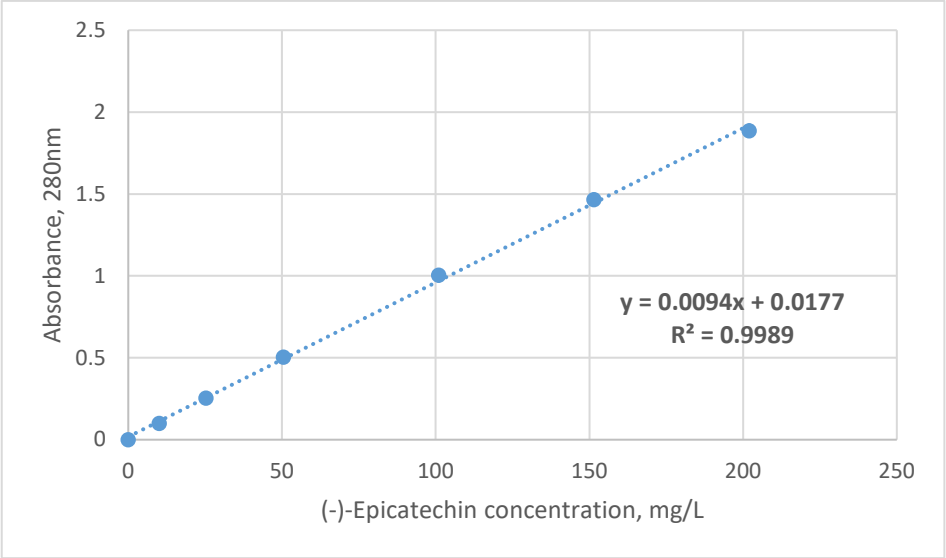


Figure S1. The standard curve of tannin concentration (epicatechin eq.) of methyl cellulose precipitable method.

CHAPTER 4

Chemical and Sensory Impacts of Accentuated Cut Edges (ACE) Grape Must Polyphenol Extraction Technique on Shiraz Wines

Wenyu Kang [1], Keren A. Bindon [2], Xingchen Wang [1], Richard A. Muhlack [1], Paul A. Smith [3], Jun Niimi [1, 4] and Susan E.P. Bastian [1,*]

[1] School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.

[2] The Australian Wine Research Institute, Hartley Grove, Urrbrae, Adelaide, SA 5064, Australia.

[3] Wine Australia, Industry House, Corner Hackney and Botanic Roads, Adelaide, SA 5000, Australia.

[4] Institute for Molecular Biosciences, Goethe University Frankfurt, Frankfurt am Main, 60438, Germany.

[*] Correspondence: sue.bastian@adelaide.edu.au; Tel.: +61-8-83136647

Foods – 2020, 9 (8), 1027.

Statement of Authorship

Title of Paper	Chemical and Sensory Impacts of Accentuated Cut Edges (ACE) Grape Must Polyphenol Extraction Technique on Shiraz Wines
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Kang, W., Bindon, K. A., Wang, X., Muhlack, R. A., Smith, P. A., Niimi, J.,& Bastian, S. E. P. (2020). Chemical and Sensory Impacts of Accentuated Cut Edges (ACE) Grape Must Polyphenol Extraction Technique on Shiraz Wines. Foods, 9 (8), 1027.

Principal Author

Name of Principal Author (Candidate)	Wenyu Kang		
Contribution to the Paper	Conceived and designed the experiments, performed the experiments, analysed and interpreted the data, as well as drafted and edited the manuscript.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	21/05/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Keren A. Bindon		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Xingchen Wang		
Contribution to the Paper	Conceived and designed the experiments, performed the experiments, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Richard A. Muhlack		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Paul A. Smith		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Jun Niimi		
Contribution to the Paper	Contributed to the data interpretation, as well as reviewed and edited the manuscript.		
Signature		Date	21/05/2020

Name of Co-Author	Susan E.P. Bastian		
Contribution to the Paper	Conceived and designed the experiments, contributed to the data interpretation, reviewed and edited the manuscript, as well as acted as corresponding author.		
Signature		Date	21/05/2020

foods

MDPI

Article

Chemical and Sensory Impacts of Accentuated Cut Edges (ACE) Grape Must Polyphenol Extraction Technique on Shiraz Wines

Wenyu Kang [1], Keren A. Bindon [2], Xingchen Wang [1], Richard A. Muhlack [1], Paul A. Smith [3], Jun Niimi [1,4] and Susan E. P. Bastian [1,*]

[1] Waite Campus, School of Agriculture, Food & Wine, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia; a1642412@adelaide.edu.au (W.K.); xingchen.wang@adelaide.edu.au (X.W.); richard.muhlack@adelaide.edu.au (R.A.M.); niimi@bio.uni-frankfurt.de (J.N.)

[2] The Australian Wine Research Institute, Hartley Grove, Urrbrae, Adelaide, SA 5064, Australia; keren.bindon@awri.com.au

[3] Wine Australia, Industry House, Corner Hackney and Botanic Roads, Adelaide, SA 5000, Australia; paul.smith@wineaustralia.com

[4] Institute for Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

* Correspondence: sue.bastian@adelaide.edu.au; Tel.: +61-8-8313-6647

Received: 26 June 2020; Accepted: 23 July 2020; Published: 31 July 2020

Abstract: Accentuated Cut Edges (ACE) is a recently developed grape must extraction technique, which mechanically breaks grape skins into small fragments but maintains seed integrity. This study was the first to elucidate the effect of ACE on Shiraz wine's basic chemical composition, colour, phenolic compounds, polysaccharides and sensory profiles. A further aim was to investigate any potential influence provided by ACE on the pre-fermentation water addition to must. ACE did not visually affect Shiraz wine colour, but significantly enhanced the concentration of tannin and total phenolics. Wine polysaccharide concentration was mainly increased in response to the maceration time rather than the ACE technique. ACE appeared to increase the earthy/dusty flavour, possibly due to the different precursors released by the greater skin breakage. The pre-fermentation addition of the water diluted the wine aromas, flavours and astringency profiles. However, combining the ACE technique with water addition enhanced the wine textural quality by increasing the intensities of the crucial astringent wine quality sub-qualities, adhesive and graininess. Furthermore, insights into the chemical factors influencing the astringency sensations were provided in this study. This research indicates that wine producers may use ACE with pre-fermentation water dilution to reduce the wine alcohol level but maintain important textural components.

Keywords: skin fragmentation; water addition; tannin; phenolics; polysaccharides; rate-all-that-apply; astringent sub-quality; progressive profiling

1. Introduction

Accentuated Cut Edges (ACE) is a new grape must processing technique that has recently received interest in the Australian and New Zealand wine sectors. The ACE technique, which is employed after conventional grape crushing, is a process whereby grape skins are mechanically cut into smaller fragments (6% of their original size) while maintaining seed integrity [1]. This technique provides more broken skin edges, with the goal of enhancing the extraction of phenolic components

from the grape skin earlier during fermentation, while avoiding the extraction of astringent or bitter compounds potentially resulting from seed damage [2]. The development of ACE started from a northern Tasmanian vineyard in Australia working on *Vitis vinifera* cv. Pinot noir grapes [3]. The work was initiated due to the recognition that wines made from this variety can have poor colour development and low pigment stability [4]. Since a more intense colour in red wines is often associated with higher quality perception by consumers [5], the finding that ACE resulted in the intensification of wine colour was promising for the future production of higher quality wine. Compared with conventional crushing, Pinot noir wines made using the ACE technique had 50% higher wine colour density and 95% higher stable pigment concentration [1]. In addition to colour, the quality of red wine is also associated with a positive mouthfeel (or textural) properties, such as the sensation of astringency, which is known to be influenced by various phenolic components such as tannins [6]. In the study on Pinot noir, wines produced by ACE were three times higher in tannin concentration than those prepared by conventional crushing [1], and had both greater astringency and bitterness intensities [2]. Recent research demonstrated that wines with the same overall astringency intensity may possess subtle mouthfeel texture sub-quality differences e.g., velvety, puckering [7,8]. Whether ACE treatment affects these more nuanced sensations is not known. ACE-treated Pinot noir wine also had a greater intensity of fruity components, notably the aromas of banana, peach, and black currant and the flavour of dark fruit [2].

Currently, Australian wine producers are faced with managing the impacts of a shortened vintage period for many grape cultivars, termed 'vintage compression'. This is thought to be due to the influence of climate change, with warmer growing seasons, a greater number of high temperature days and more days that have smaller diurnal temperature differences, resulting in the grapes harvested earlier and at higher sugar levels [9]. Management techniques to deal with the logistical disadvantages of processing the same tonnage of grapes in the face of vintage compression has increasingly gained importance within the wine industry [10]. In 2017, ACE was studied to address vintage compression, proposing the concept of Pressed Early Accentuated Cut Edges (PEACE) [11]. Based on the preliminary findings from PEACE, a two-day maceration on skins following ACE treatment was shown to be sufficient to extract a larger proportion of anthocyanin and tannin in Pinot noir wines relative to conventional crushing (eight days on un-fragmented skins). Thus, the ACE treatment allows the ferments to be pressed off skins earlier when compared with conventional crushing techniques, thereby highlighting the potential of PEACE to economise on tank space, pump-over logistics and labour requirements under the conditions of a compressed vintage [11].

However, as highlighted previously, ACE studies have thus far focused on Pinot noir, but one of the most planted red wine grape varieties globally, including in Australia, is *Vitis vinifera* cv. Shiraz [12], giving it a higher level of economic importance. Meanwhile, as vintage compression conditions lead to grapes destined for winemaking being harvested with increased sugar levels, the alcohol concentrations of wines made in Australia and elsewhere have also risen [13]. The high level of residual sugar or alcohol concentration in wines influences the sensory perception and hence reduces the perceived wine balance, quality or consumer preference [14]. While opportunities to manipulate wine alcohol through techniques such as earlier harvests, pre-fermentation water addition and reverse osmosis have been studied, these operations might also potentially lead to reduced wine quality [15–17].

Shiraz is economically important, but faces the same compressed vintage challenges. There is potential for ACE to be applied to Shiraz, however, this has not been done in a highly coloured and phenolic red grape variety before. The extent to which ACE can enhance Shiraz wine properties/sensory is unknown. Thus, the aims of the present study were to investigate, in Shiraz wine production, the impact of the ACE technique on wine chemical composition, sensory attributes and in particular, astringency and its sub-qualities. In order to determine the potential improvement provided by ACE over conventional crushing, a combination of both early pressing and water addition to wines were examined. Three treatments were investigated for both ACE and conventional crushing, whereby short skin maceration (three days on skins) was compared with a longer skin maceration time (six days). Furthermore, the longer skin contact treatments (6 days) for both standard

and ACE-processed grapes were also prepared with water addition (at the pre-fermentation stage to reduce must sugar to 13.5 Baumé (Bé)). All wine treatments were chemically characterised through a number of basic wine compositional parameters, most importantly colour, phenolic composition and polysaccharide concentration. In addition, the sensory characteristics of all treatments were profiled by rate-all-that-apply (RATA) using 61 untrained participants, and then astringency and astringent sub-qualities, assessed by modified progressive profiling (PP) using a trained panel.

2. Materials and Methods

2.1. Chemicals

Reagents and reference compounds (≥97% purity) used for the high-performance liquid chromatography (HPLC), methyl cellulose precipitable (MCP) tannin method, and the modified Somers assay were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Milli-Q water (Millipore, North Ryde, NSW, Australia) was utilised for the preparation of solutions.

For the winemaking process, potassium metabisulfite (PMS) and diammonium phosphate (DAP) were purchased from Laffort Australia (Woodville North, SA, Australia), while tartaric acid (H_2T) was purchased from Tarac Technologies (Nuriootpa, SA, Australia). Springwater (Woolworths®, Unley, SA, Australia) was used for the preparation of solutions for addition to wine as well as for must dilution, to avoid chlorine impact (potential formation of 2, 4, 6-trichloroanisole).

2.2. Vinification Protocol

Shiraz grapes were sourced from a vineyard (35°13′ S, 138°62′ E) located in the McLaren Vale region of South Australia during the 2019 vintage. The region experiences a temperate-warm Mediterranean climate, and the mean January temperature in 2019 was 31.6 °C, and the average annual rainfall from 2000 to 2019, 518.1 mm [18]. The grapes were machine harvested from 22 year-old vines holding 7.63 tonnes/hectare average yield (1 hectare is 10,000 m^2). A total of 600 kg was subsampled from the machine-harvested grapes. A portion of the grapes (300 kg) underwent a conventional crushing technique (Miller MC250) and was named NOACE treatment. The remaining 300 kg of grapes were crushed firstly by the same conventional approach and then underwent further processing through the Della Toffola Maceration Accelerator (DTMA, Della Toffola, TV, Italy; ACE treatment; minimum grape amount required by the winery). After the homogenisation of the two musts, the initial must conditions were measured by OenoFoss Type 41-01, and were not different to one another, having the following chemical compositions; sugar 14 Bé (1 Bé = 1.8 Brix = 18 g/L fermentable sugar = 1% potential alcohol), pH 3.39, 5.2 g/L titratable acidity (TA), 1.5 g/L malic acid, and 171.2 mg/L yeast assimilable nitrogen (YAN).

Thereafter, both musts were acid-adjusted by the addition of 1 g/L H_2T, 300 mg/L DAP added, followed by yeast inoculation with Enartis Ferm® red fruit (batch L.ES 736051) at a rate of 200 mg/L. As shown in Figure 1, both NOACE and ACE musts were further separated (under constant stirring to maintain the ratio between the skin and juice) into 9 by 25 kg aliquots in 30 L plastic fermenters (Brewcraft, SA, Australia). Water addition treatments were conducted by the direct addition of 500 mL of spring water (i.e., no juice run off was performed) to the NOACE- and ACE-treated musts in triplicate, to reduce the sugar concentration to 13.5 Bé, based on the Australian water addition regulatory limit. The fermentation of all six treatments (Figure 1) was conducted in triplicate, in a 20 °C temperature-controlled room, with manual plunging performed twice daily (at 10 am and 4 pm, with 10 punch downs per plunging). One day after inoculation with yeast, the lactic acid bacteria were co-inoculated by the addition of VP41 (LALLEMAND®, Edwardstown, SA, Australia, batch 314125093016) at a rate of 1.5 mg/L. After either 3 or 6 days of skin contact, the wines were pressed at 1.5 bar for 10 min using a water bag press, transferred to 10 L glass demijohns with airlocks (Ambrosio, Italy) and stored at 18 °C until dry (the total residual sugar and malic acid of all wines were below 2 g/L and 0.4 g/L, respectively). PMS was added to achieve 60 mg/L total Sulphur, then the wines stored in a 0 °C room for one week and racked off "gross lees". Thereafter, the wines were settled for a month at 0 °C and again racked from the "fine lees" before bottling. Wines were bottled

in 375 mL dark green bottles covered with carbon dioxide and screw caps, and cellared at 16 °C for a month before being analysed.

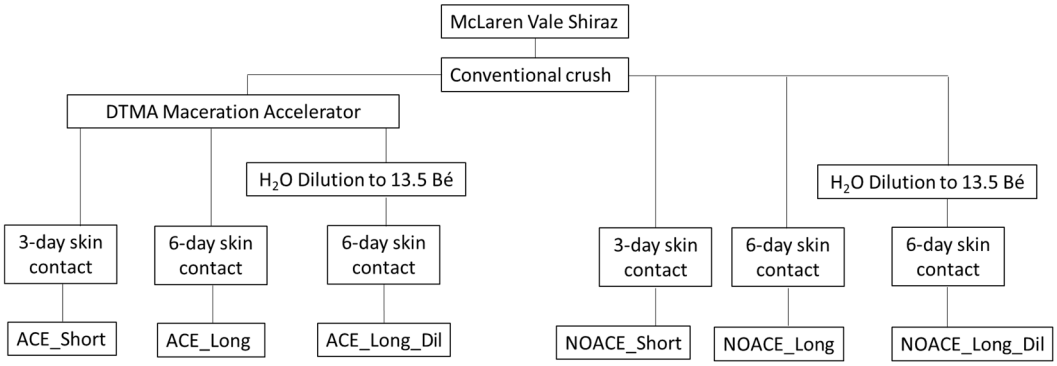


Figure 1. Summary of the treatments conducted on Shiraz musts prepared by the NOACE (conventional crush) or Accentuated Cut Edges (ACE) (conventional crush plus Della Toffola Maceration Accelerator (DTMA)) treatment, with six different treatments performed in triplicate.

2.3. Basic Wine Composition and Wine Colour Measurements

The wine samples were analysed for pH, titratable acidity (TA, as tartaric acid g/L equivalents and a TA measurement pH endpoint of 8.2), volatile acidity (VA, as g/L equivalent to acetic acid), and sulphur dioxide (SO_2, free and total) by the Australian Wine Research Institute's (AWRI) Commercial Services Laboratory (using the Winescan method and the method of sulphur dioxide free and total (the Thermo Fisher Discrete Analyser), respectively). The total residual sugars and malic acid levels were measured by Chemwell® 2910 Automated EIA and Chemistry Analyser (Awareness Technology, Palm City, FL, USA) with the Megazyme K-FRUGL (Chicago, IL, USA) and Vintessential Enzymatic L-Malic Acid (Dromana, VIC, Australia) test kits. The alcohol level of the samples was measured with the Anton Paar Alcolyzer Wine ME and DMA 4500M (North Ryde, NSW, Australia).

The wine colour was measured by both the modified Somers assay [19] and CIELab tristimulus using the Cintra 4040, (GBC Scientific Equipment, Braeside, VIC, Australia), and the results calculated and presented as the chroma and hue angle as described previously [20].

2.4. Phenolic Components and Polysaccharide Analyses in Wines

Total tannin concentration for the treatments was measured by the high-throughput MCP tannin method in technical duplicates, while the total phenolic concentration was determined by the modified Somers assay in technical triplicates [19]. Furthermore, the tannins from wine samples were isolated by solid-phase extraction [21] and analysed by HPLC (Agilent 1100) following phloroglucinolysis [22] to determine the subunit composition, mean degree of polymerisation (mDP), and molecular mass (MM (phloro)) according to the conditions outlined previously [23]. All the terminal monomer subunits had their retention times authenticated using standards before measurement [23]. The tannin molecular mass was also measured by gel permeation chromatography (MM (GPC)) on an Agilent 1200 with the modifications described previously [24]. 20 mg/mL malvidin-3-glucoside in methanol was used as a standard to validate the method; this standard was removed from the freezer, equilibrated to temperature and then diluted 1:5 with *N*,*N*-dimethylformamide prior to analysis.

For the polysaccharide analysis, the wine samples were prepared and hydrolysed as described by Li, et al. [25], but the dialysis step was replaced by a cold, pure ethanol wash [9]. The total wine soluble polysaccharides and the monosaccharide residues following acid hydrolysis were determined by HPLC (Agilent 1100) [26]. The monosaccharides were identified and quantified using commercial standards (Sigma-Aldrich, St. Louis, MO, USA).

2.5. Sensory Evaluations

2.5.1. Wine Descriptive Profiling by Naïve Wine Consumers Using Rate-All-That-Apply (RATA)

RATA is a rapid and flexible method that can profile different food or beverages products using naïve consumers as subjects [27,28]. For the characterisation of wines products, the discrimination and profiling abilities of RATA with naïve consumers have been validated against descriptive analysis using small, highly trained panellists [27]. Thus, a panel of 61 untrained participants (34 female and 27 male, average age 26 years) who had consumed red wine in the last 12 months assessed the Shiraz treatment wines in this study. The RATA assessment was conducted across two sessions (nine wine samples per session, all the samples from the triplicate of winemaking were assessed) under the same conditions as the work of Danner et al. [27] in computerized, individual booths with forced one minute breaks between each sample, and a five minute break after the first four wines. The participants used a seven-point intensity RATA scale (anchored from 1 = "extremely low" to 7 = "extremely high") to evaluate 58 attributes (Table S1, definitions were provided to the consumers) across the sensory modalities of wine colour, aroma, flavour, taste, mouthfeel, and aftertaste.

2.5.2. Astringency Profiles of Wines Assessed by a Trained Sensory Panel Using Modified Progressive Profiling (PP)

Wine astringency is a complex sensation, and is particularly hard and fatiguing for untrained individuals (normal consumers) to assess, comprehend and describe, especially the different sub-qualities of astringency perception [29]. As one study aim was to obtain an advanced understanding of the impact of ACE on the temporal perception of Shiraz wine's texture, the astringency profiles of treatment wine samples in this study were evaluated in more detail by a trained sensory panel (n = 8, 3 male and 5 female, average age 51 years) using the modified PP methodology [30]. The processes of panel recruitment, training, and sample evaluation were conducted in the same manner as our previous work [30]. Seven attributes of wine astringency were evaluated as previously determined [30] including overall astringent intensity (OAI) and 6 sub-qualities (pucker, mouth coat, dry, grippy, adhesive and graininess). The intensity of attributes in each wine were rated consecutively on 15 cm scales with low and high word anchors located at 10 and 90% of the scale, respectively. The entire attribute set were assessed in two rounds, one after the other for a given wine sample. However, the PP evaluation in this study only had 5 time periods (each lasting 10 s; the first with wine in the mouth and then 4 after expectoration, with 20 s gaps between each time period). This was because the panel training had revealed that the astringency sensation had disappeared by the fifth evaluation time period (Figure 2). All wines were presented to panellists in coded black glasses, monadically in randomised order and evaluated in computerised, individual booths. The 18 wines (6 treatments × 3 replicate of winemaking) were evaluated in duplicate across four sessions (two hours, twice weekly commencing at 10 a.m. at the University of Adelaide's Waite campus sensory facility).

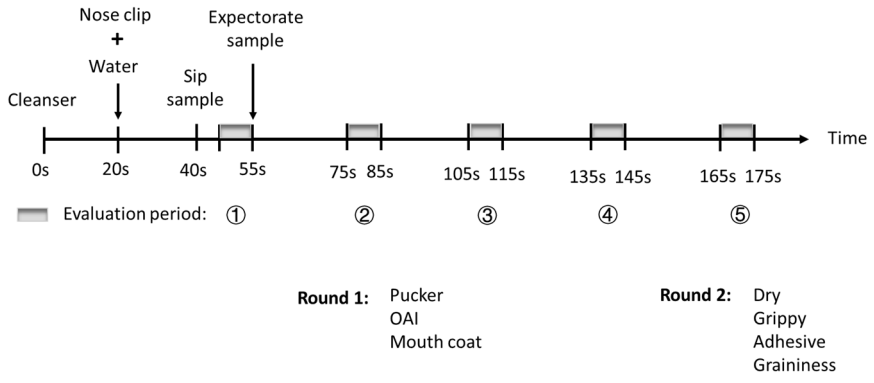


Figure 2. Schematic representation of the modified Progressive Profiling protocol. OAI is overall astringent intensity.

2.6. Data Analyses

The chemical measures were analysed by one-way analysis of variance (ANOVA) at an alpha level (α) of 5% with Fisher's least significant difference post hoc test (LSD) in XLSTAT (ver. 2016; Addinsoft SARL, Paris, France). The data from RATA were analysed by a multivariate ANOVA (at α 10%), with two-way interaction (treatment and replicate of winemaking as fixed factors, and assessor as random factor) using XLSTAT. Significantly different RATA attributes (means) were further analysed with Principal components analysis (PCA). In terms of PP assessment, the data were firstly analysed by univariate ANOVA (at α 5%) for each attribute at every single time period, with a treatment, replicate of winemaking and a replicate of sensory evaluation as fixed factors, and an assessor as a random factor using XLSTAT. Significantly different PP attributes were further analysed by the mixed assessor model canonical variate analysis (MAM-CVA) in RStudio (R ver. 3.5.1, Boston, MA, USA) with the software package CVAS (Version 1.0, written by Caroline Peltier on 3 November 2014). A partial least squares regression (PLS-R) between the significantly different attributes in PP (Y, the variables being predicted) and the significantly different chemical parameters (X, the predictor variables) were performed (stop conditions was automatic, cross-validation method used was Jackknife (LOO), and a confidence interval was 95%) using XLSTAT.

3. Results and Discussion

3.1. Basic Wine Chemical Composition and Colour

The basic wine composition resulting from the winemaking treatments is shown in Table 1. The treatments were significantly differentiated on the chemical parameters of alcohol and acid. The alcohol concentration in the water addition treatments was significantly lower in both the ACE and NOACE groups, as expected. A trend of higher pH and lower TA was found in the water addition treatments. pH is known to influence both wine colour [31] and astringency sensation [32]. The range of difference in pH was less than 0.1, which would only influence the wine colour and astringency perception negligibly, if at all.

Table 1. Basic chemical composition of the Shiraz wines prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil).

	Alcohol (% v/v)	Total residual sugar (g/L)	pH	TA (g/L)	VA(g/L)	Malic acid (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
ACE_Short	§14.83 ± 0.05 a	1.63 ± 0.12	3.54 ± 0.02 ab	6.83 ± 0.06 bc	0.72 ± 0.06	<0.40	29.67 ± 0.57	51.33 ± 2.52
ACE_Long	14.57 ± 0.15 b	1.57 ± 0.21	3.53 ± 0.02 b	6.77 ± 0.06 c	0.70 ± 0.06	<0.40	30.00 ± 1.00	48.33 ± 1.52
ACE_Long_Dil	14.23 ± 0.25 c	1.37 ± 0.12	3.57 ± 0.02 a	6.67 ± 0.21 c	0.70 ± 0.21	<0.40	28.67 ± 1.52	50.33 ± 0.57
NOACE_Short	14.83 ± 0.05 a	1.93 ± 0.15	3.48 ± 0.01 c	7.17 ± 0.06 a	0.73 ± 0.06	<0.40	27.33 ± 1.52	48.67 ± 1.53
NOACE_Long	14.93 ± 0.05 a	1.33 ± 0.23	3.52 ± 0.01 b	6.97 ± 0.06 b	0.71 ± 0.06	<0.40	29.00 ± 2.00	48.00 ± 0.00
NOACE_Long_Dil	14.50 ± 0.00 b	1.47 ± 0.25	3.57 ± 0.01 a	6.70 ± 0.06 c	0.70 ± 0.10	<0.40	31.00 ± 1.00	50.00 ± 1.73
F	8.452	2.914	8.502	6.488	1.492	N/A	1.594	1.827
p	†**0.002**	0.061	**0.002**	**0.004**	0.273	N/A	0.243	0.187

§ Data are the means (± standard deviation) of triplicate fermentations, analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. † Bold *p* values represent significant differences between treatments. A post-hoc test was run across wines within each column; values followed by the same letter in a column are not significantly different.

The effect of the ACE treatment on wine colour was firstly examined using the modified Somers method (Table 2). Similar to the previous findings from Pinot noir wines [1], the ACE treatments with longer time on skins had significantly increased wine colour density and stable pigment concentration (SO_2 resistant pigments). However, the colour enhancement in the Shiraz wines was

not as large in magnitude (approx. 17% higher wine colour density and 8% higher stable pigment concentration) relative to that observed in Pinot noir (50% higher wine colour density and 95% higher stable pigment concentration). The difference in the grape varieties may have been the cause, since the Shiraz grapes have inherently more red pigments and a darker colour than Pinot noir, and compounds which may also be more readily extractable [4]. Additionally, the colour effects were measured by CIELab (Figure 3), which is expected to better approximate the colour perceived by the human eye (the modified Somers assay was based on the measurement of spectrophotometric data for several wavelengths rather than the CIELab (a whole range, 375 to 780 nm)) [33]. The chroma, represents the intensity/depth of the wine colour, and the hue angle is identified as orange, yellow, beige, brown, pink or any of the other colours. There was no significant difference in the wine colour by CIELab across the six treatments, with the chroma of all wines being approximately 50 and the hue angle 355 (i.e., a purple to red hue). The results differed to those obtained by the Somers assay and the previous Pinot noir study. The modified Somers assay was more sensitive, however, in the case of this study, the colour differences were most likely not perceivable based on the CIELab measurements.

Table 2. Colour measurements by the modified Somers assay of Shiraz wines prepared following the NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil).

	§ Wine color density (a.u.)	Hue	Total anthocyanins (mg/L)	SO_2 resistant pigments (a.u.)
ACE_Short	†14.74 ± 0.73 a	0.56 ± 0.00	590 ± 28	2.70 ± 0.10 ab
ACE_Long	14.76 ± 0.31 a	0.56 ± 0.01	607 ± 10	2.68 ± 0.08 ab
ACE_Long_Dil	13.75 ± 0.53 ab	0.56 ± 0.01	608 ± 20	2.56 ± 0.09 bc
NOACE_Short	14.28 ± 0.61 a	0.56 ± 0.00	587 ± 13	2.72 ± 0.06 a
NOACE_Long	12.58 ± 0.43 c	0.58 ± 0.01	557 ± 21	2.48 ± 0.06 c
NOACE_Long_Dil	12.93 ± 0.80 bc	0.58 ± 0.01	573 ± 31	2.45 ± 0.06 c
F	7.261	2.860	2.391	6.225
p	‡**0.002**	0.063	0.100	**0.005**

§ Superscript represents that a.u. is the absorbance units. † Data are the means (± standard deviation) of triplicate fermentations, analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. ‡ Bold *p* values represent the significant differences between the treatments. A post hoc test was run across the wines within each column; the values followed by the same letter in a column are not significantly different.

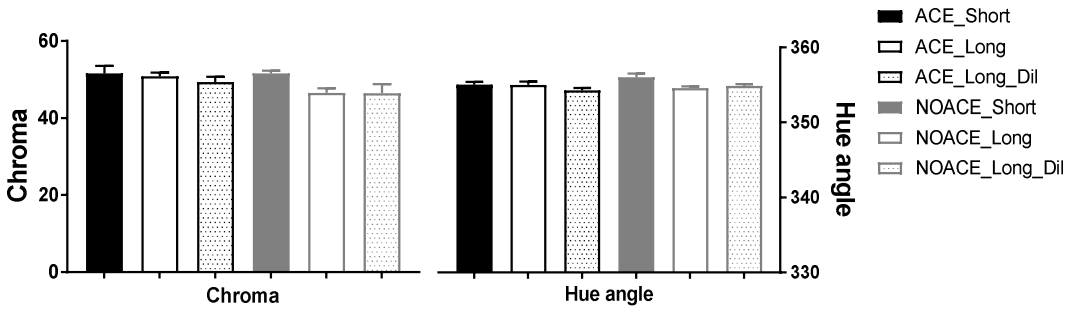


Figure 3. The colour of wines measured by CIELab. Shiraz wines were prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). Results are presented as the chroma and hue angle (mean ± standard deviation of triplicate fermentations).

3.2. Wine Total Phenolics and Total Tannin

3.2.1. ACE Effects

Wine phenolic components are important to wine colour, stability and quality, and they are considered to be primarily responsible for the sensation of astringency in wine [6,34,35]. As shown in Figure 4, with the exception of ACE_Short treatment, the total tannin concentrations in ACE-treated Shiraz wines were significantly higher than all NOACE treatments ($F = 3.72$, $p = 0.036$). Since no oak treatment was applied in this study, the tannins in wines were condensed tannins derived from the grape berries, and are located in the skin hypodermal layers, pulp, and the soft parenchyma of the seed between the cuticle and the hard seed coat [36,37]. Consistent with the previous literature [1,2], more broken skin edges provided by the ACE technique resulted in an increase in tannin extraction in Shiraz wines.

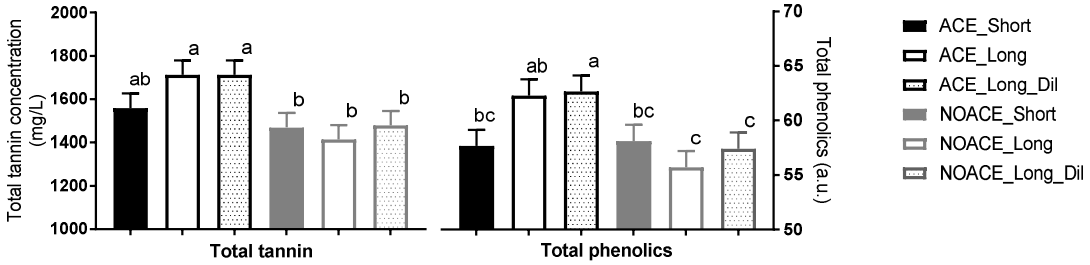


Figure 4. Mean total tannin concentrations and total phenolics (± standard error) of Shiraz wines prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). Different superscript letters above the bars indicate significant differences ($p < 0.05$) between treatments analysed by LSD. The a.u. in the right axis is absorbance units.

3.2.2. Maceration Time and Dilution Impacts

However, the enhancement of tannin extraction in the shorter skin maceration treatment was more obvious in Pinot noir wines [11] compared to the Shiraz in this study. This might due to the DTMA machine being less destructive when cutting Shiraz skins than the original ACE equipment and Pinot noir must processing. In addition, there was no significant difference in the tannin concentration between the long skin maceration and long skin maceration plus dilution treatments. This meant that the small amount of water addition before fermentation could reduce the alcohol level without significantly influencing the total tannin concentration in Shiraz wines. This is contrary to the losses in tannin concentration observed following the dilution of Shiraz in other studies [12,17], nevertheless, the amount of water addition in the current study was much lower. The total phenolics measurement indicated that the combined estimate of wine tannins together with other phenolic components, such as non-polymeric flavonoids and derived pigments, tracked similarly to the tannin concentration ($F = 3.59$, $p = 0.041$). The contents of total anthocyanins were not significantly different across the six treatments, thus the differences observed in total phenolics might mainly be caused by the proanthocyanidins and polymeric pigments (SO_2 resistant pigments).

3.3. Wine Tannin Composition

3.3.1. Maceration Time and Dilution Impacts

The tannin composition of the winemaking treatments was determined and are shown in Table 3. The mass conversion indicates the extent to which the isolated tannin was depolymerised to resolved constituent subunits by the phloroglucinolysis method, which also reflects the confidence for the interpretation of the measured subunit compositions as representative of all tannin in the

sample. Tannin mDP, which represents the average length of tannin polymers, was found to increase in the shorter skin maceration treatments relative to the longer maceration time of 6 days, independently of the treatment at crushing. The shorter skin maceration treatments also had a higher percentage of epigallocatechin subunits, indicating a greater proportion trihydroxylated material extraction from the grapes, likely reflecting a contribution from the grape skins [36,38]. On the contrary, the wines undergoing longer maceration times, including the water addition treatments, had a higher percentage of epicatechin gallate, which mainly originates from the grape seeds [36]. This indicated that when a greater proportion of grape seed tannins was transferred into wines, that this was mainly due to the maceration time, rather than the implementation of the ACE technique. It is important to highlight from the current results that the overall molecular mass (MM) of the tannin population was determined by either the phloroglucinolysis or GPC techniques had different outcomes in the current study. The MM determination by phloroglucinolysis correlated with the mDP measure, and was found to decrease in response to the extended maceration time, in agreement with an increase in seed tannin extraction. On the other hand, the MM measurement from GPC, which more accurately determines the average size of tannins as a function of their hydrodynamic volume rather than by mDP per se, was found to increase with longer maceration times. This was expected, since seed tannins are known to have a larger absolute size, or hydrodynamic volume, independent of the polymer length [23].

Table 3. The tannin composition of Shiraz wines prepared following the NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil).

	[§] **MM (Phloro) (g/mol)**	[†] **mDP**	**Epigallocatechin (%)**	**epicatechin gallate (%)**	**Mass conversion (%) of phloroglucinolysis**	[‡] **MM (GPC) (g/mol)**
ACE_Short	[δ] 2893 ± 53 a	9.58 ± 0.17 a	34.6 ± 1.0 a	4.1 ± 0.0 c	40 ± 2	1793 ± 33 b
ACE_Long	2616 ± 15 b	8.64 ± 0.06 b	32.6 ± 0.7 bc	4.9 ± 0.2 a	40 ± 1	1833 ± 32 a
ACE_Long_Dil	2691 ± 82 b	8.89 ± 0.27 b	31.8 ± 1.3 c	4.7 ± 0.2 a	38 ± 2	1811 ± 12 ab
NOACE_Short	2884 ± 46 a	9.55 ± 0.15 a	34.3 ± 0.7 ab	4.1 ± 0.1 c	38 ± 2	1778 ± 23 b
NOACE_Long	2720 ± 105 b	8.99 ± 0.34 b	31.1 ± 1.0 c	4.7 ± 0.1 a	39 ± 3	1787 ± 36 b
NOACE_Long_Dil	2583 ± 114 b	8.55 ± 0.37 b	32.0 ± 1.3 c	4.4 ± 0.0 b	39 ± 4	1673 ± 24 c
F	7.752	7.968	5.595	18.758	N/A	20.966
p	[Φ] **0.003**	**0.003**	**0.010**	**<0.0001**	N/A	**<0.0001**

[§] Tannin molecular mass determined by phloroglucinolysis. [†] Tannin mean degree of polymerisation determined by phloroglucinolysis. [‡] Tannin molecular mass determined by gel permeation chromatography at 50% elution. [δ] Data are the means (± standard deviation) of triplicate fermentations, analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. [Φ] Bold *p* values represent the significant differences between treatments. A post-hoc test was run across the wines within each column; the values followed by the same letter in a column are not significantly different.

3.3.2. ACE Effects

The observations from the tannin profiles could potentially indicate that seed integrity was maintained by ACE, since the amount of extracted tannin increased in response to ACE, but the tannins remained compositionally similar for comparable maceration times. Should ACE have disrupted the seed integrity, a decrease in mDP and an increase in proportional epicatechin gallate would have been expected earlier during maceration. According to the GPC results, the tannins derived from the ACE_Long and ACE_Long_Dil treatments were significantly larger in size than those found in the NOACE wines, suggesting more skin tannin components. Of relevance to the current study, is that larger tannins could potentially result in greater astringency perception, and this will be addressed in greater detail in the sections to follow.

3.3. Wine Polysaccharide Composition

ACE treatment and dilution had little impact on the polysaccharide composition (Table 4), while the total polysaccharide concentrations increased slightly as the maceration was prolonged from 3 to 6 days. In terms of the proportional composition of the individual monosaccharide residues, recovered following the acid hydrolysis of polysaccharides, fucose residues were significantly different across the treatments, but the levels were very low. At these equivalent levels, even in water, it is difficult to perceive the sweetness of fucose [39]. Significant differences across the treatments were also detected for rhamnose residues and the residues of galactose and arabinose, which are usually attributed to the rhamnogalacturonans (RGs) (e.g., RGII) and polysaccharides rich in arabinose and galactose (PRAGs), respectively; where both classes of polysaccharides are grape-derived. RG II and PRAGs have been shown to be important contributors to the mouthfeel of red wines and are thought to be negatively associated with bitterness and astringency [40,41]. Polysaccharides can also directly contribute to the mouthfeel properties of wines, such as the enhancement of the perception of palate fullness [41,42]. In addition, mannose (expected to be released by yeast as mannoproteins during fermentation and aging) was not significantly different across treatments.

Table 4. Concentrations (mg/L) of total polysaccharides and monosaccharide residues following acid hydrolysis. Shiraz wines prepared following the NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil).

	Mannose	Rhamnose	Glucuronic acid	Galacturonic acid	Glucose	Galactose	Xylose	Arabinose	Fucose	Total Polysaccharides
ACE_Short	[§] 114 ± 4	40 ± 2 b	8 ± 1 b	271 ± 12 c	32 ± 4 c	123 ± 4 bc	7 ± 1	126 ± 6 c	13 ± 1 c	736 ± 28 c
ACE_Long	116 ± 4	49 ± 3 a	10 ± 0 a	307 ± 9 a	50 ± 11 ab	135 ± 2 a	7 ± 2	153 ± 4 a	16 ± 2 ab	842 ± 26 a
ACE_Long_Dil	113 ± 1	49 ± 2 a	11 ± 1 a	293 ± 9 ab	57 ± 4 ab	130 ± 3 ab	8 ± 0	148 ± 2 ab	15 ± 0 ab	824 ± 9 ab
NOACE_Short	116 ± 5	38 ± 1 b	11 ± 1 a	251 ± 9 d	61 ± 2 a	121 ± 5 c	7 ± 1	125 ± 5 c	14 ± 1 bc	744 ± 23 c
NOACE_Long	117 ± 2	46 ± 1 a	11 ± 1 a	283 ± 6 bc	47 ± 12 b	131 ± 4 ab	6 ± 1	145 ± 3 b	16 ± 1 a	802 ± 26 ab
NOACE_Long_Dil	111 ± 9	46 ± 1 a	11 ± 1 a	281 ± 4 bc	50 ± 5 ab	129 ± 6 abc	7 ± 1	144 ± 7 b	17 ± 1 a	795 ± 35 b
F	0.602	15.325	3.947	14.743	5.155	3.898	1.029	19.492	5.621	8.146
p	0.700	[†] **<0.0001**	**0.024**	**<0.0001**	**0.009**	**0.025**	0.443	**<0.0001**	**0.007**	**0.001**

[§] Data are the means (±standard deviation) of triplicate fermentations, analysed with one-way analysis of variance at an alpha level of 5% and Fisher's least significant difference test. [†] Bold *p* values represent significant differences between the treatments. A post-hoc test was run across the wines within each column; values followed by the same letter in a column are not significantly different.

Foods 2020, 9, 1027

3.4. Sensory Characteristics

3.4.1. Wine Descriptive Profiling by RATA

In the current study, the ACE technique was also studied to determine the outcomes of the sensory profile of Shiraz wines. Samples were firstly evaluated by 61 untrained wine consumers to evaluate the properties of wine colour, aroma, flavour, taste, mouthfeel, and aftertaste. Among the 58 attributes of RATA, eight were significantly discriminated between the wine treatments ($p < 0.1$) by the wine consumers, and are displayed in Table 5. The eight significantly different attributes were further visualized in a PCA plot with the sample loadings (Figure 5). Encouragingly, the winemaking triplicates for each treatment appeared consistent, as a significant treatment × replicate of winemaking sensory difference was not detected. As shown, the average intensities of vanilla were higher in the ACE treatment with short maceration on both the nose and palate. The NOACE maceration with 3 and 6 days on skins had more intense "FL" (floral/perfume/musk) flavour, but the aroma of "FL" was more intense in the ACE_Short treatment wines. These observations are interesting and the profiles of volatile aromatic compounds from wines measured by head space gas chromatography (GC) with mass spectrometry would be useful to examine this further (e.g., Monoterpenoids and C13-Norisoprenoids) [43]. The intensity of sweetness in NOACE (Short and Long) wines were higher than ACE wines, but all the samples in the current study were technically dry wines (total residual sugar were all below 2 g/L). The different perceptions in sweetness were likely to be due to the different matrix effect (such as wine alcohol level and/or possibly release of yeast-derived compounds) across the treatments [44,45]. In addition, the intensity of F_ED (flavour of earthy/dusty) in the ACE treatments were higher than in the NOACE one, especially the sample of ACE_Long. A longer skin contact combined with an increase in skin breakage could account for this observation, whereby more related flavour substances (such as 3-Isopropyl-2-Methoxypyrazine) may have been released into wine [46]. In other words, the ACE technique may have accelerated the release of certain volatile substances or their precursors. However, the intensities of the flavour of herbaceous and red fruits were negatively impacted by the ACE technique, which would be worth analysing in the future by GC, such as detecting 3-sec-Butyl-2-methoxypyrazine and Nerol for the herbaceous character, as well as β-Ionone and Furaneol for the red fruits character [43]. Furthermore, the intensity improvement of dark fruits found in ACE-treated Pinot noir wines [2] was not detected in the current Shiraz wines, which might be caused by the difference in grape varieties. Wines made by Shiraz grapes are commonly associated by the sommeliers with attributes of dark fruits [47], thus, the ACE technique did not significantly affect this character. Last, but not the least, the negative dilution effects (intensity reduction in every significantly different RATA attribute) for the pre-fermentation water addition were clear to see in the RATA evaluation, which was consistent with previous literature [12].

Table 5. The significantly different attributes across the treatments from rate-all-that-apply (RATA) detected by a multivariate ANOVA (at α 10%).

Attribute	Definition	F	*p*
Aroma			
A_FL	Floral/perfume/musk	2.292	0.044
A_Van	Vanilla	2.112	0.062
Taste			
T_Sw	Sweet	2.201	0.052
Flavour			
F_RF	Red fruits (e.g., raspberry, strawberry, red cherry, and red current...)	2.036	0.071
F_ED	Earthy/dusty	3.210	0.007
F_FL	Floral/perfume/musk	1.999	0.076
F_Her	Herbaceous	1.984	0.079
F_Van	Vanilla	2.144	0.058

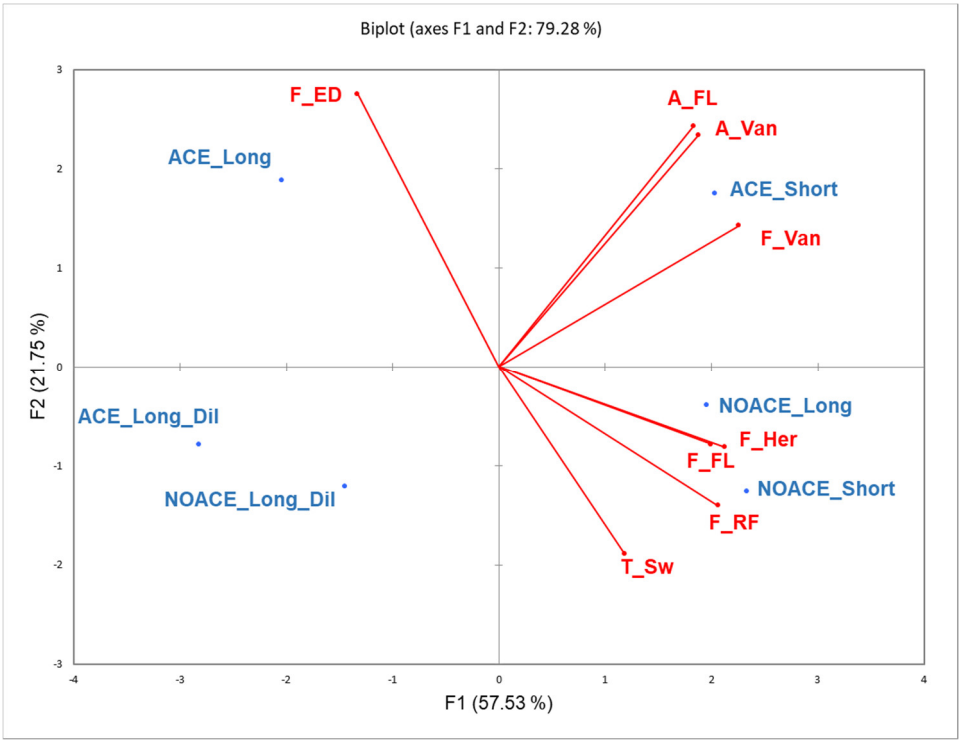


Figure 5. The PCA of six treatments for all the significantly different sensory attributes ($p < 0.1$) from RATA. Shiraz wines prepared following the NOACE and the ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). A_FL and A_Van represent the aroma of floral/perfume/musk and the aroma of vanilla, respectively. T_Sw is the taste of sweetness. In terms of flavours, "RF", "ED", "FL", "Her" and "Van" represent red fruits, earthy/dusty, floral/perfume/musk, herbaceous, and vanilla, respectively.

Wine colour differences were not detected by the consumers, in agreement with the results of the CIELab measurements taken in this study. The differences found in the chemical parameters of the acid and phenolics were not sufficiently large to elicit a sensory perception difference in the current study for either acidity or bitterness. Sixty-one untrained wine consumers did not detect a significant difference in astringency intensity between the six winemaking treatments. However, it is relevant to note that the intensity of astringency alone is insufficient to fully characterize the perception of wine astringency, that is, some wines may have a similar astringency intensity but diverse sub-qualities (textures) [48]. Meanwhile, it is also important to recognise that astringency perception is dynamic [49], in that the progression of both the intensity and sub-qualities varies depending upon the wine matrix [30,50–52]. Hence, a comprehensive astringency profile of each winemaking treatment was further evaluated by a trained sensory panel using PP.

3.4.2. Astringency Profiles of Wines Assessed by PP

The PP technique is a sensory tool for the dynamic and quantitative measurements of astringency intensity and sub-qualities [30,53]. An examination of the PP panel data in this study showed good repeatability performance by the panel, as there were no significant differences between the replicates of sensory evaluation for each attribute at five evaluation periods. Significantly different astringency profiles of the Shiraz wine treatments were perceived by the panel according to the statistical analysis of the perception data ($p < 0.05$). Six treatments significantly differed by intensity for mouth coat ($F = 70.27$, $p < 0.0001$) and adhesive ($F = 21.34$, $p < 0.0001$) at the second evaluation period (20–30 s after expectoration of wine sample). Meanwhile, the intensities of OAI ($F = 27.03$, $p < 0.0001$) and graininess ($F = 85.67$, $p < 0.0001$) were significantly different by treatment at the third evaluation period (50–60 s after expectorating wine). Although a significant influence of the assessor is common in sensory evaluation ($p < 0.0001$ in the current PP), a MAM-CVA was used to reduce the scaling effect caused by the different assessors [54]. For the ease of interpretation, a joint

Foods **2020**, *9*, 1027

presentation of the wine sample loadings (six wine treatments configuration plot) and the four significantly different PP attributes (sensory attribute configuration plot) are presented in one MAM-CVA plot (Figure 6). The first two canonical variates (CVs) accounted for 97.8% of the total variance ratio. As seen in Figure 6, the first CV was primarily related to the lower graininess and adhesive mouthfeel. The second CV is dominated by a mouth-coating mouthfeel, and to a lesser extent, the overall astringency intensity in the negative direction. The first axis strongly separated the less adhesive and grainy NOACE_Long dilution wines from all the other treatments. As illustrated by the lack of overlap between the confidence intervals, the grainier and more adhesive ACE_Long and ACE_Long dilution wines were clearly different from all NOACE wines. The astringency profiles of ACE_Short, NOACE_Short and NOACE_Long were similar, and clearly indicated that the astringency profiles of Shiraz wine made by conventional crushing were significantly influenced by the pre-fermentative implementation of water. Nevertheless, the use of the ACE technique not only reduced the impact of water addition on the astringency sensation, but also introduced obvious increases in the intensities of the adhesive and graininess textural sub-qualities. The enhancement provided by the ACE technique on the astringency profiles of Shiraz was consistent with what was found for Pinot noir (Sparrow, Holt, et al., 2016). As mentioned in the introduction, the most planted red wine grape variety in Australia is Shiraz, but this noble grape variety is also important for the wine industry globally (as it is grown and produces Shiraz or Syrah wines from for e.g., France, Portugal, Italy, Spain, South Africa, USA and New Zealand). This study confirmed others in Pinot noir revealing that ACE significantly enhanced the concentration of tannin and total phenolics. It also extended these findings in Shiraz wines showing that ACE was able to modify the astringency reduction caused by water dilution through an increased perception of adhesive and graininess sub-quality intensities. These positive influences on the textural quality of Shiraz wines could lead to a more extensive future application of ACE in the wine industry, not only for this but other red wine grape varieties in multiple wine producing countries.

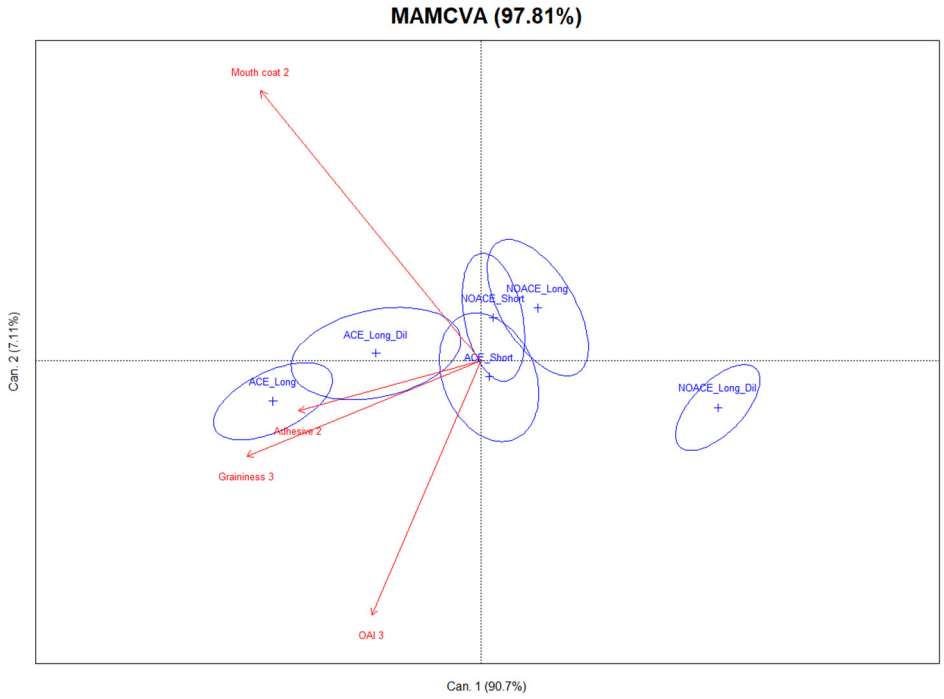


Figure 6. The mixed assessor model canonical variate analysis (MAM-CVA) of six treatments for all significantly different sensory attributes ($p < 0.05$) from five evaluation periods. Shiraz wines prepared following the NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). Hotelling Lawley stat = 4.458, F = 6.711 ($p \leq 0.001$). Ellipses indicate the confidence intervals of 90%.

To explore the underlying relationships between the significantly different mean PP sensory and wine chemistry data, the correlations between the discriminated astringency attributes (Y) and significantly different chemical parameters (X) were analysed by PLS-R (details of the first run of the PLS-R model are shown in Table S2 and Figure S1). The model was refined by a re-run of PLS-R using variables that produced variable importance in the projection (VIP) values greater than or close to 1 [55]. In the new model, the optimum number of components/latent variables required was 2, and the cross-validation index Q^2 for two components was 0.829 (and being greater than 0.5 represented the large predictive relevance of the model) [56]. This improved model (Table 6) explained 90.8% of the variation in wine chemical composition (X-variables) and 93.5% of the variation in sensory attributes (Y-variables). To extract the relevant features for the corresponding responses (mouth coat 2, adhesive 2, OAI 3, and graininess 3), the standardized regression coefficients of the selected chemical parameters (MM (GPC), total tannin, total phenolics, galacturonic acid, epicatechin gallate (%), fucose, and total polysaccharides) are displayed, respectively, in Figure 7. The intensity of the overall astringency and three discriminated sub-qualities were all significantly (standardized coefficients were all greater than 0.3) and positively associated with tannin MM (GPC), which confirmed earlier research [30,35]. The total tannin and total phenolics concentrations and the percentage of tannin galloylation also positively contributed to OAI, which was consistent with earlier findings [30,57]. However, this was the first reported observation of the contribution of these three phenolic parameters for astringency sub-qualities. On the contrary, the negative associations of OAI and three sub-qualities with fucose residues (recovered following the acid hydrolysis of polysaccharides) were found. It is difficult to perceive the sweetness of fucose at a low level which was mentioned in Section 3.3 [39], and therefore suppress the perception of astringency [58]. However, the negative associations might support the idea that the fucose in wines co-operated with other wine matrix components, eliciting a dampening of astringency perception, and this too warrants further investigation. The chemical parameters of galacturonic acid and total polysaccharides did not predominantly influence any astringency sensation, as standardised regression coefficients were <0.1 [59]. However, it should not be neglected that phenolic and polysaccharide composition in wine did not affect astringency perception alone in many cases, and their interactions are also important [25,40,41], but this needs to be meticulously investigated in the future.

Table 6. Fit statistics of the partial least squares regression (two components) analysis between the discriminated astringency attributes (Y) and significantly different chemical parameters (X).

	Mouth Coat 2	**Adhesive 2**	**Overall Astringency Intensity 3**	**Graininess 3**
Cumulative Q^2 quality index	0.721	0.918	0.757	0.924
R^2	0.897	0.971	0.899	0.974
Std. deviation	0.913	0.549	0.964	0.209
Mean-square error	0.334	0.120	0.372	0.017
Root mean squared error of prediction	0.578	0.347	0.610	0.132

Mouth Coat 2 and Adhesive 2 attributes were perceived 20–30 s after the expectoration of the wine samples. Overall Astringency Intensity 3 and Graininess 3 attributes were evaluated 50–60 s after the expectoration of the wine samples.

Foods 2020, 9, 1027

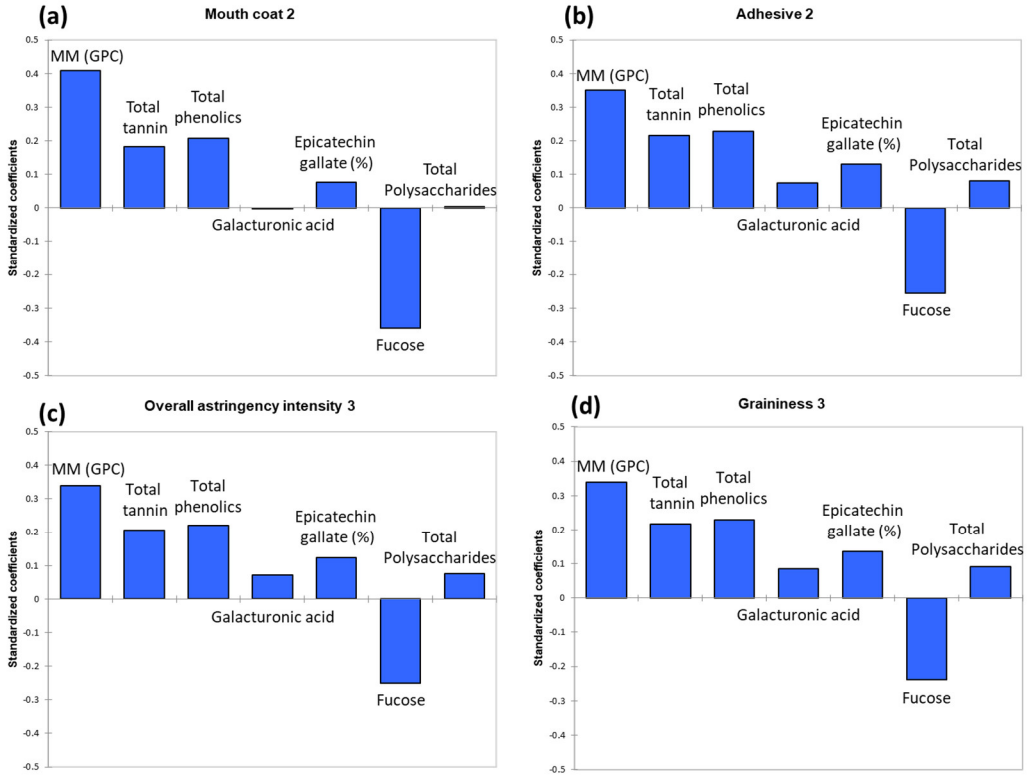


Figure 7. Standardised coefficients of the partial least squares regressions between the significantly different sensory attributes in PP (Y) and the selected (based on the variable importance in the projection) significantly different chemical parameters (X). MM (GPC) is tannin molecular mass determined by gel permeation chromatography at 50% elution. (**a**) Mouth coat 2 (20–30 s after the expectoration of wine sample) (**b**) Adhesive 2 (**c**) Overall astringency intensity 3 (50–60 s after expectorating wine) (**d**) Graininess 3.

4. Conclusions

A new grape must processing technique (ACE) was applied for the first time on Shiraz wines to elucidate the impacts on non-volatile wine chemical compositions and sensory profiles. The ACE technique did not influence the visual colour perception of Shiraz wines, but significantly increased the concentrations of total tannin and phenolics. The polysaccharide concentration in Shiraz wines was mainly influenced by the maceration time rather than ACE technique. In addition, the greater contribution of broken skin edges provided by ACE could accelerate the release of substances related to the flavour of earthy/dusty characters into wine, which should be studied further. The pre-fermentation addition of water had significant dilution effects on the consumer-perceived wine aromas and flavours. Water addition did not reduce the concentrations of tannin or phenolics significantly, but influenced the astringency profile evaluated by a trained panel. However, the ACE technique was able to moderate the perceived astringency reduction caused by dilution through an increased intensity of perception of adhesive and graininess sub-qualities. The differences on astringency sensation could be perceived by the trained panel but not in the consumers' assessment, however, more involved consumers may perceive these subtle changes. The knowledge generated by this study suggests that wine producers could utilise the ACE processing technique, in particular when winemakers need to modify the wine alcohol level by using pre-fermentative water dilution, and minimising the loss of important wine textural attributes. Insights into the compositional factors affecting the astringency sensation (overall intensity and sub-qualities) were provided in this study.

Supplementary Materials: The following are available online at www.mdpi.com/2304-8158/9/8/1027/s1, Table S1: List of sensory attributes scored in the rate-all-that-apply (RATA) assessment, Table S2: The model performance for the first run of partial least squares regression, and Figure S1: The plot of the first run of Partial Least Squares regression between the significantly different sensory attributes from PP (in blue) and significantly different chemical parameters (in red).

Author Contributions: W.K., K.A.B., X.W., S.E.P.B. conceived and designed the experiments. W.K. and X.W. performed the experiments. W.K. analysed the data and drafted the manuscript. W.K., X.W., K.A.B., R.A.M., P.A.S., J.N., S.E.P.B. contributed to the data interpretation, as well as reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the University of Adelaide (The Adelaide Graduate Research Scholarship) and Wine Australia (AGW Ph1605, WA Ph1803 and AWR1701). Wine Australia invests in and manages research, development, and extension on behalf of Australia's grape growers and winemakers and the Australian government.

Acknowledgments: We thank Coriole wines (Mark Lloyd and Duncan Lloyd) for the donation of uncrushed grapes and ACE-treated musts, and Richard E. Smart, the developer of the commercialised ACE technique, for guidance and useful discussion. Luke Qi (AWRI) is acknowledged for technical support in winemaking, and the analysis of the composition of wine tannin and polysaccharide. The sensory assessment was approved by the Human Research Ethics Committee of the University of Adelaide (project number: H-2016-084). We are thankful for the assistance of Lukas Danner, Trent Johnson, David Jeffery, Dimitra Capone, Claire Armstrong, Pietro Previtali and all participants of the sensory evaluations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sparrow, A.M.; Smart, R.E.; Dambergs, R.G.; Close, D.C. Skin particle size affects the phenolic attributes of Pinot noir wine: Proof of concept. *Am. J. Enol. Viticult.* **2016**, *67*, 29–37.
2. Sparrow, A.M.; Holt, H.E.; Pearson, W.; Dambergs, R.G.; Close, D.C. Accentuated cut edges (ace): Effects of skin fragmentation on the composition and sensory attributes of Pinot Noir wines. *Am. J. Enol. Viticult.* **2016**, *67*, 169–178.
3. Smart, R.; Sparrow, A. New winemaking process conceived in a northern Tasmania pilot winery-the beginnings of ACE. *Wine Vitic. J.* **2016**, *31*, 9.
4. Sacchi, K.L.; Bisson, L.F.; Adams, D.O. A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Viticult.* **2005**, *56*, 197–206.
5. Parpinello, G.P.; Versari, A.; Chinnici, F.; Galassi, S. Relationship among sensory descriptors, consumer preference and color parameters of Italian Novello red wines. *Food Res. Int.* **2009**, *42*, 1389–1395.
6. Harrison, R. Practical interventions that influence the sensory attributes of red wines related to the phenolic composition of grapes: A review. *J. Food Sci. Technol.* **2018**, *53*, 3–18.
7. Gawel, R.; Iland, P.G.; Francis, I.L. Characterizing the astringency of red wine: A case study. *Food Qual. Prefer.* **2001**, *12*, 83–94.
8. Ferrer-Gallego, R.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. *Food Res. Int.* **2014**, *62*, 1100–1107.
9. Schelezki, O.J.; Smith, P.A.; Hranilovic, A.; Bindon, K.A.; Jeffery, D.W. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition. *Food Chem.* **2018**, *244*, 50–59.
10. Petrie, P. Quantifying the advancement and compression of vintage. *Aust. N. Z. Grapegrow. Winemak.* **2016**, *5*, 40.
11. Sparrow, A.M.; Smart, R.E. Pinot noir wine processing and quality improved by skin fragmentation. *Catal. Discov. Pract.* **2017**, *1*, 88–98.
12. Schelezki, O.J.; Antalick, G.; Šuklje, K.; Jeffery, D.W. Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis. *Food Chem.* **2020**, *309*, 125698.
13. Schelezki, O.J.; Šuklje, K.; Boss, P.K.; Jeffery, D.W. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food Chem.* **2018**, *259*, 196–206.

14. King, E.S.; Dunn, R.L.; Heymann, H. The influence of alcohol on the sensory perception of red wines. *Food Qual. Prefer.* **2013**, *28*, 235–243.
15. Longo, R.; Blackman, J.W.; Torley, P.J.; Rogiers, S.Y.; Schmidtke, L.M. Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *J. Sci. Food Agric.* **2017**, *97*, 8–16.
16. Petrie, P.; Teng, B.; Smith, P.A.; Bindon, K.A. Sugar reduction: Managing high Baume juice using dilution. *Wine Vitic. J.* **2019**, *34*, 36.
17. Teng, B.; Petrie, P.R.; Smith, P.A.; Bindon, K.A. Comparison of water addition and early-harvest strategies to decrease alcohol concentration in *Vitis vinifera* cv. Shiraz wine: Impact on wine phenolics, tannin composition and colour properties. *Aust. J. Grape Wine Res.* **2020**, *26*, 158–171.
18. Australian Government, Bureau of Meteorology. Available online: http//www.bom.gov.au (accessed on 11 October 2019).
19. Mercurio, M.D.; Dambergs, R.G.; Herderich, M.J.; Smith, P.A. High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *55*, 4651–4657.
20. Kang, W.; Niimi, J.; Bastian, S.E.P. Reduction of red wine astringency perception using vegetable protein fining agents. *Am. J. Enol. Viticult.* **2018**, *69*, 22–31.
21. Kassara, S.; Kennedy, J.A. Relationship between red wine grade and phenolics. 2. Tannin composition and size. *J. Agric. Food Chem.* **2011**, *59*, 8409–8412.
22. Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746.
23. Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A* **2003**, *995*, 99–107.
24. Bindon, K.A.; Kennedy, J.A. Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. *J. Agric. Food Chem.* **2011**, *59*, 2696–2707.
25. Li, S.; Bindon, K.; Bastian, S.E.; Jiranek, V.; Wilkinson, K.L. Use of winemaking supplements to modify the composition and sensory properties of shiraz wine. *J. Agric. Food Chem.* **2017**, *65*, 1353–1364.
26. Ruiz-Garcia, Y.; Smith, P.A.; Bindon, K.A. Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydr. Polym.* **2014**, *114*, 102–114.
27. Danner, L.; Crump, A.M.; Croker, A.; Gambetta, J.M.; Johnson, T.E.; Bastian, S.E.P. Comparison of rate-all-that-apply (RATA) and descriptive analysis (DA) for the sensory profiling of wine. *Am. J. Enol. Viticult.* **2018**, *69*, 12–21.
28. Oppermann, A.; de Graaf, C.; Scholten, E.; Stieger, M.; Piqueras-Fiszman, B. Comparison of rate-all-that-apply (RATA) and descriptive sensory analysis (DA) of model double emulsions with subtle perceptual differences. *Food Qual. Prefer.* **2017**, *56*, 55–68.
29. Vidal, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. How do consumers describe wine astringency? *Food Res. Int.* **2015**, *78*, 321–326.
30. Kang, W.; Niimi, J.; Muhlack, R.A.; Smith, P.A.; Bastian, S.E. Dynamic characterization of wine astringency profiles using modified progressive profiling. *Food Res. Int.* **2019**, *120*, 244–254.
31. Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age". *J. Sci.I Food Agric.* **1977**, *28*, 279–287.
32. Payne, C.; Bowyer, P.K.; Herderich, M.; Bastian, S.E.P. Interaction of astringent grape seed procyanidins with oral epithelial cells. *Food Chem.* **2009**, *115*, 551–557.
33. McGuire, R.G. Reporting of objective color measurements. *HortScience* **1992**, *27*, 1254–1255.
34. Sun, B.; Sá, M.d.; Leandro, C.a.o.; Caldeira, I.; Duarte, F.L.; Spranger, I. Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *J. Agric. Food Chem.* **2013**, *61*, 939–946.
35. McRae, J.M.; Schulkin, A.; Kassara, S.; Holt, H.E.; Smith, P.A. Sensory properties of wine tannin fractions: Implications for in-mouth sensory properties. *J. Agric. Food Chem.* **2013**, *61*, 719–727.
36. Adams, D.O. Phenolics and ripening in grape berries. *Am. J. Enol. Viticult.* **2006**, *57*, 249–256.
37. Bindon, K.A.; Kassara, S.; Smith, P.A. Towards a model of grape tannin extraction under wine-like conditions: The role of suspended mesocarp material and anthocyanin concentration. *Aust. J. Grape Wine Res.* **2017**, *23*, 22–32.
38. Bindon, K.A.; Smith, P.A.; Holt, H.; Kennedy, J.A. Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. *J. Agric. Food Chem.* **2010**, *58*, 10736–10746.

39. Moskowitz, H.R. The sweetness and pleasantness of sugars. *Am. J. Psychol* **1971**, *84*, 387–405.
40. Quijada-Morín, N.; Williams, P.; Rivas-Gonzalo, J.C.; Doco, T.; Escribano-Bailón, M.T. Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chem.* **2014**, *154*, 44–51.
41. Chong, H.H.; Cleary, M.T.; Dokoozlian, N.; Ford, C.M.; Fincher, G.B. Soluble cell wall carbohydrates and their relationship with sensory attributes in Cabernet Sauvignon wine. *Food Chem.* **2019**, *298*, 124745.
42. Vidal, S.; Francis, L.; Noble, A.; Kwiatkowski, M.; Cheynier, V.; Waters, E. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta* **2004**, *513*, 57–65.
43. Unterkofler, J.; Muhlack, R.A.; Jeffery, D.W. Processes and purposes of extraction of grape components during winemaking: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 1–19.
44. Cretin, B.N.; Dubourdieu, D.; Marchal, A. Influence of ethanol content on sweetness and bitterness perception in dry wines. *LWT* **2018**, *87*, 61–66.
45. Hufnagel, J.C.; Hofmann, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008**, *56*, 9190–9199.
46. Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. *Understanding Wine Chemistry*; John Wiley & Sons: Chichester, West Sussex, United Kingdom, 2016.
47. Pearson, W.; Schmidtke, L.; Francis, L.; Blackman, J. Sensory analysis: Provenance, preference and pivot: Exploring premium Shiraz with international sommeliers and Australian winemakers using a new rapid sensory method. *Wine Vitic. J.* **2018**, *33*, 35.
48. Bajec, M.R.; Pickering, G.J. Astringency: Mechanisms and perception. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 858–875.
49. Guinard, J.-X.; Pangborn, R.M.; Lewis, M.J. The time-course of astringency in wine upon repeated ingestion. *Am. J. Enol. Viticult.* **1986**, *37*, 184–189.
50. Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Dynamic characterization of red wine astringency: Case study with Uruguayan Tannat wines. *Food Res. Int.* **2016**, *82*, 128–135.
51. Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Sensory characterization of the astringency of commercial Uruguayan Tannat wines. *Food Res. Int.* **2017**, *102*, 425–434.
52. Kemp, B.; Trussler, S.; Willwerth, J.; Inglis, D. Applying temporal check-all-that-apply (TCATA) to mouthfeel and texture properties of red wines. *J. Sens.* **2019**, *34*, e12503.
53. Jack, F.R.; Piggott, J.R.; Paterson, A. Analysis of textural changes in hard cheese during mastication by progressive profiling. *J. Food Sci.* **1994**, *59*, 539–543.
54. Peltier, C.; Visalli, M.; Schlich, P. Enhancing canonical variate analysis by taking the scaling effect into account. *Food Qual. Prefer.* **2018**, *64*, 88–93.
55. Lattey, K.A.; Bramley, B.; Francis, I. Consumer acceptability, sensory properties and expert quality judgements of Australian Cabernet Sauvignon and Shiraz wines. *Aust. J. Grape Wine Res.* **2010**, *16*, 189–202.
56. Cramer, R.D. Partial least squares (PLS): Its strengths and limitations. *Perspect. Drug Discov. Des.* **1993**, *1*, 269–278.
57. Bindon, K.A.; Kassara, S.; Solomon, M.; Bartel, C.; Smith, P.A.; Barker, A.; Curtin, C. Commercial *Saccharomyces cerevisiae* yeast strains significantly impact Shiraz tannin and polysaccharide composition with implications for wine colour and astringency. *Biomolecules* **2019**, *9*, 466.
58. Courregelongue, S.; Schlich, P.; Noble, A.C. Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food Qual. Prefer.* **1999**, *10*, 273–279.
59. Nguyen, A.N.; Johnson, T.E.; Jeffery, D.W.; Capone, D.L.; Danner, L.; Bastian, S.E. Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing Ganoderma lucidum extract as a novel ingredient. *Foods* **2020**, *9*, 224.

Supplementary Materials (Foods, 2020, 9 (8), 1027):

Table S1. List of sensory attributes scored in the rate-all-that-apply (RATA) assessment.

	No.	Attribute abbreviation	Definition
Color	1	C_R	Intensity of red color
	2	C_P	Intensity of purple color
	3	C_B	Intensity of brown color

	No.	Attribute abbreviation		No.	Attribute abbreviation	Definition
Aroma	4	A_DF	**Flavor**	26	F_DF	Dark fruit (e.g. blackberry, blackcurrant, plum, and dark cherry...)
	5	A_RF		27	F_RF	Red fruit (e.g. raspberry, strawberry, red cherry, and red current...)
	6	A_DrF		28	F_DrF	Dried fruit (e.g. prune, raisins, fig and dried apricote...)
	7	A_Ja		29	F_Ja	Jammy
	8	A_Con		30	F_Con	Confectionery (e.g. candy, lolly, fruit drops...)
	9	A_Choc		31	F_Cho	Chocolate
	10	A_CN		32	F_Co	Coconut
	11	A_CV		33	F_CV	Cooked vegetables (e.g. cabbage and beans...)
	12	A_ED		34	F_ED	Earthy / Dusty
	13	A_EM		35	F_EM	Eucalypt / Mint
	14	A_FL		36	F_FL	Floral / Perfume / Musk
	15	A_FFM		37	F_FFM	Forest floor / Mushrooms
	16	A_GP		38	F_GP	Green pepper / Capsicum
	17	A_Her		39	F_Her	Herbaceous
	18	A_Le		40	F_Le	Leather
	19	A_Pep		41	F_Pep	Pepper (black and white pepper)
	20	A_Sav		42	F_Sav	Savoury / Meaty / Gamey
	21	A_Sp		43	F_Sp	Spice (e.g. anise, clove, cinnamon, liquorice, and nutmeg...)
	22	A_SS		44	F_SS	Stemmy / Stalky
	23	A_TS		45	F_TS	Toasty / Smoky
	24	A_Van		46	F_Van	Vanilla
	25	A_Wo		47	F_Wo	Woody (e.g. cedar, pencil shavings, and cigar box...)
Taste	48	T_B				Bitterness
	27	T_Sw				Sweet
	28	T_A				Sour / Acidity
Mouthfeel	51	MF_B				Wine body
	52	MF_OH				Alcohol level / Heat
	53	MF_Ast				Astringency / Tannin
	54	MF_Sm				Smoothness
	55	MF_Ro				Roughness
	56	MF_Vis				Viscosity (the resistance of the wine when you move it around on the palate)
Aftertaste	57	AT_F				Length of the aftertaste of fruit flavors
	58	AT_NF				Length of the aftertaste of non-fruit flavors

Table S2. The model performance for the first run of Partial Least Squares regression. The discriminated astringency attributes are Y-variables and significantly different chemical parameters are X-variables.

Model quality of Partial Least Squares regression		
Statistic	Comp1	[a]Comp2
[b]Q^2 cum	0.185	0.748
[c]R^2Y cum	0.637	0.944
[c]R^2X cum	0.417	0.750

Variable Importance in the Projection (VIP):		
Variable	VIP for Comp1	VIP for Comp2
GPC MM (g/mol)	2.073	**1.849**
Total tannin	1.886	**1.555**
Total phenolics	1.824	**1.502**
Galacturonic acid	1.181	**1.037**
%gall	1.179	**1.021**
Fucose	0.666	**0.995**
Total Poly	0.978	**0.928**
Galactose	0.907	0.862
Rhamnose	0.739	0.855
Arabinose	0.738	0.828
HPLC MM (g/mol)	0.031	0.799
%Tri-OH	0.283	0.796
mDP	0.012	0.787
pH	0.240	0.772
Alcohol	0.589	0.705
Titratable acid pH 8.2	0.322	0.689
Glucuronic acid	0.335	0.539
Glucose	0.148	0.172

[a] The optimum number of components/latent variables required for this model was 2 (determined by the automatic function in the XLSTAT).

[b] The Q^2 cumulative index measures the global goodness of fit and the predictive quality of the models.

[c] The cumulative R^2Y and R^2X cum that corresponds to the correlations between the explanatory (X) and dependent (Y) variables with the components are very close to 1 with 2 components.

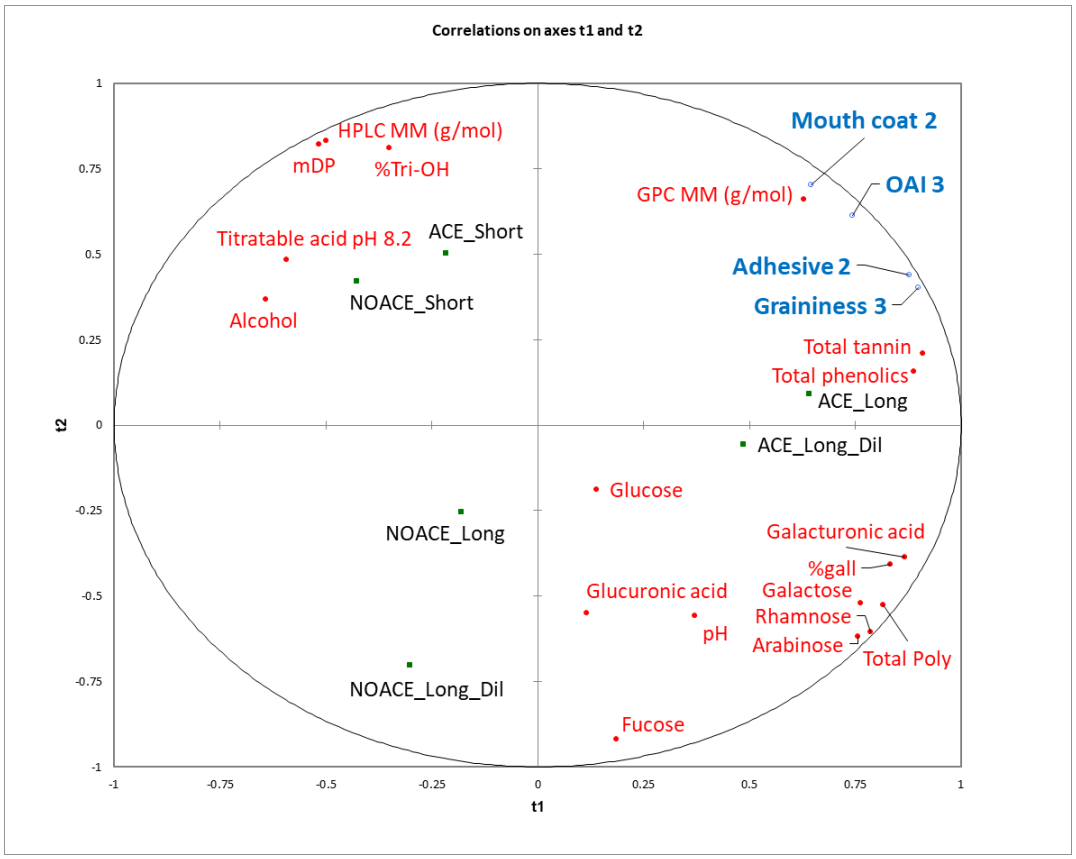


Figure S1. The plot of the first run of Partial Least Squares regression between the significantly different sensory attributes from PP (in blue) and significantly different chemical parameters (in red). Shiraz wines (in black) prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). %Tri-OH, %gall and total poly are epigallocatechin(%), epicatechin gallate (%) and total polysaccharides, respectively.

CHAPTER 5

A preliminary chemical and sensory study of the Accentuated Cut Edges (ACE) technique on Shiraz wines produced at an industry scale

The Shiraz wines reported on in Chapter 4, were all produced on a small (25kg ferments) and replicated research scale. We wished to examine whether findings for the research scale wines would translate to wines made on an industry scale. Therefore, two pilot commercial wines produced in 2018 by the Coriole winery at industry scale (approximately 2.45 tonne ferments) were analysed in 2019 for chemical composition and sensory measures alongside the research wines and the results presented in this Chapter (in a short research communication format based on the research note requirement of American Journal of Enology and Viticulture).

Abstract: A new grape-must polyphenol extraction technique, Accentuated-Cut-Edges (ACE), previously investigated in a limited number of studies using research wines revealed its capacity for modifying wine astringency. However, since Shiraz wines examined previously were produced in small scale fermentation batches, a follow-up investigation was needed to validate the ACE technique for commercial use. Thus, two pilot commercial wines (average 2.45 tonnes for each treatment) ACE (conventional crushing plus treatment of Della Toffola Maceration Accelerator) with 5-day skin contact and NOACE (conventional crushing only) with 8 days on skins were produced in this study and analysed for basic wine chemical composition, colour, phenolic compounds, polysaccharides and sensory profiles. It was encouraging to observe again, that similar to research scale wines, even at industry volumes the ACE technique could improve the extraction of phenolic compounds. This indicated that ACE could potentially assist the wine industry to combat one of the challenges caused by climate change, namely vintage compression, by pressing red wine ferments earlier, freeing up tank space for other wines.

Keywords: Commercial winemaking, skin fragmentation, tannin, phenolics, polysaccharides, sensory characterization.

1. Introduction

Accentuated Cut Edges (ACE) is a new grape must processing technique by which grape skins are mechanically broken down into smaller fragments while maintaining seed integrity (Sparrow et al. 2016b). This innovative technique provides more broken skin edges and therefore improves the extraction of phenolic components during fermentation, while avoiding the extraction of astringent or bitter compounds potentially resulting from seed damage (Sparrow et al. 2016a). The ACE was originally created to address the dilemma of poor colour development and low pigment stability in wines made by *Vitis vinifera* cv. Pinot

noir (Smart and Sparrow 2016). Compared with conventional crushing, the ACE technique was able to increase wine colour density by 50% and stable pigment concentration by 95% in Pinot noir wines (Sparrow et al. 2016b). In addition to colour, wines produced by the ACE technique were three times higher in tannin concentration (Sparrow et al. 2016b), and following sensory assessment demonstrated both greater astringency and bitterness intensities (Sparrow et al. 2016a). The ACE technique also improved the fruity characters in Pinot noir wine, notably the aromas of banana, peach, and black currant along with the flavour of dark fruit (Sparrow et al. 2016a).

Vintage compression represents a shortened vintage period for many grape cultivars, which increases pressure on winery logistics including tank space, pump-over regimes and labour needs for the wine industry (Petrie et al. 2019). Since the ACE technique might enable the extraction of phenolic components from the grape skin earlier than usual during fermentation, the feasibility of this processing technique to address vintage compression was studied in 2017 (Sparrow and Smart 2017). According to the preliminary findings in 2017, the ACE treatment with two-day maceration on skins was sufficient to extract a larger proportion of anthocyanin and tannin in Pinot noir wines relative to conventional crushing (eight days on un-fragmented skins). Therefore, the ACE treatment allows ferments to be pressed off skins earlier when compared with conventional crushing techniques, thereby has the capacity to permit better compressed vintage management (Sparrow and Smart 2017).

Prior to this PhD thesis and specifically the research within Chapters 4 and the current chapter, limited studies had evaluated ACE and only in Pinot Noir grapes. However, the most planted red wine grape variety in Australia is Shiraz (*Vitis Vinifera*), and having its origins in Ancient Persia, this noble grape variety is important for the wine industry globally as well, and it is grown and produces Shiraz or Syrah wines from France, Portugal, Italy, Spain, South Africa, USA and New Zealand (Robinson and Harding 2015). Therefore, in Chapter 4 of this

thesis, a study was conducted to investigate the effects of ACE on Shiraz wines. A total of six experimental treatments were produced in triplicate, where wines were produced following NOACE (conventional crushing) and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). Wine samples underwent detailed colour, chemical composition, and sensory profiling and a more in depth examination of astringency and its sub-qualities. In addition, insights into chemical factors influencing the astringent sensations were elucidated. The observations in Chapter 4 were the first to illustrate that the ACE technique did not affect perceived wine colour, but significantly enhanced the concentration of tannin and total phenolics. Polysaccharide concentration in wine was primarily increased in response to maceration time rather than the ACE technique. For wine flavour, the greater contribution of broken skin edges provided by ACE promoted the release of substances related to earthy/dusty flavour into wine. Although pre-fermentation addition of water significantly diluted wine aromas, flavours and astringency profiles, the ACE technique was able to reduce the impacts of dilution through an increase in the intensity of adhesive and graininess astringency sub-quality perception.

As the six experimental Shiraz wines examined in Chapter 4 were produced in small scale fermentation batches (25kg), it was recognised that a follow-up investigation was needed to validate the ACE technique for commercial use. In this Chapter, two pilot commercial wines (ACE with 5-day skin contact and NOACE with 8 days on skins) produced by a commercial winery at industry scale in the vintage of 2018, were analysed in 2019. This preliminary experiment conducted a detailed analysis of the polyphenolic and polysaccharide content of these two commercial wines plus generated detailed sensory and dynamic astringency sub-quality profiles in order to investigate the feasibility of the ACE technique on Shiraz wines at an industry scale.

2. Materials and methods

2.1 Vinification protocol of commercial wine samples

The Shiraz grapes for commercial wine samples were sourced from the same vineyard in the McLaren Vale region (35°13′S, 138°62′E) as the six experimental treatment wines in Chapter 4. However, these grapes were harvested and processed in the vintage of 2018. The mean January temperature of this region in 2018 was 30.1°C with average annual rainfall 485.4 mm, which was 1.5°C lower and 20 mm higher compared to 2019 (Australian Government, Bureau of Meteorology, 2018).

The grapes were machine-harvested at 13 Bé (pH 3.7 and 4.6 g/L titratable acidity), separated into two parcels and underwent two different treatments; Commercial_ACE_Short (5 days skin maceration) and Commercial_NOACE_Long (8 days skin maceration) (2.3 and 2.6 tonnes of grapes, respectively). The NOACE and ACE fruit underwent conventional crushing (Miller MC250), but the ACE parcel was further processed through a Della Toffola Maceration Accelerator. Both musts were acid-adjusted by the addition of 1.5 g/L tartaric acid, followed by yeast inoculation with Enartis Ferm® Vintage Red (200 mg/L). The fermentations were conducted in 4-tonnes open stainless steel fermenters (the fermentation temperature ranged from 20 to 28°C) with hand plunging twice daily (full submersion of the cap), and the malolactic fermentation (MLF) occurred naturally. After either 5 or 8 days of skin contact, wines were pressed and then transferred into neutral French oak hogsheads (5-7th fill). After the MLF, 80 mg/L total sulphur dioxide were added into wines. After a six-month maturation in oak, these two commercial wine samples were blended from oak barrels respectively, and then hand-bottled (750ml black Bordeaux-shape bottles) without fining and filtration and cellared at 16 °C for a year before being analysed.

2.2 Chemicals and measurements

Reagents, reference compounds (⩾ 97% purity) and monosaccharides standards (for both identification and quantification) used for the high-performance liquid chromatography (HPLC), methyl cellulose precipitable (MCP) tannin method, and the modified Somers assay were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Milli-Q water (Millipore, North Ryde, NSW, Australia) was utilized for the preparation of solutions.

2.2.1 Chemical measurements

Wine samples were analysed for pH, titratable acidity (as tartaric acid g/L equivalents, and the measurement endpoint was pH 8.2), volatile acidity (as g/L equivalent to acetic acid), and sulfur dioxide (free and total) by the Australian Wine Research Institute's (AWRI) Commercial Services Laboratory. Total residual sugars and malic acid levels were measured by Chemwell® 2910 Automated EIA and Chemistry Analyser (Awareness Technology, Palm City, FL, USA) with the Megazyme K-FRUGL (Chicago, Illinois, USA) and Vintessential Enzymatic L-Malic Acid (Dromana, VIC, Australia) test kits. The alcohol level of the samples was measured with an Anton Paar Alcolyzer Wine ME and DMA 4500M (North Ryde, NSW, Australia). The colour of wine samples was measured by both the modified Somers assay (Mercurio, Dambergs, Herderich, & Smith, 2007) and CIELab tristimulus (GBC Scientific Equipment Cintra 4040).

Total tannin concentration was measured by the high throughput MCP tannin method, and total phenolic concentration was determined by the modified Somers assay (Mercurio et al. 2007). In addition, tannins from the commercial wine samples were isolated by solid-phase extraction (Kassara and Kennedy 2011) and analysed by HPLC (Agilent 1100) following phloroglucinolysis (Kennedy and Jones 2001) to determine the subunit composition, mean degree of polymerization (mDP), and molecular mass (MM (phloro) according to the

conditions outlined previously (Kennedy and Taylor 2003). The tannin molecular mass was also measured by gel permeation chromatography (MM (GPC)) on an Agilent 1200 with the modifications described previously (Bindon and Kennedy 2011). The polysaccharides in wine samples were prepared and hydrolysed as in the work of Schelezki et al. (2018). Total wine soluble polysaccharides and the monosaccharide residues following acid hydrolysis were determined by HPLC (Agilent 1100) (Ruiz-Garcia et al. 2014).

All chemical parameters were measured in technical triplicate.

2.2.2 Sensory evaluations

Sensory evaluations of commercial wine samples were conducted in the same manner and at the same times as the six experimental wine treatments reported in Chapter 4. The wine descriptive profiling was characterised by using rate-all-that-apply (RATA, using a seven-point intensity scale) for 58 common red wine attributes (across the sensory modalities of wine colour, aroma, flavour, taste, mouthfeel, and aftertaste) by a panel of 61 untrained participants (34 female and 27 male, average age 26 years) (Danner et al. 2018). Furthermore, astringency profiles of wines were assessed by a trained sensory panel (n=8, 3 male and 5 female, average age 51 years) using modified progressive profiling (PP) for seven wine astringency attributes (overall astringent intensity (OAI), pucker, mouth coat, dry, grippy, adhesive and graininess) (Kang et al. 2019). The PP evaluation consisted of 5 time periods (each lasting 10 sec), the first with wine in the mouth and then 4 after expectoration, with 20-second gaps between each time period. The intensity of attributes in each wine were rated consecutively on 15 cm scales with low and high word anchors located at 10 and 90% of the scale, respectively.

All sensory profiles of each wine were evaluated in duplicate.

2.3 Data analyses

The chemical measurements of two commercial wine samples were analysed by independent samples t-test with the assumption of Levene's Test for Equality of Variances in SPSS statistics (ver. 26; IBM Corporation, Chicago, IL, USA). The sensory data of these two wines were analysed and presented together with the six experimental treatments of chapter 4. Since the commercial wine samples' fermentations were not replicated, data for the experimental treatments were firstly averaged across triplicate ferments. The data from RATA was analysed by a multivariate ANOVA (at α 5%) using XLSTAT, with treatment as fixed factors, and assessor as a random factor. Significantly different RATA attributes (means) were further analysed with Principal components analysis (PCA). In terms of PP assessment, the data was firstly analysed by univariate ANOVA (at α 5%) for each attribute at every single time period, with treatment and replicate of sensory evaluation as fixed factors, and assessor as random factor using XLSTAT. Significantly different PP attributes were further analysed by the mixed assessor model canonical variate analysis (MAM-CVA) in RStudio (R ver. 3.5.1, Boston, MA, USA) with the software package CVAS (Version 1.0, written by Caroline Peltier on 3rd November 2014).

2. Results and discussion

2.1 Chemical composition

The chemical composition of the two commercial wine samples are displayed in Table 1. There were significant chemical differences between the two wines for alcohol concentration, total residual sugar, pH and volatile acidity, however the differences of mean values were small and would likely not result in perceptible differences between the two wines. The levels of sulphur dioxide (free and total) were significantly different between these two commercial wines, which might be caused by various degrees of oxidation occurring in

the wines in the oak barrels. Sulphur dioxide in wine is vital for wine colour and preventing oxidation (Waterhouse et al. 2016). As a likely consequence of the obvious differences in sulphur levels between two commercial wines, the wine colour outcomes were different to the six experimental wines. There was no significant difference for neither hue (regardless if measured by the modified Somers assay or CIELab) nor total anthocyanin in the six experimental treatments, but significant differences (2-tailed Sig. <0.05) were observed within the commercial wine samples. In the work of Sparrow and Smart (2017) (likewise at an industry scale), ACE technique with shorter skin maceration had higher colour density and hue than the conventional crushing, but the wines they made was using Pinot noir. In addition, of note was the observation in commercial Shiraz wines of an increase in SO_2 resistant pigments in the ACE wines, which was the same as the small scale fermentations in Chapter 4. This indicated that the ACE technique enables a significant increase in SO_2 resistant pigments, probably because more proanthocyanidins were extracted from grapes into wines to combine with anthocynins to form the polymeric pigments (SO_2 resistant pigments) (Casassa and Harbertson 2014). Additionally, as seen for the six experimental treatments, total phenolics and tannin concentrations of Commerical_ACE_Short (5 days on skins) were similar to the Commercial_NOACE_Long (8 days on skins), which means that the ACE technique can also meaningfully enhance the phenolic extraction from grapes into wine at an industry scale. This 3-day reduction in skin maceration time, but resultant similar phenolic extraction profile indicated once again the potential of using the ACE technique for the wine industry to combat one of the challenges caused by climate change, vintage compression. This means that by being able to press wine ferments earlier, that this would free up tank space in order to process other wines.

However, the results of polysaccharide composition were different from those in Chapter 4, whereby the concentrations increased with increasing maceration. In the

commercial wine samples, only the concentration of the arabinose residues significantly increased in response to maceration time, but the remaining polysaccharide composition did not. The arabinose residue arises generally from the polysaccharides rich in arabinose and galactose (PRAGs), and the PRAGs are important contributors to the mouthfeel of red wines and are thought to be negatively associated with bitterness and astringency (Chong, Cleary, Dokoozlian, Ford, & Fincher, 2019; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014). The concentration of xylose reduced with increasing maceration. The possible reasons behind the differences in polysaccharide observations between commercial and experimental wines, such as different must condition, rate of fermentation or fermentation temperature etc., need to be further verified in the future.

Table 1. Chemical composition (means ± standard deviation) of two commercial Shiraz wines analysed with independent samples t-test (assumption of Levene's Test for Equality of Variances was applied).

	Commercial_ACE_ Short	Commercial_ NOACE_Long	t	Sig. (2-tailed)
Alcohol (% v/v)	14.2±0.1	14.5±0.0	-8.000	[b]**0.001**
Total residual sugar (g/L)	0.9±0.1	1.3±0.1	-5.500	**0.005**
pH	3.66±0.01	3.71±0.01	-8.000	**0.001**
[a]Titratable acid (g/L, at pH 8.2)	6.1±0.0	5.8±0.0	N/A	N/A
Malic acid (g/L)	<0.40	<0.40	N/A	N/A
Sulfur Dioxide (free) (mg/L)	12.3±0.6	31.7±1.5	-20.506	**<0.0001**
Sulfur Dioxide (total) (mg/L)	52.3±0.6	85.0±1.0	-49.000	**<0.0001**
Volatile acidity (g/L)	0.42±0.01	0.48±0.01	-12.728	**<0.0001**
Wine colour density (a.u.)	14.11±0.20	13.48±0.74	1.432	0.225
Hue	0.710±0.001	0.700±0.001	10.877	**<0.0001**
Total Anthocyanins (mg/L)	496.9±0.1	554.8±17.5	-5.745	**0.005**
SO_2 resistant pigments (a.u.)	4.35±0.04	4.11±0.99	3.982	**0.016**
Chroma (CIELab)	35.89±0.00	33.43±0.03	147.572	**<0.0001**
Hue angle(CIELab)	6.360±0.028	6.937±0.215	-4.592	**0.010**
Total phenolics (a.u.)	58.1±0.1	59.74±2.2	-1.303	0.263
Total tannin (mg/L)	1283±71	1351±173	-0.626	0.565
MM (HPLC) (g/mol)	2513±93	2874±24	-6.518	**0.003**
mDP	8.4±0.3	9.597±0.1	-6.499	**0.003**
Epigallocatechin(%)	29.33±0.64	29.67±0.43	-1.400	0.234
Epicatechin gallate (%)	2.77±0.08	2.88±0.05	-2.095	0.104
Mass conversion (%) of phloroglucinolysis	16.03±0.39	17.53±0.41	N/A	N/A
MM (GPC) (mg/L)	1457±4	1475±2	-7.291	**0.002**
Mannose (mg/L)	125.1±1.0	124±6.0	0.317	0.767
Rhamnose (mg/L)	45.5±1.4	46.4±2.0	-0.606	0.577
Glucuronic acid (mg/L)	13.3±0.5	12.2±1.6	1.144	0.316
Galacturonic acid (mg/L)	145.1±6.2	149.3±5.6	-0.874	0.432
Glucose (mg/L)	59.2±21.5	42.6±1.2	1.334	0.253
Galactose (mg/L)	140.2±3.5	139.3±4.6	0.262	0.806
Xylose (mg/L)	7.4±0.6	5.9±0.4	3.492	**0.025**
Arabinose (mg/L)	103±2.8	121±5.7	-4.886	**0.008**
Fucose (mg/L)	11.0±0.4	9.9±0.8	2.217	0.091
Total Polysaccharide (mg/L)	649.9±30.4	650.6±23.1	-0.034	0.975

[a] t cannot be computed because the standard deviations of both groups are 0.

[b] Bold significance values represent significant differences between treatments.

2.2 Sensory evaluations

2.2.1 Wine descriptive profiling by RATA

Descriptive profiling of wines was firstly achieved by RATA, which is a rapid method that can profile different food or beverage products using naïve consumers as subjects (Danner et al. 2018; Oppermann et al. 2017). The RATA evaluation of these two commercial wines was conducted, analysed and presented together with the six experimental treatments of chapter 4. 30 out of 58 attributes were discriminated ($p < 0.05$) by 61 untrained participants/naïve consumers (Table 2). An exploration of the data was carried out through a Principle Component Analysis (PCA) (Figure 1) across the two commercial wines and the six experimental treatments of Chapter 4. As shown in the figure, the wine samples clustered into two groups, either commercial (which appeared on the right of the PCA biplot) or experimental (in the middle) wines. The commercial wines associated with more intense peppery, spicy, savoury, earthy, toasty and woody characters, which was most likely due to a cooler growing season in 2018 and the usage of oak barrels (Rankine 2007; Zhang et al. 2015). The experimental wines were higher intensity in the characters of red fruit and confectionery characters, which might be reduced in intensity by oak usage in the commercial wines and which were a year older (Robinson and Harding 2015). Examining the commercial wines, the Commericial_ACE_Short wine had a higher intensity of stemmy and herbaceous flavours on the palate, which might be due to the ACE technique enhancing the release of substances related to these two flavours (e.g. 3-sec-Butyl-2-methoxypyrazine) (Unterkofler et al., 2020). On the contrary, the Commericial_NOACE_Long wine associated with more cooked vegetables and savoury aromas on the nose, possibly because substances/precursors generating these two aromas were mainly released from skin into the wine at a later stage of fermentation (when the alcohol level is relatively high) or because this wine sample was more reductive (higher in sulphur levels) (Rankine 2007). However, these speculations should be

studied further by head-space gas chromatography (GC) with mass spectrometry. In addition, the difference in astringency perception as well as roughness were discriminated by the naïve wine consumers where the experimental wines had higher intensities of both attributes. The observation of this mouthfeel perception corresponded with the results of chemical analysis, whereby the experimental wines had approximately 300 mg/L more and larger sized tannins than the commercial wines (McRae et al. 2013). The astringency profile was further evaluated in detail by a trained sensory panel (n=8) using PP.

Table 2. The significantly different attributes across two commercial and six experimental wine samples from RATA detected by a multivariate ANOVA (at α 5%).

Attribute		Definition	F	*p*
Colour				
	C_P	Intensity of purple colour	12.994	< 0.0001
	C_B	Intensity of brown colour	7.578	< 0.0001
Aroma				
	A_RF	Red fruit (e.g. raspberry, strawberry, red cherry, and red current...)	5.415	< 0.0001
	A_Con	Confectionery (e.g. candy, lolly, fruit drops...)	2.442	0.018
	A_CV	Cooked vegetables (e.g. cabbage and beans...)	3.233	0.002
	A_ED	Earthy / Dusty	3.750	0.001
	A_FL	Floral / Perfume / Musk	7.543	< 0.0001
	A_Le	Leather	4.128	0.000
	A_Pep	Pepper (black and white pepper)	4.735	< 0.0001
	A_Sav	Savoury / Meaty / Gamey	5.333	< 0.0001
	A_Sp	Spice (e.g. anise, clove, cinnamon, liquorice, and nutmeg...)	3.263	0.002
	A_SS	Stemmy / Stalky	2.320	0.025
	A_TS	Toasty / Smoky	7.128	< 0.0001
	A_Wo	Woody (e.g. cedar, pencil shavings, and cigar box...)	5.730	< 0.0001
Flavour				
	F_RF	Red fruit (e.g. raspberry, strawberry, red cherry, and red current...)	6.215	< 0.0001
	F_DrF	Dried fruit (e.g. prune, raisins, fig and dried apricote...)	2.549	0.014
	F_Con	Confectionery (e.g. candy, lolly, fruit drops...)	4.679	< 0.0001
	F_CV	Cooked vegetables (e.g. cabbage and beans...)	2.863	0.006
	F_ED	Earthy / Dusty	3.151	0.003
	F_FL	Floral / Perfume / Musk	3.322	0.002
	F_Her	Herbaceous	2.324	0.025
	F_Le	Leather	2.519	0.015
	F_Pep	Pepper (black and white pepper)	4.277	< 0.0001
	F_Sav	Savoury / Meaty / Gamey	4.192	< 0.0001
	F_SS	Stemmy / Stalky	2.737	0.009
	F_Wo	Woody (e.g. cedar, pencil shavings, and cigar box...)	5.783	< 0.0001
Taste				
	T_Sw	Sweet	2.635	0.011
Mouthfeel				
	MF_Ast	Astringency / Tannin	5.996	< 0.0001
	MF_Ro	Roughness	2.138	0.039
Aftertaste				
	AT_NF	Length of the aftertaste of non-fruit flavours	2.713	0.009

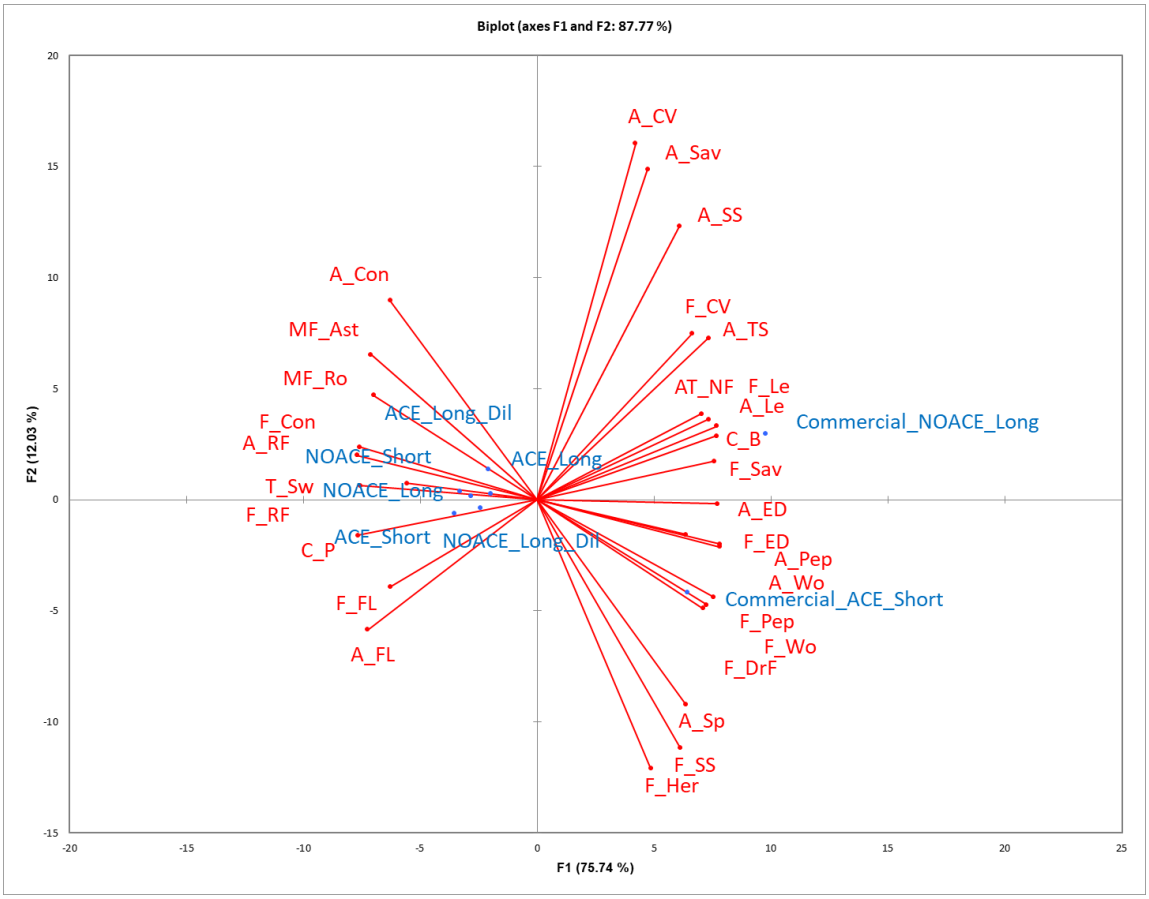


Figure 1. Principal Component Analysis of two commercial and six experimental wine samples for all significantly different sensory attributes ($p < 0.05$) from RATA. Experimental Shiraz wines prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). The data present in this figure were average of triplicate fermentation and duplicate evaluations for experimental wines and duplicate evaluations of commercial wines.

2.2.2 Astringency profiles of wines assessed by PP

A comprehensive dynamic astringency profile was generated by progressive profiling which evaluated the attributes of overall astringency intensity (OAI) and astringent sub-qualities, and the discriminated attributes ($p < 0.05$) are displayed in a mixed assessor model canonical variate analysis plot (MAM-CVA, Figure 2). Similar to the results of RATA

evaluation, there was a relatively clear separation between commercial and experimental wine samples on the MAM-CVA plot, with the two commercial wines associated with lower intensities of astringency attributes. This was likely due to the differences in total tannin concentration and the size of tannins (MM) between commercial and experimental wines as mentioned above (McRae et al. 2013). Similar to the observations of astringency profile in Chapter 4, the experimental wines treated by ACE plus long skin maceration (ACE_Long and ACE_Long_Dil) could still be separated from other experimental wines, because they had significantly higher tannin concentration and lager tannin size than others. While the astringency profiles were observed to be similar for the ACE_Short and NOACE_Long treatments for the experimental wines, these two treatments could be differentiated with the commercial wines. The Commercial_ACE_Short wine had higher intensity of graininess 2 (20-30 sec after expectoration of wine sample) but less intensity of OAI 3 (50-60 sec after expectorating wine). Greater differentiation between commercial wines based on astringency intensity and astringent sub-qualities might be caused by the different vinification protocols between experimental and commercial wines. For example, times on skins for commercial wines were 5 or 8 days, but were 3 and 6 days for experimental wines. In addition to maceration time, oak barrel maturation applied in the commercial wines and different dimensions of the fermenters cap surface to volume ratio could be other contributors to the differences in the astringency sensation (Chira and Teissedre 2015; Setford et al. 2017). Lastly, the polysaccharide composition could also explain the greater differentiation in astringency between commercial wines. Although the polysaccharide composition between the two commercial wines were relatively similar, the experimental NOACE_Long had significantly more total polysaccharides than ACE_Short. The total polysaccharides have negative effects on wine astringency perception (Chong, Cleary, Dokoozlian, Ford, &

Fincher, 2019; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014), thus the differences of astringency sensation in experimental wines were decreased.

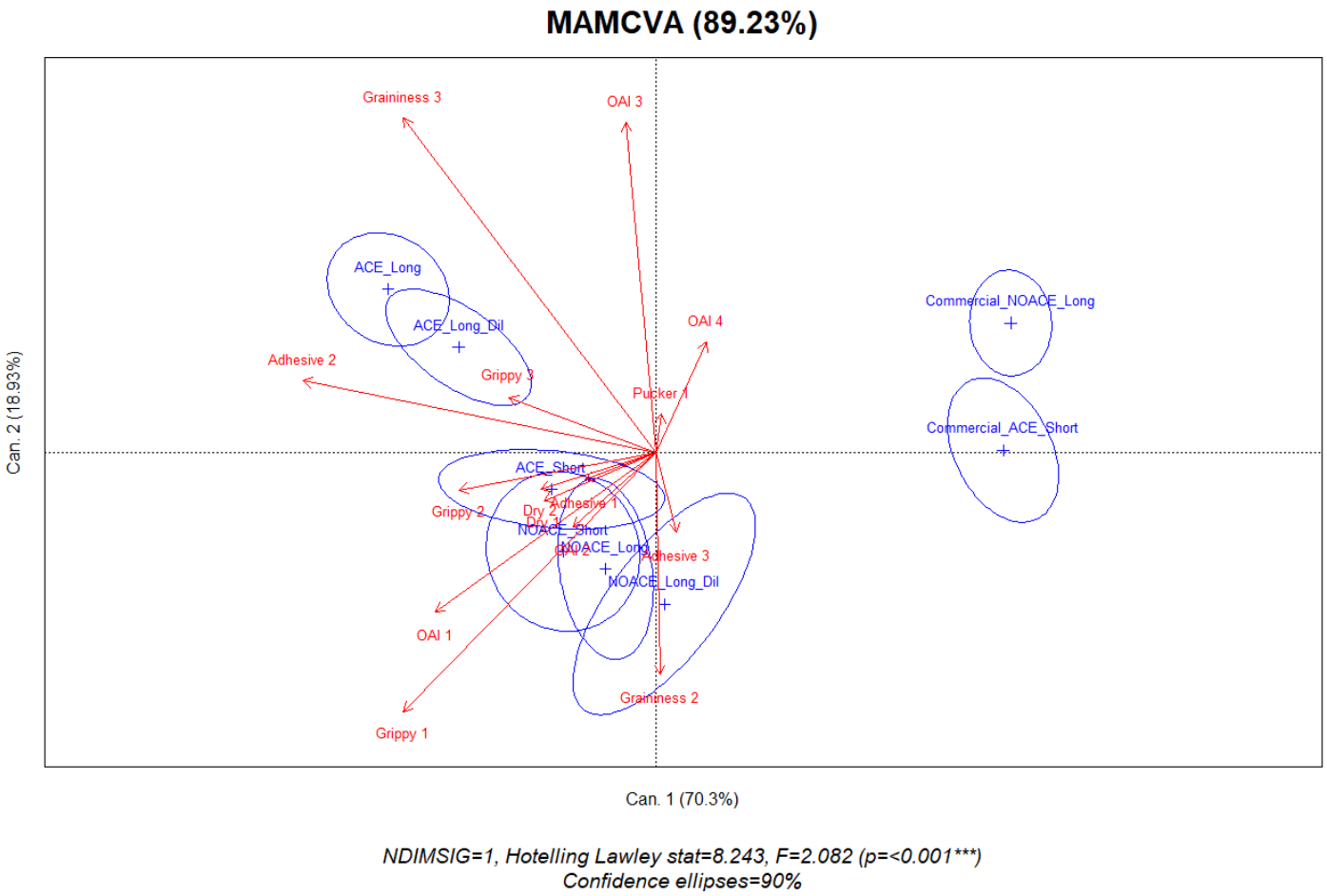


Figure 2. The MAM-CVA of two commercial wine samples and six experimental treatments for all significantly different astringency attributes ($p < 0.05$) from five evaluation periods. Experimental Shiraz wines prepared following NOACE and ACE maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation water dilution to 13.5 Bé (Long_Dil). Ellipses indicate the confidence intervals of 90%.

3. Conclusion and limitation

This preliminary experiment investigated the influence of the ACE grape must extraction technique with a shorter maceration period compared to conventional crushing only (NOACE) but with more time on skins in Shiraz wines at an industry scale. It was inspiring to observe again at an industry level that the ACE technique could enhance the extraction of phenolics (3-days less skin maceration, but similar phenolic extraction). This indicated that ACE could be used the wine industry to face the challenge of vintage compression caused by climate change. In future, the impacts of ACE on wine colour and sensory at an industry scale should be further explored by a separate but more comprehensive experiment, including volatile flavour analysis, research on other grape varieties (red and white varieties), replicate fermentations, and using wines without oak influence i.e. matured in inert and closed vessels (e.g. stainless steel) after fermentation to remove this confounding factor.

Reference:

Bindon, K.A., and J.A. Kennedy. 2011. Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. J. Agr. Food Chem. 59:2696-2707.

Casassa, L.F., and J.F. Harbertson. 2014. Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. Annu. Rev. Food Sci. Technol. 5:83-109.

Chira, K., and P.-L. Teissedre. 2015. Chemical and sensory evaluation of wine matured in oak barrel: effect of oak species involved and toasting process. Eur. Food Res. Technol. 240:533-547.

Danner, L., A.M. Crump, A. Croker, J.M. Gambetta, T.E. Johnson, and S.E.P. Bastian. 2018. Comparison of Rate-All-That-Apply (RATA) and Descriptive Analysis (DA) for the sensory profiling of wine. Am. J. Enol. Viticult. 69:12-21.

Kang, W., J. Niimi, R.A. Muhlack, P.A. Smith, and S.E. Bastian. 2019. Dynamic characterization of wine astringency profiles using modified progressive profiling. Food Res. Int. 120:244-254.

Kassara, S., and J.A. Kennedy. 2011. Relationship between red wine grade and phenolics. 2. Tannin composition and size. J. Agr. Food Chem. 59:8409-8412.

Kennedy, J.A., and G.P. Jones. 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J. Agr. Food Chem. 49:1740-1746.

Kennedy, J.A., and A.W. Taylor. 2003. Analysis of proanthocyanidins by high-performance gel permeation chromatography. J. Chromatogr. A 995:99-107.

McRae, J.M., A. Schulkin, S. Kassara, H.E. Holt, and P.A. Smith. 2013. Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. J. Agr. Food Chem. 61:719-727.

Mercurio, M.D., R.G. Dambergs, M.J. Herderich, and P.A. Smith. 2007. High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. J. Agr. Food Chem. 55:4651-4657.

Oppermann, A., C. de Graaf, E. Scholten, M. Stieger, and B. Piqueras-Fiszman. 2017. Comparison of Rate-All-That-Apply (RATA) and Descriptive sensory Analysis (DA) of model double emulsions with subtle perceptual differences. Food Qual. Prefer. 56:55-68.

Petrie, P., B. Teng, P.A. Smith, and K.A. Bindon. 2019. Sugar reduction: Managing high Baume juice using dilution. Wine & Viticulture Journal 34:36.

Rankine, B. 2007. Making good wine. Macmillan Publishers, Australia.

Robinson, J., and J. Harding. 2015. *The Oxford companion to wine*. American Chemical Society.

Ruiz-Garcia, Y., P.A. Smith, and K.A. Bindon. 2014. Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. Carbohydrate polymers 114:102-114.

Schelezki, O.J., P.A. Smith, A. Hranilovic, K.A. Bindon, and D.W. Jeffery. 2018. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from

Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition. Food Chem 244:50-59.

Setford, P.C., D.W. Jeffery, P.R. Grbin, and R.A. Muhlack. 2017. Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process modelling. Trends in Food Science & Technology 69:106-117.

Smart, R., and A. Sparrow. 2016. New winemaking process conceived in a northern Tasmania pilot winery-the beginnings of ACE. Wine and viticulture journal:9.

Sparrow, A.M., H.E. Holt, W. Pearson, R.G. Dambergs, and D.C. Close. 2016a. Accentuated Cut Edges (ACE): Effects of Skin Fragmentation on the Composition and Sensory Attributes of Pinot noir Wines. Am. J. Enol. Viticult. 67:169-178.

Sparrow, A.M., and R.E. Smart. 2017. Pinot noir wine processing and quality improved by skin fragmentation. Catalyst: Discovery into Practice:catalyst. 2017.17004.

Sparrow, A.M., R.E. Smart, R.G. Dambergs, and D.C. Close. 2016b. Skin particle size affects the phenolic attributes of Pinot noir wine: Proof of concept. Am. J. Enol. Viticult. 67:29-37.

Waterhouse, A.L., G.L. Sacks, and D.W. Jeffery. 2016. *Understanding wine chemistry*. John Wiley & Sons.

Zhang, P., K. Howell, M. Krstic, M. Herderich, E.W.R. Barlow, and S. Fuentes. 2015. Environmental factors and seasonality affect the concentration of rotundone in Vitis vinifera L. cv. Shiraz wine. PloS one 10.

CHAPTER 6

Concluding remarks and future perspectives

Astringency is an essential element driving wine quality, style, complexity and consumer preferences, and therefore has been the focus of much investigation by wine researchers globally. This thesis examined novel, alternative technologies to modify red wine astringency and aimed to measure its perception along with that of its more subtle sub-qualities, which has to date proved quite challenging. Furthermore, attempts were made to investigate the relationships between the wine matrix and the wine chemical composition, particularly the polyphenolic and polysaccharide profiles, with red wine texture and overall sensory perception. In contrast to methods used in past studies, a modified progressive profiling (PP) methodology was utilised to examine wine astringency and astringent sub-quality perception. This sensory methodology enabled dynamic and quantitative intensity measurement of astringent attributes. Thus, PP provided enhanced understanding of the chemical basis of subtle wine astringent sub-quality differences because of wine style; intentional manipulation of the astringent profile using systematic matrix component or physical changes; or novel grape processing techniques. In a response to more recent consumer expectations and demands, a substitute to animal-derived processing aids was explored and thus the efficacy of potato proteins to manipulate astringent compounds in red wine was examined, along with gaining insights into their optimal use. To aid with efficient extraction of grape polyphenol compounds, a new grape processing technology, Accentuated Cut Edges (ACE) had been developed by scientists in conjunction with industry. Although this technology had been studied with Pinot noir wine production, this thesis project explored ACE to elucidate its effect on Shiraz wine chemical composition and sensory profiles of both replicated research wines and industry scale wine samples for the first time.

6.1 Concluding remarks

6.1.1 Develop methodologies for improved sensory assessment and measurement of wine astringency and astringent sub-quality perception.

Reports of numerous sensory methodologies designed to evaluate temporal oral sensations exist. Progressive profiling is a sensory method originally developed to monitor perceived texture changes of cheese products by humans and had not been used in wine sensory evaluation prior to this thesis. Originally it involved simultaneous sensory assessment of the intensity of a specific set of pre-defined sensory attributes at discrete time intervals, from the onset of chewing through to swallowing. As such, progressive profiling enables evaluation of more attributes at one time than Time Intensity (TI), which is essential for decreasing the number of evaluations (rounds) of a product and therefore avoids panellist fatigue. This is particularly pertinent for astringency, which characteristically builds in intensity and duration upon repeated ingestion. Modified progressive profiling as utilised in this thesis, enabled measurement of the intensity of wine astringency profiles rather than the categorical data that would be measured had Temporal Dominance of Sensations (TDS) or Temporal Check-All-That-Apply (TCATA) been used.

Since wines have multiple dynamic sensory attributes that vary over the time the wine is held in the mouth (especially the astringent sensation) and after expectoration or swallowing, after examination of the sensory methodology literature, the decision was made to utilise a modified progressive profiling panel (n=8) for the assessment of astringent qualities in a set of commercially available red wines made from different grape varieties. The method was modified for these wines incorporating six by 10 sec time periods to replace single time points, and panellists wore nose clips during the evaluation to focus their attention on mouthfeel.

Universally used reference standards for astringent sub-qualities, have not been reported in the texture literature. Thus, an unambiguous vocabulary used for texture attributes is not readily available and has made astringency sub-quality assessments difficult. Previous published literature has largely relied on the feel of different cloth materials by the touch of the hand to define these sub-qualities. Nevertheless, in the experiments outlined in Chapter 2, an agreement on seven red wine astringency attributes and the method of their evaluation were clearly defined: overall astringent intensity (OAI) and 6 sub-qualities; pucker, mouth coat, dry, grippy, adhesive and graininess. The food grade reference standards defining these seven attributes were also generated (details of these standards may be found from Table 2 in Chapter 2).

Based on the observations in Chapter 2, the intensities of the astringent sub-qualities in wines were highly correlated with OAI. However, the sub-quality profiles at a specific evaluation period varied depending on the wine, as did the dynamic progression of all attributes. In addition, a better understanding of the relationships between the subtle astringent sub-qualities in wine and chemical parameters were gained by modified progressive profiling. This was achieved by establishing statistical correlations between sensory attributes at time periods with the highest effect sizes and chemical measures of the wine (which ensures that the data set of sensory measurement is as quantitative as the chemical data). In the studies of Chapter 4, modified progressive profiling was further used to examine 18 wines (6 treatments × triplicate wine ferments) made from the same grape variety (*Vitis vinifera* cv. Shiraz) to understand typical textural characters. Even though these research wines were made from the same batch of grapes (albeit with different treatments) under the same conditions, the subtle differences in astringency profiles could still be detected using modified progressive profiling.

Thus, the astringency attribute nuances and level of discrimination between these found in the studies of distinct wine styles in Chapter 2 and more similar research wines in

Chapter 4 indicated that modified PP provided an alternative tool for the quantitative measurements of astringency profiles, especially for the subtle differences of wine astringency sub-qualities.

6.1.2 Understand the drivers of different astringency 'texture or mouthfeel' qualities using chemistry and sensory evaluations.

Astringency sensations are elicited by several stimuli such as various phenolic components, organic acids, multivalent salts, and charged polysaccharides. However, the influencing factors behind astringent sub-quality perception are likely to be intricate and have not yet been fully elucidated. In the experiments of Chapter 2, a range of chemical measures of 13 Australian commercial red wines and 2 rosés produced from 11 grape varieties were made. These included density, alcohol concentration, pH, titratable acidity, residual sugars (glucose and total), total phenolics, and total tannin concentration. A more in depth analysis of the individual profiles of isolated tannins from each of these wines including subunit composition, mean degree of polymerization (mDP), and molecular mass was also undertaken. To understand any relationships between these chemical parameters and wine astringent sub-qualities, the chemical measures were correlated with perceived astringency responses defined by progressive profiling. The attribute pucker, negatively correlated to wine sugars. This was in agreement with the literature, which has revealed that sugars could suppress the overall astringent sensation. The correlation analysis also indicated that wine alcohol concentration positively associated with the sub-qualities of mouth coat and adhesive. OAI correlated highly and positively with wine tannins (including total concentration, percentage of epigallocatechin, tannin mDP and molecular mass). The intensity of the mouthfeel attribute dry positively correlated with total tannin concentration, and tannin mDP and molecular mass. The tannin molecular mass measured by gel permeation chromatography (GPC) was positively associated with all seven astringency attributes.

However, wine is a complex matrix, and consists of other components that are possibly unknown or not measured in Chapter 2, such as polysaccharides. Hence, the profiles of polysaccharides in wines (total polysaccharide concentrations as well as the proportional composition of the individual monosaccharide residues recovered following acid hydrolysis) were measured in Chapter 4 together with other wine chemical parameters. The relationships between these wine chemical components and astringency perceptions were also analysed by partial least squares regression. Similar to the observations in Chapter 2, the OAI and subtle, textural sub-qualities (mouth coat, adhesive and graininess) were positively driven by tannin molecular mass measured by GPC, total tannin, total phenolics and the percentage of tannin galloylation. Additionally, negative associations of OAI and these three sub-qualities with fucose residues were found.

This thesis research has now produced deeper understanding of factors that impact these specific astringency sensations. Thus if one wanted to manipulate these specific sensations in wine, one has models to test if that is possible. Furthermore, whether this knowledge could then be applied in industry to modify wine mouthfeel for enhanced consumption satisfaction needs to be explored.

6.1.3 Investigate alternative fining agents (natural & non-animal derived proteins) which have the capacity to modify wine astringency, and gain insights into how to optimise their use.

Wine astringency is modifiable by fining; the addition of reactive agents that bind to astringent compounds and results in a concentration reduction of these constituents, thus controlling the astringent sensation. Conventionally, to remove phenolic compounds in wine, animal-based and/or synthetic products are used as fining agents within the wine industry. However, using alternatives (such as vegetable proteins) has gained increased interest due to a

strong global shift in consumers' food and beverage requirements (for allergenic or ethical reasons). As a succedaneum to animal-derived fining agents, potato proteins were investigated in Chapter 3 because they have the potential to replace gelatin, a traditional protein fining agent derived from animal collagen (according to the outcomes of our published research paper in the appendix of this thesis, American Journal of Enology and Viticulture. 69:1, 2018). Firstly, the kinetics of tannin and phenolic removal (measured at10 time points across 48 hours) by fining using potato proteins at different doses (125, 250, 500 and 1000 mg/L) were investigated on two unfined Cabernet Sauvignon (*Vitis vinifera*) wines. The fining efficacy of potato proteins was simultaneously compared with gelatin. The concentration of total phenolics and tannin mostly declined as the dose of fining agents was increased, but gelatin consistently induced a greater decrease than potato proteins at all time points. One hour of fining time was sufficient for both fining agents to adsorb the majority of phenolics and tannin in the unfined wines. Nevertheless, the fining response differed between the two wines for both fining agents.

In addition, based on the different response between the two wines from the kinetic study, a more detailed comparison of the wine phenolic profiles was made. A similar phenolic composition indicated that the different fining response observed for the two wines was not predominantly due to differences in tannin sub-units or size, but was more likely because of the influence of other chemical parameters within the wine matrix.

Furthermore, a systematic examination of the impact of the wine matrix components (including pH, ethanol concentration and sugar concentration) and physical factors (temperature and agitation) on the efficacy of the fining process was performed. The approach taken was a Design of Experiments (DoE, a fractional 2^{5-1} factorial design) on one of the Cabernet Sauvignon wines (selected due to the observation that a reduction in polyphenols occurred more readily in response to potato protein fining) using potato proteins.

The results from the DoE suggested that potato protein fining was significantly impacted by the wine pH, ethanol concentration, fining temperature and an interaction (pH × ethanol), but not by sugar content or agitation. Insights for the improvement of fining were gained from the findings of Chapter 3, elucidating that potato protein fining efficacy could be improved by treating wines at higher temperatures (20°C, rather than the conventional 10-15°C), and at both a lower pH, and/or alcohol concentration.

6.1.4 Examine a novel wine process technology for the modification of wine astringency and astringent sub-quality perception.

Accentuated Cut Edges (ACE), a newly developed grape extraction technique, mechanically breaks grape skins into small fragments but maintains seed integrity, potentially enhancing the concentration and composition of polyphenols in finished wine. This technique was used previously on Pinot noir grapes (*Vitis vinifera*) in order to improve wine colour and phenolic extraction because wines made from this variety can have poor colour development and low pigment stability. Nevertheless, the most economically important grape variety for Australia and many other wine regions globally is *Vitis vinifera* cv. Shiraz. Australian wine producers are facing the challenges of vintage compression that increases tank space, needs pump-over logistics and labour requirements and also leads to grapes destined for winemaking being harvested with increased sugar levels. The ACE technique is considered to have the potential to help address vintage compression, whereby it allows wine to be pressed off skins earlier compared with conventional crushing techniques. The work of Chapter 4 was the first to use the ACE technique on Shiraz grapes and examine the impacts of the combination of ACE and pre-fermentation water addition on wine composition and sensory, and this work was also the first to elucidate the impacts of ACE on wine polysaccharide composition and astringent sub-quality perception. Six treatments of Shiraz wines were produced in triplicate in the vintage of 2019 from grapes sourced in the McLaren Vale region of South Australia; i.e. NOACE

(conventional crushing) or ACE (crushing followed by further cutting of skins) as well as maceration with either 3 days (Short) or 6 days (Long) on skins, or 6 days on skins with pre-fermentation dilution to 13.5 Bé (Long_Dil). The chemical composition (alcohol concentration, residual sugar, pH, titratable acid, volatile acid, malic acid, sulfur conditions, colour measurements by modified Somers assay and CIELab, and the profiles of phenolic compounds and polysaccharides) and sensory profiles (using rate-all-that-apply for 58 common red wine attributes and modified progressive profiling for seven wine astringency attributes) of treatment wines were determined.

The ACE technique did not influence the sensorial perception of Shiraz wine colour (based on both CIELab and rate-all-that-apply measurements), but significantly enhanced the concentration of tannin and total phenolics. Polysaccharide concentration in wine was mainly affected by maceration time rather than ACE. In terms of wine sensory outcomes, ACE appeared to increase earthy/dusty flavour and aroma in wine, possibly due to more precursors released by greater skin breakdown. The pre-fermentation addition of water had significant impacts on wine aromas and flavours, such as a reduction of floral/perfume/musk intensity on the nose and red fruit intensity on the palate. Water addition before fermentation also significantly reduced the wine's overall astringency intensity and astringent sub-quality profiles, but in combination with the ACE technique increased the intensities of adhesive and graininess which partly overcame the impact of water addition on mouthfeel. This knowledge suggests that the wine industry might use the ACE processing technique when winemakers have a need to modify wine alcohol level by using pre-fermentation water addition.

The six experimental Shiraz wines were produced in small scale fermentation batches (25kg), and it was therefore recognised that a follow-up investigation was needed to validate the ACE technique for commercial use. Therefore, in Chapter 5, two pilot

commercial wines (ACE with 5-day skin contact and NOACE with 8 days on skins) were produced by the Coriole winery at industry scale (averaged 2.45 tonnes for each treatment) in the vintage of 2018 and analysed in 2019. This preliminary experiment investigated the feasibility of the ACE grape extraction technique on Shiraz wines at an industry scale. Due to the wine's scale, the design and conditions of this experiment were not replicated, as was the case for the experimental wines. Nevertheless, it was encouraging to observe again even at industry volumes that the ACE technique could improve the extraction of phenolics. This indicated that ACE could potentially be used by the wine industry to combat one of the challenges of climate change and vintage compression caused by climate change, by pressing wine ferments earlier, freeing up tank space for other wines.

6.2 Future directions

Modified progressive profiling presents a sensory approach able to measure the intensity of astringency and its sub-qualities' profiles in wines, but so far this method has only been used in studies with small numbers (15, and 20) of wines. Hence, to conduct modelling, for e.g. to examine if a given sub-quality usually appears at a certain time period, evaluation of a larger number of different wines by PP would be required in further studies. In addition, wine astringency sensations are complex and require more time to evaluate (particularly due to the sub-qualities), therefore modified progressive profiling is still limited by the need to conduct several rounds of evaluations per sample (2 rounds for 7 attributes in this thesis, only one round required for other temporal methods but they are qualitative in nature). Thus, it would be relevant to improve the current protocol in future (such as by changing the length of evaluation period and/or time between evaluations). This thesis did not conduct a side by side comparison between the different sensory methods currently available to characterize wine astringency profiles, so it would be of interest to compare the data between modified progressive profiling and Temporal Dominance of Sensations (TDS) in the future, i.e.

investigating whether the attribute with the highest effect size from modified progressive profiling is the same as the dominant attribute from TDS at each evaluation period.

The drivers of different astringency sub-qualities were investigated in this thesis by identifying relationships between chemical parameters (basic wine composition and phenolic and polysaccharide components) and sensory evaluation datasets. However, the exploration of other wine matrix compositions (such as metal ions) for astringency sub-qualities is warranted. In addition to the "correlation" analysis of perceived astringency with analytical measures of wine chemical composition used in this thesis, two other approaches could be used to understand the drivers of astringency sub-qualities, e.g. "fractionation" and "synthesis". For instance, different phenolic fractions could be isolated from wine and then further fractionated. In turn, these fractions could be added into a neutral white or model wine, and a sensory evaluation conducted to elucidate relationships between specific phenolic fractions/compounds and their respective astringent mouthfeel qualities.

This thesis studied an alternative, non-animal derived fining agent, potato protein. Insights into the optimal conditions for its use in fining astringent compounds were revealed in Chapter 3. The physical factor of agitation during fining did not significantly impact either the adsorption of total phenolics or tannin. However, certain positive effects of agitation were detected which revealed that agitation might become a major effect on fining response if the contact time for fining were reduced (the data observed in Chapter 3 were at 48 hours after fining addition). This hypothesis should be confirmed in further studies, for example decrease the fining time to 10 hours or less. Furthermore, the current research showed that by utilising potato or gelatin a maximum fining response was achieved at approximately 1 hour. This offers the possibility to conduct astringency fining using an in-line dosing system in the future as opposed to batch fining and racking off fining lees. In addition, fractional factorial designs (as used in the Chapter 3) are not designs that will reveal all possible optimum conditions, but

do help to identify important variables that have an effect on whatever is measured in an efficient way. Hence, future work should be expand the factorial designs on a greater range of red wines, with greater compositional variation (such as metal ions and polysaccharides) to continue searching for the factors that do have an effect on the potato proteins fining. Then, once all the variables that do have an effect are identified, optimisation designs can be performed.

Further research is required to screen other potential wine astringency fining agents by using both instrumental (including volatile profiles) and sensory analyses. The use of different types of agents combined at one time for astringency modification is also a future research direction.

Up until now, the ACE technique has been used in the production of Pinot noir and Shiraz, thus, research on other grape varieties (including the impacts on aromas/flavours on white varieties) is required in future. Two pilot commercial wines were produced by a winery in Chapter 4, but a more comprehensive experiment in industrial scale should be conducted. In addition, it would be worthwhile conducting consumer tests to elucidate the consumers' preference for (e.g. preference tests) and attitude (e.g. online-survey) towards ACE wines in future.

In summary, the research contained in this thesis provides advanced insights and alternative tools for researchers and the wine industry. Uncovering what components impact wine astringency, knowing how to better evaluate perceived wine astringency along with its sub-qualities and modify this important wine sensory attribute with a more informed approach, will enhance the capability of wine producers to better cope with some of the ramifications of climate change such as higher alcohol levels and vintage compression, target product style and quality plus meet consumer expectations.

APPENDIX

These is an additional peer-reviewed paper generated during candidature, which is written using data from experiments of Mr Kang's Master of Viticulture and Oenology research project.

Reduction of red wine astringency perception using vegetable protein fining agents

Wenyu Kang [a], Jun Niimi [a] and Susan E.P. Bastian [a,*]

[a] School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia.

[*] Correspondence: sue.bastian@adelaide.edu.au; Tel.: +61-8-83136647

American Journal of Enology and Viticulture – 2018, 69:1, 22-31.

Reduction of Red Wine Astringency Perception Using Vegetable Protein Fining Agents

Wenyu Kang,[1] Jun Niimi,[1] and Susan Elaine Putnam Bastian[1]*

Abstract: The use of vegetable proteins to fine astringent compounds in wine has gained increased interest due to the pressure of consumer demand. The objective of this study was to compare the ability of alternative vegetable proteins (derived from rice, soy, pea, or potato) to reduce tannin and thereby astringency, relative to that of traditional fining agents (gelatin and polyvinylpolypyrrolidone [PVPP]) in a commercial wine with added grape seed extract. Total tannin and phenolics, SO_2-resistant pigments, pH, and color of the treated wines were determined, and astringency intensity perception was evaluated by a trained sensory panel (n = 9). Potato, pea, soy, and gelatin proteins similarly reduced total tannin concentration. Similar to PVPP, addition of rice or soy protein reduced total phenolics. These alternative vegetable proteins also influenced the chroma, which may change the depth of wine color. Furthermore, this study was the first to evaluate the change in astringency sensation resulting from the use of rice and soy proteins as alternative fining agents. The type of vegetable proteins used appeared to fine different types of polyphenolic compounds, an observation that was reflected on astringency perception and requires further investigation. The chemical and sensory measures showed that rice and potato proteins have the potential to replace PVPP and gelatin, respectively.

Key words: astringency, fining, sensory, tannin, vegetable proteins, wine color

Astringency is an important contributor to the tactile (touch) sensation of wine (Gawel 1998), and is characterized by drying, roughing, and puckering sensations felt in the oral cavity (Lee and Lawless 1991, Vidal et al. 2015). The generally accepted mechanism of astringency perception is primarily due to the lack of lubrication of the oral epithelium caused by the interactions of astringent stimuli (e.g., polyphenols) with salivary proteins, such as proline-rich proteins (PRP) and histatins, and the subsequent precipitation of these complexes (Jöbstl et al. 2004, Poncet-Legrand et al. 2007, Scollary et al. 2012). Astringency is considered an important factor that drives wine quality, complexity (Peynaud and Blouin 1996), and consumer liking (Bastian et al. 2010, Lattey et al. 2010). Because of the impact of astringency on wine hedonics, processing aids such as fining agents are used during the winemaking process to control wine perception.

Astringency fining is the process of adding reactive substances (agents) that bind to polyphenolic compounds to reduce the concentration of these constituents and control the astringency of wine (Rankine 2004). Fining agents used to remove these astringent components are typically proteinaceous or protein-like, and are either derived from animals or synthetically produced (Laborde et al. 2006, Cosme et al. 2009, Boulton et al. 2013). Traditionally, proteinaceous fining agents are used in the wine industry, and these include gelatin, egg albumen, isinglass, and casein (Zoecklein et al. 1995, Margalit 2012). Although the efficacy of the fining agents will vary for each wine depending on the internal wine conditions (such as wine pH, temperature, polyphenol types, and others) and the molecular weight (MW) of the individual agent, per the same unit weight, gelatin is the most effective in tannin removal, followed by egg albumen, isinglass, and casein, probably because gelatin has more potential hydrogen-binding sites (Zoecklein et al. 1995). A nonproteinaceous fining agent commonly used in the wine industry is polyvinylpolypyrrolidone (PVPP), a synthetic, high-MW polymer of polyvinylpyrrolidone (Margalit 2012). It can remove total and flavonoid phenolic compounds in wine, but preferentially binds to low-MW phenolic compounds (Ribéreau-Gayon et al. 2006). Therefore, PVPP finds its major application in removing precursors for browning in white wines and browning and bitterness in red wines.

There are numerous limitations to the use of traditional wine fining agents. One major hurdle is to cater to the wide range of consumers' dietary requirements, some of which are by choice (for example vegetarians and vegans), whereas others are by necessity (such as potential fish and dairy food allergies) (Mills et al. 2009). In addition, consumers are becoming more aware of synthetic additives used in food processing and generally prefer natural to synthetic additives in wines (Saltman et al. 2015). Traditional agents may influence wine quality. Gelatin can change wine color (Somers

[1]School of Agriculture, Food & Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA, Australia, 5064.

*Corresponding author: (sue.bastian@adelaide.edu.au; tel: +61 8 8313 6647)

Acknowledgments: This research was funded by the School of Agriculture, Food and Wine, the University of Adelaide. The sensory study was approved by the Human Research Ethics Committee of the University of Adelaide (project number: H-2016-084). The authors are thankful for the assistance given by Dr. Lukas Danner, Liang Chen, Hélène Bossan, and all members of the sensory panel. Tarac Technologies Pty. Ltd. is greatly appreciated for the donation of grape tannin extracts and Laffort Australia for the gratuitous potato proteins.

Supplemental data is freely available with the online version of this article at www.ajevonline.org.

Manuscript submitted June 2017, revised Oct 2017, accepted Oct 2017

doi: 10.5344/ajev.2017.17054

and Verette 1988), and anecdotally, it has been observed to reduce the overall intensity of aroma (Voilley et al. 1990). Residual amounts of egg albumen and gelatin can dissolve in the wine, which can leave undesirable precipitates in the finished wine (Rankine 2004), and require a cofining agent such as silica dioxide (Kieselsol) or hydrolyzable tannins to remove the residual, dissolved protein (Hahn and Possmann 1977). Isinglass is often used in beer and wine clarification, but has the main disadvantage of being expensive and also may impart minor fishy character in wines (Zoecklein et al. 1995). To address these considerations, studies have investigated the potential for vegetable proteins to substitute these traditional fining agents (Maury et al. 2003).

A range of vegetable proteins have shown potential to reduce phenolic content in wine, including those from corn/maize (Tschiersch et al. 2008, 2010, Simonato et al. 2009, 2013), potato (Tschiersch et al. 2010, Gambuti et al. 2012), grape seed (Vincenzi et al. 2013), wheat (Maury et al. 2003, 2016, Tschiersch et al. 2010, Granato et al. 2014), white lupin (Maury et al. 2003), rice (Tschiersch et al. 2010), pea (Cosme et al. 2012), lentil and soy (Granato et al. 2014), and sorghum (Hagerman and Butler 1980). Of these proteins, only the effects of corn, potato, grape seed, and pea protein on the astringency perception of wine have been tested with sensory analyses, albeit some in a nonrobust way. Unfortunately, the relative effectiveness of numerous vegetable proteins in reducing wine astringency is poorly understood. Furthermore, the relative fining abilities of vegetable proteins compared with those of traditional fining agents has not been fully examined.

The objective of this research was to compare the abilities of vegetable proteins sourced from rice, soy, pea, or potato to reduce tannin and thereby astringency, plus alter bitterness and overall flavor intensity perception with those of the traditional astringency fining agents gelatin and PVPP in model, unfined Cabernet Sauvignon (*Vitis vinifera*) red wine.

Materials and Methods

Materials. Chemicals used for analysis were ammonium sulfate, (+)-catechin hydrate (≥98%), methylcellulose, food-grade pectin, potassium metabisulfite, and food-grade quinine sulfate (all from Sigma-Aldrich); compressed, food-grade nitrogen gas (Coregas Pty. Ltd.); hydrochloric acid 37% and sodium hydroxide (Merck Pty. Ltd.); and grape seed extract (GSE) (Supplemental Table 1) and tartaric acid (Tarac Technologies). Milli-Q water (Millipore) was used for the preparation of fining agent stock solutions.

Four alternative vegetable protein powders were used for fining: rice protein isolate (total protein 82.8 g/100 g [Nutrients Direct Pty Ltd.]), pea protein isolate (total protein 81.7 g/100 g [MyoPures]), soy protein isolate (total protein 91 g/100 g [Bulk Nutrients]), and Patatin P (protein content undisclosed [Laffort]). Protein isolates of rice, pea, and soy were obtained as commercial nutrient supplements. Two fining agents, powdered gelatin (porcine-derived and bloom number of zero [Laffort]) and PVPP (Sigma-Aldrich), were used as the standard fining agents that are traditionally used in the wine industry and served as reference points for comparison.

Cabernet Sauvignon bag-in-box wine (2015 vintage, region of South Australia, Yalumba) was used as the base wine, which had the following original chemical composition: pH 3.60, 5.9 g/L total acidity, 14.3% alcohol, 0.4 g/L residual sugar, 41 mg/L free SO_2, 66 mg/L total SO_2, and 0.11 g/L copper. The wine also had oak contact (2 g/L French oak chips) during the six-day ferment process according to the producer. This base wine was used to add GSE and simulate an unfined astringent wine from the winemaking process. It was determined, through bench-top tasting by three experts, that the addition of 0.5 g/L GSE (stirred for 2 hrs at 20°C, covered by inert food-grade compressed N_2 gas) best simulated the astringency of an unfined wine.

Experimental design. The experiment for the chemical measurements was designed to cover a wide range of fining concentrations and a selection of powdered vegetable proteins and conventional industry fining agents. A 6 × 12 fining agent type by concentration design, respectively, was used in the present study (Table 1). The concentrations were selected after preliminary trials, as we hypothesized that fining would not necessarily reduce tannin in a linear manner. Thus, logarithmic increments were made for the fining concentrations. The concentrations of gelatin and rice proteins were identical, with ~0.13 logarithmic increments within the range of 50 to 1000 mg/L. The concentrations of the remaining four agents were identical, with ~0.1 logarithmic increments from 100 to 1000 mg/L. The maximum concentration of fining agent used was uniform across all fining agents (1000 mg/L) according to the standards of the International Organisation of Vine and Wine (Issue 2016; International code of oenological practices). Base and control wine samples were also added to the experimental design, in which the base wine was original Cabernet Sauvignon wine and the control was the wine with 0.5 g/L GSE added (i.e., model unfined wine). This gave a total of 74 samples for chemical measurements.

Table 1 Fining agent concentration ranges and corresponding fining levels for the chemical measurements.

	Fining concentration (mg/L)[a]					
Fining level	Gelatin	PVPP[b]	Pea protein	Rice protein	Potato protein	Soy protein
1	15	30	30	15	30	30
2	50	100	100	50	100	100
3	70	125	125	70	125	125
4	90	160	160	90	160	160
5	120	200	200	120	200	200
6	170	250	250	170	250	250
7	220	315	315	220	315	315
8	300	400	400	300	400	400
9	405	500	500	405	500	500
10	550	630	630	550	630	630
11	740	795	795	740	795	795
12	1000	1000	1000	1000	1000	1000

[a]The fining concentrations were determined by log increments based on the results of the preliminary tests.
[b]PVPP, polyvinylpolypyrrolidone.

The experimental design for the sensory study was chosen on the basis of the results of tannin measurements, since tannins are considered to be primarily responsible for the sensation of astringency in wine (McRae et al. 2013). Wines fined with every agent at three concentrations (50, 315, and 1000 mg/L) were evaluated by the panel. As 50 mg/L is a common starting dose for bench-top fining trials in the wine industry, this rate was selected as the lowest fining concentration. Including base, control, and treatments, all 20 wine samples were prepared in duplicate.

Fining trials. To accurately measure the amounts of fining agents used for addition, each of the six fining agents was solubilized in Milli-Q water as stock solutions (50 g/L, stirred for 12 hrs at 20°C) before use (Simonato et al. 2013). Each stock solution was serially diluted to produce 12 concentrations for addition to the unfined wine. Solubilized fining agents (200 µL) were added to model unfined wines (10 mL) and mixed with an IKA MS 1 shaker. Base and control wine sample additions were made with the same volume of water but without fining agents to maintain a consistent volume. All 74 wine samples were settled for 48 hrs after the addition, under N_2 gas at 20°C, followed by centrifugation at 1900*g* for 10 min (Maury et al. 2003) in a Hettich Universal 320 R centrifuge. The fining trials were carried out in duplicate.

The 20 wine sample preparations for sensory analysis were prepared in duplicate following the same procedure as described above, but scaled up to 500 mL per sample (centrifuged in a Beckman Coulter Beckman J2-21 centrifuge).

Chemical analyses. To measure the wine treatments for chemical analyses, the supernatants from the fining trials were recovered. Samples were analyzed for pH (Iland 1980), and total tannins were measured with the 1 mL methylcellulose-precipitable method (Mercurio and Smith 2006, Sarneckis et al. 2006); total phenolics and SO_2-resistant pigments were determined with the modified Somers assay (Somers and Evans 1974, 1977, Mercurio et al. 2007). All spectrophotometric measures were performed with the Thermo Multiskan spectrophotometer (Thermo Fisher Scientific), and the tannin concentrations were calculated as epicatechin equivalents (g/L) by extrapolation from an epicatechin standard curve (Supplemental Figure 1). The colors of treatments were measured through CIELab tristimulus using GBC Scientific Equipment Cintra 4040 (10 degree observer angle, in a wavelength range of 375 to 780 nm with 2 nm slit widths and at a scan speed of 1000 nm/min), and were presented as chroma = $\sqrt{a*^2 + b*^2}$ and hue angle = $\frac{1}{\tan \frac{b*}{a*}}$.

Sensory evaluation. The sensory evaluation consisted of a series of training sessions and formal evaluations, held twice weekly at the University of Adelaide's Waite campus sensory facility. A panel comprising four female and five male assessors (23 to 27 years old) from the University of Adelaide were recruited on the basis of availability for the sensory testing. The assessors were trained in the use of scales, detection, and discrimination of astringency and bitterness in water solutions and wines for a total of 6 hrs. The base wines with 0, 10, or 20 mg/L quinine were used as standards representing 30, 50, and 75% bitterness intensities on the scale, respectively, and were used for the training. Analogously, base wines with 0, 0.3, or 0.6 g/L GSE, representing 30, 50, and 80% astringency intensity on the scale, respectively, were used for the training. No standard for overall flavor intensity was provided, but the panelists had extensive experience in rating this attribute. Formal evaluation commenced upon satisfactory performance of the assessors, including agreement, repeatability, and discrimination of astringency and bitterness.

The trained panel assessed wine samples for the intensity of astringency, bitterness, and overall flavor on 150 mm line scales, anchored at 10 and 90% as "low" and "high", respectively. Forty samples (i.e., 20 duplicate samples) were evaluated across three sessions. Prior to each session, the base and control wines were used to refresh each panelist's memory of astringency. The data were collected with the web-based data collection software RedJade. Wine samples (25 mL) were served at room temperature (25°C) in black ISO wine-tasting glasses with four-digit codes and in a randomized order. Wine samples were assessed in individual booths under white light. Interstimulus breaks of 2 min were provided along with water, crackers, and 1 g/L pectin solution as palate cleansers to minimize astringency carryover.

Data analyses. The chemical measures were analyzed by one-way analysis of variance (ANOVA). The sensory data was analyzed with a general linear model ANOVA (GLM-ANOVA), with two-way interaction (panelists as random factor and fining agents, fining concentrations, and replicates as fixed factors). A further one-way ANOVA on astringency level within the fining agents was also performed. All ANOVA models were conducted with SPSS statistics (ver. 23, IBM Corporation), analyzed at an alpha level of 5%, and significantly different parameters were further analyzed with Fisher's least significant difference (LSD) post-hoc test in XLSTAT (ver. 2016, Addinsoft SARL).

Results and Discussion

The effects of fining agents on total tannin concentration. We determined total tannin concentrations in wines as a function of increasing concentrations of six fining agents. Tannin levels were significantly ($p < 0.05$) reduced with increasing fining concentration, and this was the case for all fining agent types (Figure 1). Gelatin reduced total tannin the most, followed by potato protein (~1 and 0.7 g/L reduction, respectively), and pea and soy proteins reduced tannin similarly to each other (~0.45 g/L reduction). Gelatin and potato protein showed a similar trend of tannin reduction across similar concentration ranges. However, gelatin consistently reduced tannin more than did potato protein (~0.3 g/L), and hence required a lower concentration to achieve the tannin level that is equivalent to base wine (1.8 g/L). In addition, the current findings differed from the literature, in which gelatin and potato protein at 100, 200, and 300 mg/L could remove tannin with similar efficiencies (Gambuti et al. 2012). Despite using gelatin with the same bloom number of zero, differences in fining procedures may have been the reason for the discrepancies in these results; Gambuti et al. (2012) fined for

1 wk at 14°C, whereas in the current study, the wines were fined for two days at 20°C.

High rice protein concentrations decreased tannin concentrations, unlike in previous reports (Tschiersch et al. 2010), where rice was as effective as potato. These authors used a specified MW fraction of rice proteins (10 to 32 kDa), which might explain this difference in results from the current study in which rice protein was not fractionated. In addition, pea and soy proteins at similar concentrations (~400 mg/L) reduced the tannin concentrations close to that of base wine. PVPP was the least effective fining agent in removing tannin, consistent with literature; PVPP has a rigid tertiary structure and therefore is limited in interacting with large polyphenolic compounds (Laborde et al. 2006).

The effects of fining agents on total phenolics. Increases in the concentrations of PVPP and rice and soy proteins each significantly ($p < 0.05$, $p < 0.001$, and $p < 0.01$, respectively) reduced total phenolics in wine (Figure 2). Gelatin, pea, and potato proteins yielded no significantly different results, most likely owing to the large variation in standard deviations. The ability of PVPP to remove total phenolics was consistent with previous studies (Sims et al. 1995, Baron et al. 1997, Donovan 1998). Pea proteins at 400 mg/L reduced total phenolics by 4.48% in the current study, which was less than previous reports of 9.04% of phenolics (Cosme et al. 2012). Different fractions of pea proteins might explain this difference as well.

Tannin concentration and total phenolics measures varied with fining agent type. For instance, potato proteins decreased a large amount of total tannins, but did not significantly reduce total phenolics. In contrast, rice protein was effective in reducing total phenolics, but reduced little tannin. It is possible that rice proteins have a higher affinity to phenolic

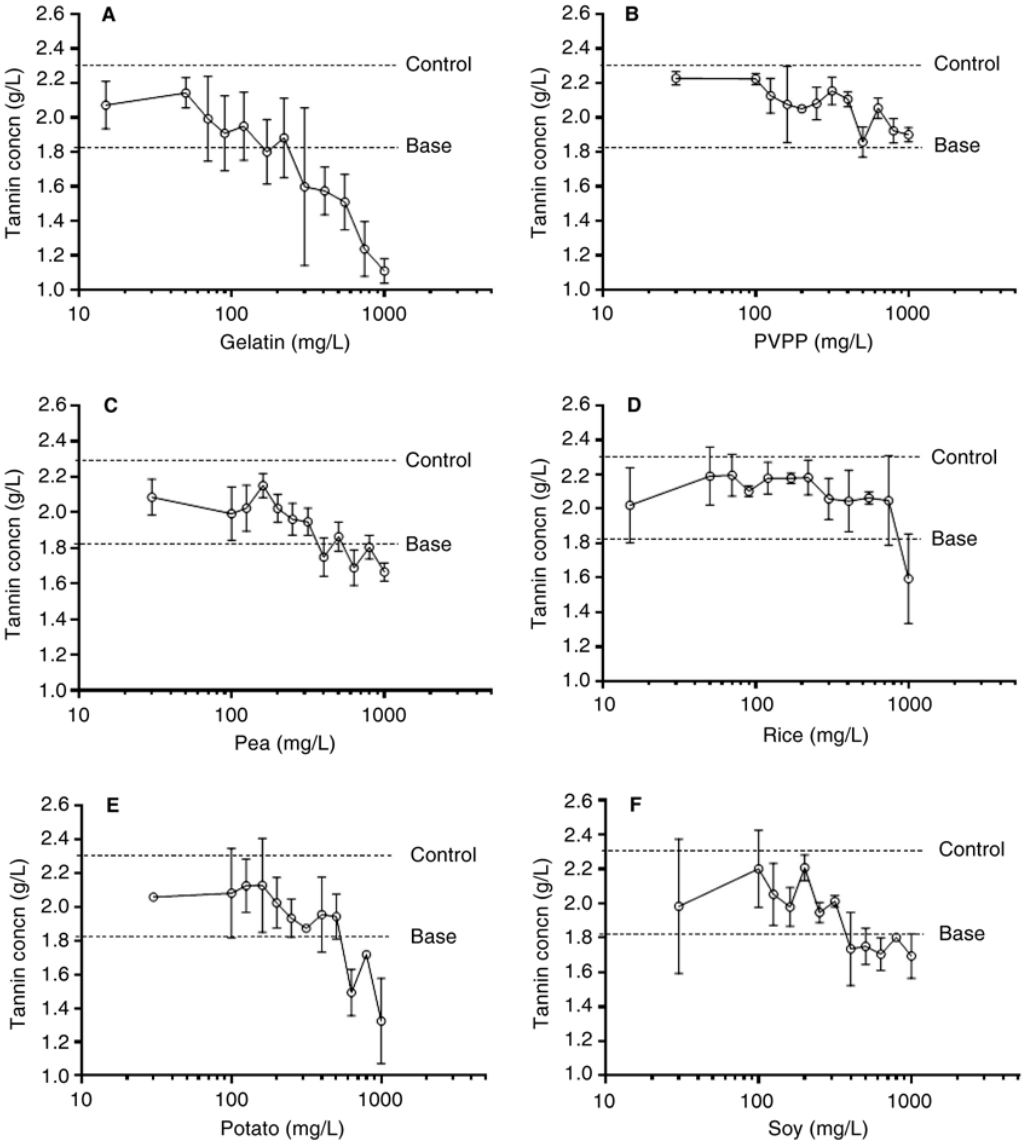


Figure 1 Mean tannin concentrations (± standard deviation) as a function of log fining concentrations of (**A**) gelatin or (**B**) PVPP, or (**C**) pea, (**D**) rice, (**E**) potato, or (**F**) soy protein. The dotted lines denote mean tannin concentrations of control and base wine samples. PVPP, polyvinylpolypyrrolidone.

monomers, but this requires further verification. These fining agents are thought to interact with phenolic compounds through the same principles of hydrophobic interactions and hydrogen bonding. The phenolic compounds in wine are hydrophobic because they have aromatic ring structures and interact with hydrophobic pockets of proteins, and phenolic hydroxyl groups share a proton with the keto-imide carbonyl of peptides (Scollary et al. 2012). However, the different MW fractions and tertiary structures of proteins provide different binding sites (Sarni-Manchado et al. 1999). Hence, the MW fractions and tertiary structures of proteins need to be measured and determined in future studies to decipher the different characteristics of phenolic compound removal by these fining agents.

The influence of fining agent on pH and color. pH is known to affect both wine color (Somers and Evans 1977) and astringency perception (Payne et al. 2009). Low-level addition of soy protein resulted in a significantly lower pH ($p < 0.05$) (data not shown). However, the range of difference in pH was at most 0.02, which would not profoundly affect color and astringency perception. The minimal fining effect of PVPP on pH was consistent with literature (Gómez-Plaza et al. 2000).

The effect of fining agents on wine color was also determined. The chroma represents the degree of deviation from gray toward pure chromatic color, and is similar to color saturation or intensity in the tristimulus spectrum (McGuire 1992). All fining agents significantly reduced chroma as a function of fining concentration ($p < 0.001$ for gelatin and PVPP and for rice, potato, and soy protein, and $p < 0.01$ for pea protein), with gelatin reducing chroma the most (Figure 3). A decrease in wine chroma by pea protein was also reported by Cosme et al. (2012).

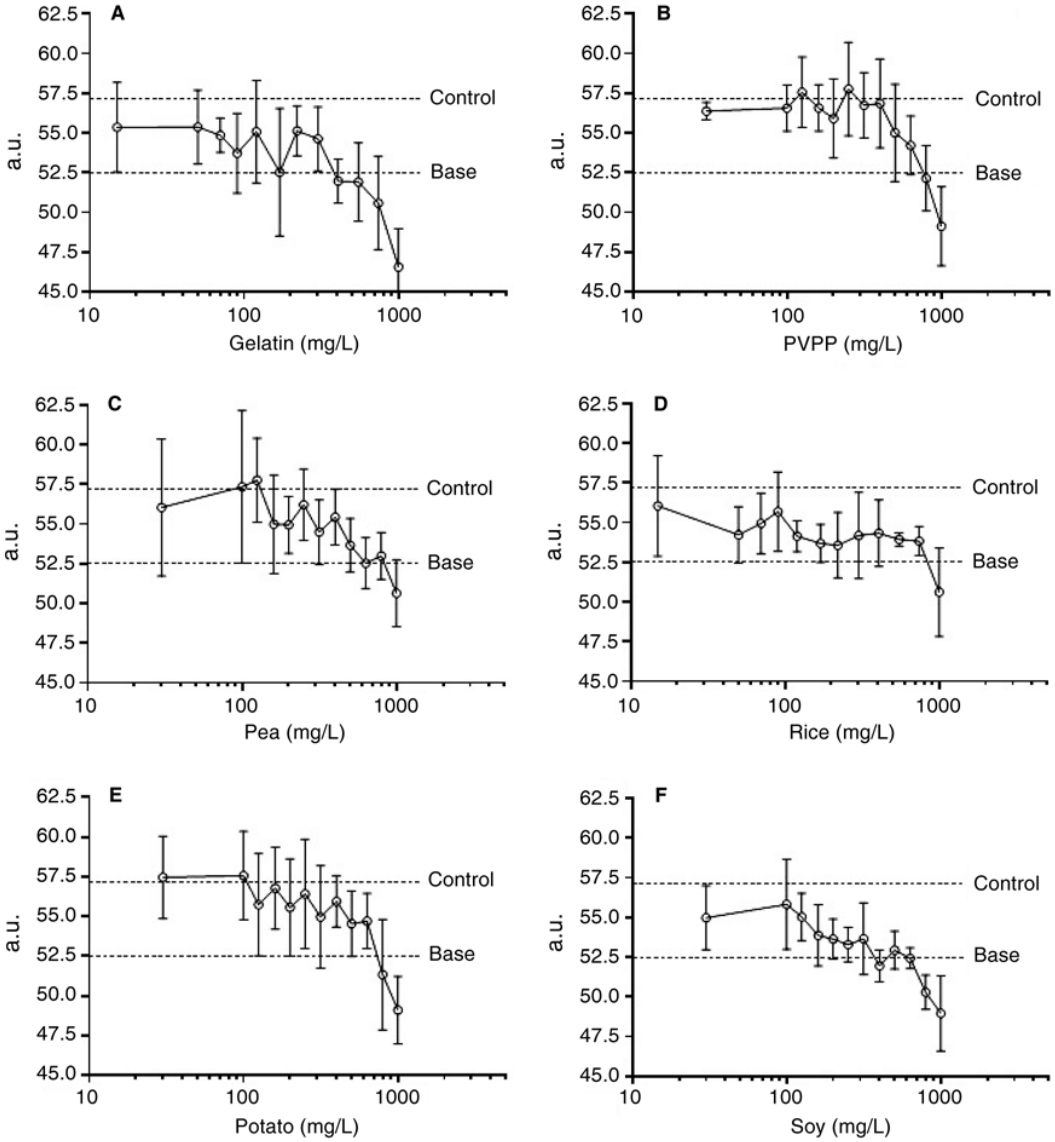


Figure 2 Mean total phenolics values (± standard deviation) as a function of log fining concentrations of (**A**) gelatin or (**B**) PVPP, or (**C**) pea, (**D**) rice, (**E**) potato, or (**F**) soy protein. The dotted lines denote mean tannin concentrations of control and base wine samples. PVPP, polyvinylpolypyrrolidone. a.u., absorbance units.

Hue was calculated from the CIELab measures, which are colors that are visible to the human eye, such as orange, yellow, beige, brown, pink, or any of the other colors (McGuire 1992). Potato and soy proteins significantly ($p < 0.01$ and $p < 0.05$, respectively) decreased hue with increased concentration (Figure 4), with the four remaining agents showing only trends in reduction. This means that the fining treatments with potato and soy proteins could change wine color from yellow-red toward more red hue. The principal compounds contributing to the color of young and aged wines differ (Somers and Verette 1988), so it is likely that fining agents could produce different results in a given wine at different maturation ages. Since the wine used in the current study was aged for only a year, the results for hue indicated that potato and soy proteins could influence those representative compounds, and thus influence the hue in young red wines. However, this finding differed from those in previous reports, which used young red wines as well (albeit less than 3 yrs aging), and suggested that potato proteins have fewer effects on wine hue (Tschiersch et al. 2010, Gambuti et al. 2012). Differences in phenolic composition in the wines across these studies may have been the reason for this discrepancy. Further, the conclusion of these previous studies was based on the results of spectrometric measurement of several wavelengths, rather than a whole range (375 to 780 nm) from CIELab.

The compounds mainly representing the color in aged wines are polymeric pigments resistant to SO_2 bleaching (Somers and Verette 1988). In our study, SO_2-resistant pigments were significantly decreased by gelatin and pea and potato proteins (all $p < 0.001$) and soy protein ($p < 0.01$) (Table 2). This suggested that these four fining agents have the potential to change color in aged wines, and by contrast, that rice proteins could be possibly utilized in older wine without causing any color change. The performance of gelatin in the current study confirmed the color change that mainly stems from polymeric pigments and has been observed in previous reports (Somers and Verette 1988). In addition, when we integrated the results of CIELab in the current study, both potato and soy proteins were found to influence the color of both young and aged, equivalent wines. Intensely colored red wines are associated with high quality by consumers (Parpinello et al. 2009). Therefore, the implications of using potato and soy proteins as fining agents in industry could consequently influence consumers' preference.

Sensory evaluation. Wines fined with three concentrations per fining agent were evaluated for astringency, bitterness, and overall flavor intensity. The mean astringency intensities for each fining agent and the control and base wine samples were determined (Figure 5). Gelatin and potato protein significantly ($p < 0.05$) reduced the astringency intensity from the control (wine with added GSE and unfined). Fining wine with 315 mg/L gelatin had a significantly lower astringency intensity than that of the control. Further, fining with 1000 mg/L of gelatin and potato protein both yielded significantly lower astringency intensities than the base wine (a reduction of 31.3% and 31.6% on the scale, respectively). In agreement with the research by Gambuti et al. (2012), potato protein appeared to be a suitable alternative to conventional fining agents. These authors also reported that 300 mg/L potato protein reduces more astringency than does gelatin at the same concentration, but this was not observed in the current study. Differences in the fining procedures used between Gambuti et al. (2012) and the current study may explain these divergent results. In addition, the remaining four fining agents (PVPP and pea, rice, and soy proteins) did not significantly reduce astringency. Nonetheless, there was a trend in astringency reduction ($p = 0.055$) in the treatments fined by soy proteins.

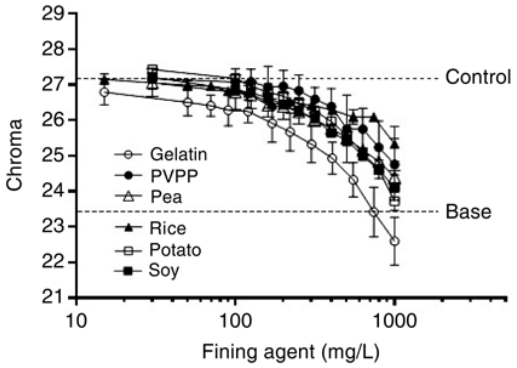


Figure 3 Mean chroma (± standard deviation) as a function of log fining concentrations. The dotted lines denote mean chroma of control and base wine samples. PVPP, polyvinylpolypyrrolidone.

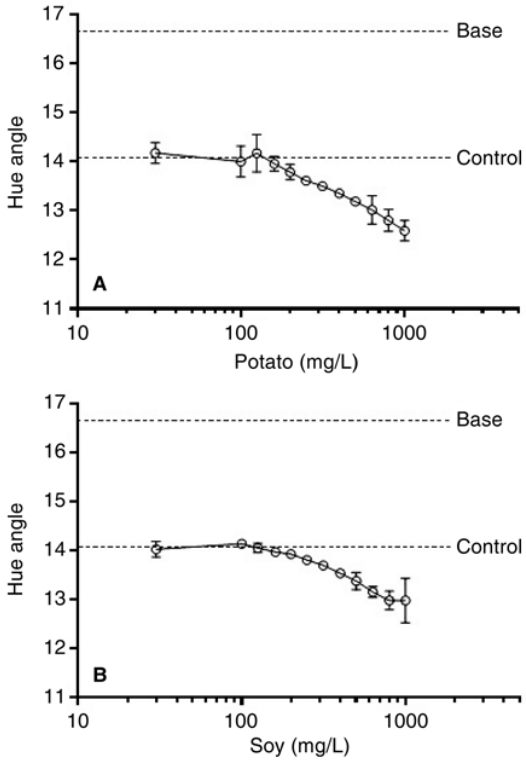


Figure 4 Mean hue angle (± standard deviation) of wines fined as a function of log (**A**) potato or (**B**) soy protein concentration. The dotted lines denote mean chroma of control and base wine samples.

To compare the influences of fining agent type and concentration on sensory attributes, we analyzed the data with GLM-ANOVA with two-way interactions. Astringency intensity significantly differed by fining agent type overall and by fining concentration overall (both $p < 0.001$) (Figure 6). Rice protein overall showed a fining ability similar to that of PVPP. The remaining three vegetable proteins overall did not significantly differ in fining ability from gelatin. Of all the vegetable proteins tested, that from potato reduced astringency the most. This was followed by pea and soy proteins, which both reduced astringency similarly, and lastly by rice protein. The comparison of mean fining concentrations across the fining agents showed that 315 and 1000 mg/L were more effective than 50 mg/L, which was not surprising. However, a fining concentration >315 mg/L overall did not significantly reduce astringency any further. No significant fining agent by concentration interaction was detected, which suggested that observations due to the effect of concentration were independent of the fining agent used. There was a significant influence of assessor on astringency perception, which is a common observation in sensory evaluation that stems from differences in individual scale use as well as sensitivities (Meilgaard et al. 2006). Encouragingly, the assessors could consistently rate the astringency intensity of each sample across sessions, as a significant replicate × assessor interaction was not detected.

In the current study, the changes in astringency perception resulting from fining with rice, pea, or soy protein were determined and have not been reported previously. Pea and soy proteins reduced tannin and astringency similarly. In addition, rice proteins reduced astringency very little, an observation similar to that for PVPP. It must be noted that the vegetable proteins (pea, rice, and soy) used in this study were unpurified mixtures. That is, they were not enological processing aids, but were able to reduce astringency and demonstrated their potential function as fining agents. Further experiments could be conducted to refine or purify the protein fractions from these supplements or other vegetable extracts to repeat these studies and examine their efficacy relative to gelatin. The positive findings from the vegetable protein fining on astringency reduction suggest that more studies are required to assess the effects of vegetable proteins from other sources, such as wheat, sorghum, carrot, and onion peel or even ocean-derived algal proteins, on sensory perception.

Bitterness did not significantly differ by fining agent or fining agent concentration. It has been reported that low-MW phenolics such as flavan-3-ols might influence the potential bitterness sensation of wines (Hufnagel and Hofmann 2008, Sáenz-Navajas et al. 2010). However, as differences in bitterness in the current study were small (51.2 to 59.7% on the scale), fining may not have influenced the concentration of phenolic compounds sufficiently to also affect bitterness. The compounds for bitterness need to be measured and compared with known values in further research to confirm this observation. All combinations of two-way interactions were not significant for bitterness.

The sensory panel did not detect significant differences in overall flavor intensity. This suggested that all six agents did not cause a noticeable change in flavor intensity at the three fining concentrations used in the current study. Previous literature also reported that fining with pea proteins did not lead to discernible differences in flavor intensity (Cosme et al. 2012). These authors suggested that the addition of pea

Table 2 SO_2-resistant pigments of control, base, and fined wine samples.[a]

Fining levels[b]	Gelatin	PVPP[c]	Pea protein	Rice protein	Potato protein	Soy protein
0[d]	2.59 ± 0.12 a	2.59 ± 0.12	2.59 ± 0.12 a	2.59 ± 0.12	2.59 ± 0.12 ab	2.59 ± 0.12 ab
1	2.53 ± 0.05 a	2.57 ± 0.12	2.54 ± 0.06 a	2.71 ± 0.13	2.67 ± 0.03 a	2.53 ± 0.15 abc
2	2.54 ± 0.03 a	2.58 ± 0.13	2.54 ± 0.04 a	2.71 ± 0.08	2.61 ± 0.04 ab	2.53 ± 0.07 abc
3	2.47 ± 0.05 ab	2.58 ± 0.11	2.56 ± 0.07 a	2.68 ± 0.06	2.59 ± 0.04 abc	2.61 ± 0.02 a
4	2.47 ± 0.12 ab	2.58 ± 0.08	2.51 ± 0.05 ab	2.69 ± 0.10	2.53 ± 0.01 abcd	2.49 ± 0.09 abcd
5	2.53 ± 0.00 a	2.58 ± 0.00	2.51 ± 0.04 ab	2.68 ± 0.09	2.55 ± 0.10 abc	2.49 ± 0.07 abcd
6	2.41 ± 0.03 abc	2.57 ± 0.13	2.56 ± 0.01 a	2.63 ± 0.10	2.51 ± 0.03 abcd	2.54 ± 0.02 abc
7	2.43 ± 0.19 abc	2.58 ± 0.11	2.50 ± 0.00 ab	2.61 ± 0.04	2.47 ± 0.04 bcde	2.50 ± 0.03 abcd
8	2.30 ± 0.10 bcd	2.56 ± 0.06	2.41 ± 0.00 bc	2.59 ± 0.04	2.42 ± 0.05 cdef	2.42 ± 0.02 cd
9	2.26 ± 0.05 cd	2.43 ± 0.19	2.42 ± 0.00 bc	2.62 ± 0.14	2.38 ± 0.08 def	2.43 ± 0.07 bcd
10	2.17 ± 0.03 d	2.46 ± 0.01	2.35 ± 0.00 c	2.61 ± 0.09	2.32 ± 0.05 ef	2.34 ± 0.04 de
11	2.11 ± 0.07 de	2.40 ± 0.11	2.33 ± 0.01 c	2.58 ± 0.14	2.30 ± 0.09 f	2.25 ± 0.08 e
12	1.92 ± 0.09 e	2.24 ± 0.11	2.23 ± 0.01 d	2.38 ± 0.14	2.09 ± 0.14 g	2.24 ± 0.06 e
F	9.598	1.290	1.779	10.185	7.908	4.654
p value	<0.001	ns[e]	<0.001	ns	<0.001	<0.01

[a]Data are mean (± standard deviation) absorbance units, analyzed with one-way analysis of variance and Fisher's least significant difference test. A post-hoc test was run across fining concentrations within each fining agent; values followed by the same letter in a column are not significantly different. The value of resistant pigments in the base wine was 2.73 ± 0.02 absorbance units.
[b]Each fining level represents the different fining concentrations for each agent, and level increase means fining concentration increase. Further details are shown in Table 1.
[c]PVPP, polyvinylpolypyrrolidone.
[d]Control wine with added grape seed extract.
[e]ns: no significant difference.

protein also has no sensorial impact on visual, aroma, and taste characters. All combinations of two-way interactions were not significant for overall flavor intensity.

The relationships between chemical and sensory data. Although all six fining agents significantly reduced total tannin concentration, significant reductions in astringency intensity were detected only for gelatin and potato protein. The cause for this could be that gelatin and potato protein had a greater affinity for the higher polymerized tannins or galloylated tannin forms than the other fining agents, thereby removing tannins that were stronger elicitors of astringency perception. Tannins with higher polymerization levels are known to elicit higher astringency intensity (Noble 1994). Galloyl aromatic rings enhance the hydrophobic interactions with the PRPs' proline ring, and a higher percentage of galloylation leads to further precipitation of salivary proteins (Zhu et al. 1997, Charlton et al. 2002, Vidal et al. 2004). Whether this was the case here is unknown, as tannin concentration was determined in the current study with the methylcellulose-precipitable method, which is a general tannin assay. To confirm this speculation, further investigation is required using targeted tannin analysis methods such as high-performance liquid chromatography (Kennedy and Jones 2001). Ideally, the choice of fining concentration for both the chemical and sensory experiments should be the same to enable direct correlations between the two data sets. The preliminary data reported here provide directions for future studies. Further studies that also determine the MW fractions and tertiary structure of vegetable proteins would enhance further understanding of their mode of action in the

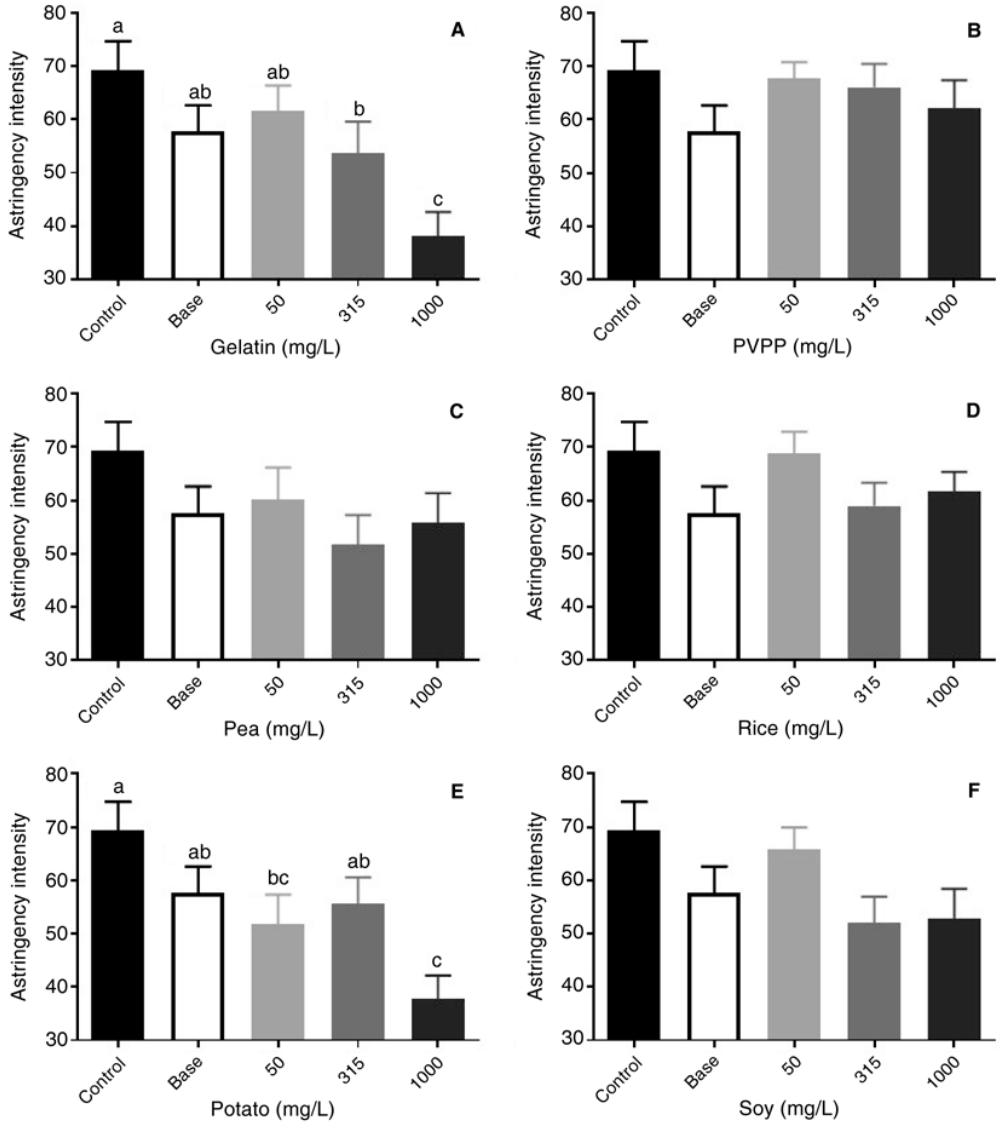


Figure 5 Mean astringency intensities (± standard error) of wines fined with (**A**) gelatin or (**B**) PVPP, or (**C**) pea, (**D**) rice, (**E**) potato, or (**F**) soy proteins, each compared with control and base wine sample. The different superscript letters indicate significant differences ($p < 0.05$), determined by analysis of variance with Fisher's least significant difference test. PVPP, polyvinylpolypyrrolidone.

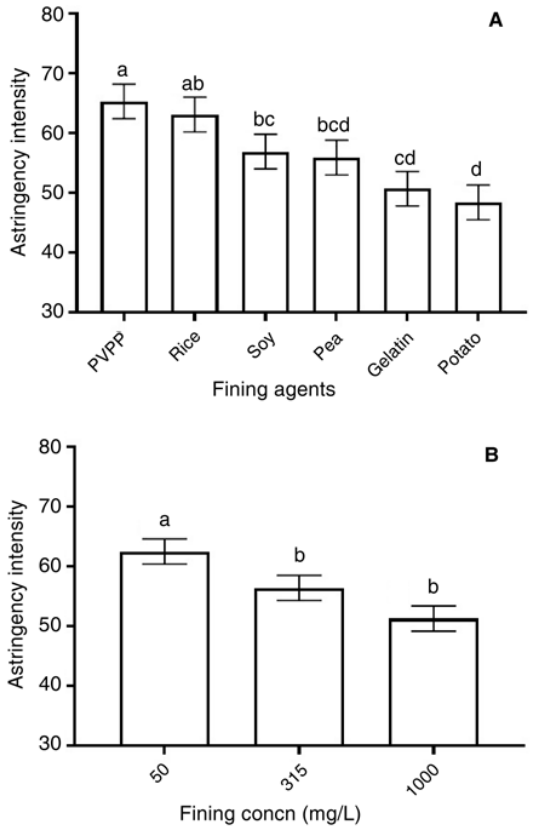


Figure 6 Mean astringency intensities (± standard error) by (**A**) fining agent or (**B**) fining concentration. The different superscript letters indicate significant differences ($p < 0.05$), determined by analysis of variance with Fisher's least significant difference test.

interactions with tannin. As the current study was conducted on a simulated unfined wine with the addition of GSE, testing on commercial, unfined wine is necessary to confirm the industrial relevance of the vegetable proteins.

Conclusion

Tannins in wine can be fined with alternative vegetable proteins. The possibility of using rice or soy protein to fine wine and reduce astringency has been demonstrated for the first time. The similar effects observed for rice protein and PVPP on the reduction of total phenolics and chroma and marginal impacts on astringency perception, suggest the potential for these two fining agents to be used interchangeably. Further, potato proteins have the potential to substitute for gelatin because of its similar ability to decrease tannins and astringency perception. However, potato proteins can influence wine color; thus, the wine industry should use this protein with caution.

Literature Cited

Baron R, Mayen M, Merida J and Medina M. 1997. Changes in phenolic compounds and color in pale Sherry wines subjected to fining treatments. Z Lebensm Unters F A 205:474-478.

Bastian SEP, Collins C and Johnson TE. 2010. Understanding consumer preferences for Shiraz wine and Cheddar cheese pairings. Food Qual Prefer 21:668-678.

Boulton RB, Singleton VL, Bisson LF and Kunkee RE. 2013. Principles and Practices of Winemaking. Springer Science & Business Media, New York.

Charlton AJ, Baxter NJ, Khan ML, Moir AJG, Haslam E, Davies AP and Williamson MP. 2002. Polyphenol/peptide binding and precipitation. J Agric Food Chem 50:1593-1601.

Cosme F, Ricardo-da-Silva JM and Laureano O. 2009. Effect of various proteins on different molecular weight proanthocyanidin fractions of red wine during wine fining. Am J Enol Vitic 60:74-81.

Cosme F, Capao I, Filipe-Ribeiro L, Bennett RN and Mendes-Faia A. 2012. Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics. LWT–Food Sci Technol 46:382-387.

Donovan JL, McCauley JC, Tobella NT and Waterhouse AL. 1998. Effects of small-scale fining on the phenolic composition and antioxidant activity of Merlot wine. *In* Chemistry of Wine Flavor. Waterhouse AL and Eberler SE (eds.), pp. 142-155. ACS Symposium Series, Washington, DC.

Gambuti A, Rinaldi A and Moio L. 2012. Use of patatin, a protein extracted from potato, as alternative to animal proteins in fining of red wine. Eur Food Res and Technol 235:753-765.

Gawel R. 1998. Red wine astringency: A review. Aust J Grape Wine Res 4:74-95.

Gómez-Plaza E, Gil-Muñoz R, López-Roca JM, De La Hera-Orts ML and Martínez-Cultíllas A. 2000. Effect of the addition of bentonite and polyvinylpolypyrrolidone on the color and long-term stability of red wines. J Wine Res 11:223-231.

Granato TM, Nasi A, Ferranti P, Iametti S and Bonomi F. 2014. Fining white wine with plant proteins: Effects of fining on proanthocyanidins and aroma components. Eur Food Res Technol 238:265-274.

Hagerman AE and Butler LG. 1980. Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28:947-952.

Hahn GD and Possmann P. 1977. Colloidal silicon dioxide as a fining agent for wine. Am J Enol Vitic 28:108-112.

Hufnagel JC and Hofmann T. 2008. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. J Agric Food Chem 56:9190-9199.

Iland P. 1980. Practical considerations in pH measurements of grape juice and wine. Aust NZ Grapegr Winemaker 196:36-38.

Jöbstl E, O'Connell J, Fairclough JPA and Williamson MP. 2004. Molecular model for astringency produced by polyphenol/protein interactions. Biomacromolecules 5:942-949.

Kennedy JA and Jones GP. 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J Agric Food Chem 49:1740-1746.

Laborde B, Moine-Ledoux V, Richard T, Saucier C, Dubourdieu D and Monti JP. 2006. PVPP-polyphenol complexes: A molecular approach. J Agric Food Chem 54:4383-4389.

Lattey KA, Bramley BR and Francis IL. 2010. Consumer acceptability, sensory properties and expert quality judgements of Australian Cabernet Sauvignon and Shiraz wines. Aust J Grape Wine Res 16:189-202.

Lee CB and Lawless HT. 1991. Time-course of astringent sensations. Chem Senses 16:225-238.

Margalit Y. 2012. Concepts in Wine Chemistry. 3rd ed. Board and Bench Publishing, San Francisco.

Maury C, Sarni-Manchado P, Lefebvre S, Cheynier V and Moutounet M. 2003. Influence of fining with plant proteins on proanthocyanidin composition of red wines. Am J Enol Vitic 54:105-111.

Maury C, Sarni-Manchado P, Poinsaut P, Cheynier V and Moutounet M. 2016. Influence of polysaccharides and glycerol on proanthocyanidin precipitation by protein fining agents. Food Hydrocolloid 60:598-605.

McGuire RG. 1992. Reporting of objective color measurements. HortScience 27:1254-1255.

McRae JM, Schulkin A, Kassara S, Holt HE and Smith PA. 2013. Sensory properties of wine tannin fractions: Implications for in-mouth sensory properties. J Agric Food Chem 61:719-727.

Meilgaard MC, Carr BT and Civille GV. 2006. Sensory Evaluation Techniques. 4th ed. CRC Press, Boca Raton FL.

Mercurio M and Smith PA. 2006. New formats for the methyl cellulose precipitable (MCP) tannin assay allow high throughput measurement of grape and wine tannin by industry. AWRI Tech Rev 164:1-10.

Mercurio MD, Dambergs RG, Herderich MJ and Smith PA. 2007. High throughput analysis of red wine and grape phenolics – adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. J Agric Food Chem 55:4651-4657.

Mills EN, Sancho AI, Rigby NM, Jenkins JA and Mackie AR. 2009. Impact of food processing on the structural and allergenic properties of food allergens. Mol Nutr Food Res 53:963-969.

Noble AC. 1994. Bitterness in wine. Physiol Behav 56:1251-1255.

Parpinello GP, Versari A, Chinnici F and Galassi S. 2009. Relationship among sensory descriptors, consumer preference and color parameters of Italian Novello red wines. Food Res Int 42:1389-1395.

Payne C, Bowyer PK, Herderich M and Bastian SEP. 2009. Interaction of astringent grape seed procyanidins with oral epithelial cells. Food Chem 115:551-557.

Peynaud E and Blouin J. 1996. The Taste of Wine: The Art and Science of Wine Appreciation. John Wiley & Sons, New Jersey.

Poncet-Legrand C, Gautier C, Cheynier V and Imberty A. 2007. Interactions between flavan-3-ols and poly (L-proline) studied by isothermal titration calorimetry: Effect of the tannin structure. J Agric Food Chem 55:9235-9240.

Rankine B. 2004. Making Good Wine. Pan Macmillan Australia Pty Limited, Sydney.

Ribéreau-Gayon P, Glories Y, Maujean A and Dubourdieu D. 2006. Phenolic compounds. *In* Handbook of Enology: The Chemistry of Wine Stabilization and Treatments, vol. 2, 2nd ed. pp. 141-203. John Wiley & Sons, Ltd, Chichester, UK.

Sáenz-Navajas MP, Ferreira V, Dizy M and Fernández-Zurbano P. 2010. Characterization of taste-active fractions in red wine combining HPLC fractionation, sensory analysis and ultra performance liquid chromatography coupled with mass spectrometry detection. Anal Chim Acta 673:151-159.

Saltman Y, Johnson TE, Wilkinson KL and Bastian SEP. 2015. Australian wine consumers' acceptance of and attitudes toward the use of additives in wine and food production. Int J Wine Res 7:83-92.

Sarneckis CJ, Dambergs RG, Jones P, Mercurio M, Herderich MJ and Smith PA. 2006. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. Aust J Grape Wine Res 12:39-49.

Sarni-Manchado P, Deleris A, Avallone S, Cheynier V and Moutounet M. 1999. Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agent in enology. Am J Enol Vitic 50:81-86.

Scollary GR, Pásti G, Kállay M, Blackman J and Clark AC. 2012. Astringency response of red wines: Potential role of molecular assembly. Trends Food Sci Tech 27:25-36.

Simonato B, Mainente F, Suglia I, Curioni A and Pasini G. 2009. Evaluation of fining efficiency of corn zeins in red wine: A preliminary study. Ital J Food Sci 21:97-105.

Simonato B, Mainente F, Selvatico E, Violoni M and Pasini G. 2013. Assessment of the fining efficiency of zeins extracted from commercial corn gluten and sensory analysis of the treated wine. LWT–Food Sci Technol 54:549-556.

Sims CA, Eastridge JS and Bates RP. 1995. Changes in phenols, color, and sensory characteristics of muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. Am J Enol Vitic 46:155-158.

Somers TC and Evans ME. 1974. Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. J Sci Food Agric 25:1369-1379.

Somers TC and Evans ME. 1977. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2,"chemical age." J Sci Food Agric 28:279-287.

Somers TC and Verette E. 1988. Phenolic composition of natural wine types. *In* Wine Analysis. Modern Methods of Plant Analysis, vol 6. Linskens HF and Jackson JF (eds), pp. 219-257. Springer, Berlin, Heidelberg.

Tschiersch C, Pour Nikfardjam MSP, Schmidt O and Schwack W. 2008. Comparison of seventeen different fining agents used with wine. Mitt Klosterneuburg 58:123-131.

Tschiersch C, Nikfardjam MP, Schmidt O and Schwack W. 2010. Degree of hydrolysis of some vegetable proteins used as fining agents and its influence on polyphenol removal from red wine. Eur Food Res and Technol 231:65-74.

Vidal L, Giménez A, Medina K, Boido E and Ares G. 2015. How do consumers describe wine astringency? Food Res Int 78:321-326.

Vidal S, Francis L, Williams P, Kwiatkowski M, Gawel R, Cheynier V and Waters E. 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chem 85:519-525.

Vincenzi S, Dinnella C, Recchia A, Monteleone E, Gazzola D, Pasini G and Curioni A. 2013. Grape seed proteins: A new fining agent for astringency reduction in red wine. Aust J Grape Wine Res 19:153-160.

Voilley A, Lamer C, Dubois P and Feuillat M. 1990. Influence of macromolecules and treatments on the behavior of aroma compounds in a model wine. J Agric Food Chem 38:248-251.

Zhu M, Phillipson JD, Greengrass PM, Bowery NE and Cai Y. 1997. Plant polyphenols: Biologically active compounds or non-selective binders to protein? Phytochemistry 44:441-447.

Zoecklein BW, Fugelsang KC, Gump BH and Nury FS. 1995. Fining and fining agents. *In* Wine Analysis and Production, pp. 242-271. Springer, Boston, MA.